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Infertility, Mendelian randomization, Circulating plasma metabolites

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This study used a two-sample Mendelian randomization design,utilizing circulating plasma metabolite genome wide association study data as an exposure factor and FinnGen Consortium R10 genome wide association study data for infertility in men and women as an outcome. The causal relation between plasma metabolites and infertility in men and women was assessed using five methods: inverse variance weighted, Egger regression, weighted median, maximum likelihood estimation, and simple mode.

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This analysis identified 17 and 10 metabolites positively and negatively associated with infertility in women, respectively. Similarly, 22 and 30 metabolites were positively and negatively associated with infertility in men, respectively. Galactonate and glycerate levels were identified as risk factors for infertility in both men and women. In addition, sphingomyelin exerts protective effects against infertility in both men and women. Metabolic pathway analysis revealed enrichment of critical metabolic pathways related to infertility.

Conclusions
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Genetic causal associations between serum metabolites and infertility: a Mendelian randomization study

Jiahao Shi[1+], Jiamei Zhu[3+], Tian Tao[2*]

[1]Department of Urology, The Affiliated Wuxi People's Hospital of Nanjing Medical University, Wuxi, China

[2]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Soochow University, Suzhou, China

[3]Department of Obstetrics and Gynecology, Jingjiang People's Hospital Affiliated to Yangzhou University, Taizhou, China

*** Corresponding author:**

Tian Tao; Email: taotian_1123@163.com

[+]these authors contributed equality to this work

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Introduction

Infertility, a prevalent reproductive system disorder with rising global incidence, impacts individual well-being, family dynamics, and societal demographics. Defined as the failure to achieve clinical pregnancy after 12 months of regular unprotected intercourse[1]. It affects 14.3% and 25% of reproductive-age couples in developed and developing countries, respectively[2]. Notably, male factors contribute to nearly 50% of cases, highlighting the need for early prevention, diagnosis, and targeted management strategies.

Integrative omics (transcriptomics, proteomics, metabolomics) has emerged as a pivotal tool in life sciences. Evidence indicates that metabolic disorders (e.g. hypertension, diabetes, obesity) are strongly linked to infertility in both sexes[3,4]. Metabolomics, extending beyond genomics and proteomics[5, 6], identifies metabolic biomarkers and elucidates pathogenic pathways by analyzing circulating metabolite profiles between infertile patients and healthy controls, offering novel therapeutic targets for infertility prevention and treatment.

Mendelian randomization (MR) is an epidemiological method that utilizes single-nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to infer causal relationships between exposures and diseases, circumventing limitations of observational studies[7]. Leveraging genome-wide association study (GWAS) data, we applied a two-sample MR approach to investigate causal effects of serum metabolites on infertility and identify underlying metabolic pathways.

Materials and methods

Study design

We used a two-sample MR approach based on GWAS summary statistics to investigate causal associations between circulating metabolites in humans and the risk of infertility in both men and women. This is a genetic epidemiological approach for investigating causality and addressing confounders by using IVs under three assumptions: (i) IVs are not associated with confounders; (ii) IVs are associated with exposure factors; and (iii) IVs are not associated with outcome variables, with IVs influencing outcomes only through exposure. Genetic information on circulating metabolites and infertility in humans was obtained from separate GWAS databases, and the flowchart of this MR study is shown in Figure 1.

GWAS data

Chen et al. obtained a genome-wide association database of 870 blood metabolites [8]. This is the most comprehensive analysis of blood metabolites in humans and is included in the GWAS catalog. After applying the appropriate exclusion criteria, the Canadian Longitudinal Study of Aging finalized the identification of 8,299 participants between the ages of 45 and 85 years and performed genome-wide typing, identifying 248 linked loci. They were also tested for 1,091 circulating plasma metabolites and 309 metabolite ratios. Of the 1,091 metabolites, 850 had a known identity (including lipids, amino acids, xenobiotics, nucleotides, cofactors and vitamins, carbohydrates, peptides, and energy-related molecules), whereas the remaining 241 were defined as unknown or partially characterized molecules; metabolite ratios and unknown molecules were excluded from this study. Information on these metabolites is provided in Supplementary Table S1.

FinnGen is a large public-private cooperation project covering the entire territory of Finland to determine the etiology of various diseases and assess the role of genetics in health and disease. We downloaded GWAS data related to infertility in both men and women from the R10 version of the FinnGen consortium, which yielded 14,759 infertility cases in women, 111,583 controls, 1,429 infertility cases in men, and 130,139 controls (https://r10.finngen.fi/).

IV selection

In this study, we selected SNPs as genetic IVs. We rigorously screened the IVs associated with serum metabolites using the following steps to ensure the robustness of the MR analyses (Figure 1). First, SNPs with MAF > 0.01 in exposure and outcome were selected, followed by screening for SNPs satisfying the locus-wide significance threshold ($P < 1 \times 10^{-5}$) associated with exposure. Subsequently, SNPs were removed by performing the LD clumping procedure with $r^2 < 0.001$ and a window size = 10,000 kb to eliminate variants in linkage disequilibrium. Genetic variants with F > 10 were selected as strong IVs to avoid bias from weak IVs [9].

MR analysis

Five methods were used to evaluate the causal relation between exposure (plasma metabolites) and outcome (infertility), with the random-effects inverse variance weighted (IVW) model as the primary analytical method, along with the MR–Egger, weighted median, maximum likelihood, and simple mode methods [10]. If only one IV was available for the plasma metabolites, the Wald ratio was used. If two or more IVs were present, the IVW method was used. Results with significant P-values for the IVW method and beta values for the remaining four methods in the same direction as the IVW method were considered meaningful. In the heterogeneity test, Cochran's Q statistic had a P-value > 0.05, indicating no heterogeneity between SNPs at the statistical level. Second, horizontal pleiotropy was assessed using MR–Egger regression. A P-value < 0.05 for the

intercept term indicated the presence of statistically significant horizontal pleiotropy. We detected reverse causality using the Steiger test of directionality, with TRUE indicating the absence of statistically significant reverse causality. The results show that all MR methods yielded results in the same direction and that the MR–Egger method did not detect statistically significant pleiotropy, supporting our main finding. Statistical analyses were conducted using R software (version 4.2.3), and MR analysis was performed using the TwoSampleMR package.

Metabolic pathway analysis

The RaMP database of Web-based Metaconflict 6.0 was used to analyze metabolic pathways leading to infertility (https://www.metabcanalest.ca/), and pathways with FDR < 0.05 were considered significantly enriched.

Results

MR results for infertility in men and women

After excluding unknown metabolites and metabolite ratios, 870 plasma metabolite-associated SNPs with known structures and functions were used. The number of IVs selected for plasma metabolites ranged from 3 to 32, and we derived causal associations between 870 metabolites and infertility in both men and women

(Supplementary Tables S2 and S3). The IVW method showed a positive correlation between increased levels of 17 metabolites and the risk of infertility in women, including eight from the lipid metabolism pathway, three from the amino acid metabolism pathway, two from the cofactor and vitamin pathways, two from the carbohydrate pathway, one from the xenobiotic pathway, and one from a partially characterized molecular pathway. The top five of these metabolites were as follows: 7-methylxanthine (odds ratio [OR] = 1.187, 95% confidence interval [CI] = 1.076–1.311, P = .0007); octadecadienedioate (C18:2-DC) (OR = 1.086, CI = 1.041–1.134, P = .0002); galactonate (OR = 1.192, CI = 1.069–1.329, P = .0016); metabolonic lactone sulfate (OR = 1.067, CI = 1.026–1.109, P = .0011); sphingomyelin (d18:0/18:0, d19:0/17:0) (OR = 1.112, CI = 1.037–1.193, P = .0030). Furthermore, we observed 10 metabolites that were negatively associated with infertility in women. The top five of these included oleate/vaccenate (18:1) (OR = 0.865, CI = 0.784–0.954, P = .0037); glycoursodeoxycholate (OR = 0.892, CI = 0.827–0.963, P = .0032); sphingomyelin (d18:2/23:1) (OR = 0.894, CI = 0.819–0.975, P = .0116); glycocholate (OR = 0.923, CI = 0.862–0.987, P = .0196); glycohyocholate (OR = 0.869, CI = 0.772–0.979, P = .0208) (Figure 2 and Supplementary Table S4).

A total of 22 metabolites were identified as positively associated with infertility in men. The top five metabolites were as follows: 1-oleoyl-GPI (18:1) (OR = 1.429, CI = 1.151–1.773, P = .0012); 11β-hydroxyandrosterone glucuronide (OR = 1.382, CI =

1.134–1.683, P = .0013); tauro-β-muricholate (OR = 1.178, CI = 1.051–1.319, P = .0048); 4-vinylguaiacol sulfate (OR = 1.408, CI = 1.110–1.788, P = .0049); trans-4-hydroxyproline (OR = 1.372, CI = 1.095–1.718, P = .0059). Concurrently, 30 metabolites were negatively associated with infertility in men. Of these, the top five included 5α-androstan-3β, 17β-diol monosulfate (2) (OR = 0.852, CI = 0.767–0.947, P = .0029); 1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (p-16:0/18:2) (OR = 0.742, CI = 0.608–0.904, P = .0032); sphingomyelin (d18:1/24:1, d18:2/24:0) (OR = 0.659, CI = 0.498–0.873, P = .0036); carnitine C14 (OR = 0.704, CI = 0.543–0.914, P = .0083); androsterone sulfate (OR = 0.903, CI = 0.835–0.976, P = .0100) (Figure 3 and Supplementary Table S5).

Infertility in men and women share several causal metabolites. Pyridoxal was positively associated with infertility in both women (OR = 1.096, CI = 1.027–1.170, P = .0058) and men (OR = 1.257, CI = 1.042–1.518, P = .0171). Galactonate and glycerate levels were risk factors for infertility in both men and women. Furthermore, sphingomyelin exerted a protective effect against infertility and was inversely correlated with infertility in women (d18:2/23:1). By contrast, in infertility in men, six sphingomyelin metabolites were identified: sphingomyelin (d18:0/18:0, d19:0/17:0); sphingomyelin (d18:0/20:0, d16:0/22:0); sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0); sphingomyelin (d18:1/18:1, d18:2/18:0); sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2);

sphingomyelin (d18:1/24:1, d18:2/24:0).

Sensitive analysis

The directionality of the MR results was consistent across all methods (Supplementary Materials S1 and S2). MR–Egger results showed that no horizontal pleiotropy was detected for 27 metabolites associated with infertility in women and 52 metabolites related to infertility in men (Supplementary Table S1). Scatter plots depict the effect of each SNP locus on infertility, with the slopes representing the magnitude of impact. Leave-one-out analysis showed no significant outliers (Supplementary Materials S3 and S4).

Metabolic pathway analysis

A study of metabolic pathways revealed that the five most prevalent metabolic pathways in infertility in women were glycerolipid metabolism, glycine and serine metabolism, valine/leucine/isoleucine degradation, bile acid biosynthesis, and thiamine metabolism (Figure 4A). The five metabolic pathways strongly associated with infertility in men were de novo triacylglycerol biosynthesis, malate-aspartate shuttle, glycerol phosphate shuttle, cardiolipin biosynthesis, and bile acid biosynthesis. Additionally, five metabolic pathways were common to infertility in both men and women (Figure 4B): bile acid biosynthesis, vitamin B6 metabolism, glycerolipid metabolism, transfer of acetyl groups into

mitochondria, and valine/leucine/isoleucine degradation.

Discussion

In this two-sample MR analysis utilizing largescale GWAS data, we systematically investigated the potential causal associations between 871 plasma metabolites and infertility in both men and women. Multiple risk and protective factors associated with infertility, as well as key metabolic pathways, were identified. The results provide new evidence and insights into infertility pathogenesis and potential targets for prevention and treatment. Metabolomics, a widely used method for studying reproduction-related diseases, effectively identifies biomarkers in blood, semen, and follicular fluid. Among these, blood samples are particularly valuable because of their accessibility and rich metabolic profile. This study not only validated characteristic metabolites but also further elucidated key metabolic pathways leading to infertility, laying an important foundation for a deeper understanding of infertility's molecular mechanisms and the development of precise therapeutic strategies.

A decline in semen quality is a major cause of infertility in men. In some countries, semen quality problems have become a primary issue for young men with fertility problems [11]. One study found differences in the serum metabolic profiles of men with different sperm concentrations and identified the markers as peptides related to the

protein complement C3f [12]. Zhang et al. identified 24 potential biological markers of serum metabolites in patients with azoospermia and in healthy controls. Taurine exhibits high levels in healthy individuals and is enriched in one of the metabolic pathways, gluconeogenesis, which is consistent with the results of the present study [13]. Androgen secretion-stimulating factors play a pivotal role in sperm production, potentially enhancing sperm viability and count [14]. Alipour et al. demonstrated that pyruvate and taurine enhanced sperm viability through a correlation between seminal plasma metabolomic profile and ejaculation-abstinence period length in normozoospermic men [15]. Tauro-β-muricholate is a compound that combines taurine and bile acids, exhibiting dual functionality as both bile acids and taurine. Bile acids are associated with idiopathic infertility. Furthermore, FXR binds to endogenous bile acids and impedes spermatozoa fertilization [16]. This study indicates that serum metabolites of tauro-β-muricholate may be a risk factor for infertility in men. This may be related to the biological function of bile acids, and further investigation is required to elucidate the specific biological mechanisms. In the present study, carnitine C18:2 was identified as a potential biomarker of infertility in men, whereas carnitine C14 was found to have a protective effect against this condition. The carnitine family encompasses a range of active forms with distinct and significantly varying functions among the various metabolite members. Urine metabolomic analyses have shown that acylcarnitines, carnitine C8, and carnitine C10:2 are strongly associated with infertility in men [17]. However, other studies have indicated that carnitine, a

naturally occurring antioxidant in mammals, is a potential therapeutic agent for improving infertility in men. In vitro experiments have demonstrated that L-carnitine significantly improves sperm quality in patients with severe spermatopenia [18]. These findings suggest that the roles of carnitine and its metabolites in infertility in men are complex and varied and that their specific functional mechanisms require further in-depth study.

A comprehensive literature review indicated that the principal metabolic pathways implicated in infertility in men are the tricarboxylic acid cycle, oxidative phosphorylation, glycolysis, and lipid metabolism pathways [19]. Our findings indicate that the metabolic pathways associated with infertility in men, including fatty acid biosynthesis, gluconeogenesis, and the Warburg effect, are linked to the tricarboxylic acid cycle, glycolysis, and lipid metabolism. Spermatocyte motility and capacitation are dependent on glycolysis. It has been proposed that the pathogenesis of infertility in men may be related to a decrease in ATP due to a blockage of the glycolytic pathway in spermatocytes [20]. Lipid metabolism plays an important role in fertility in men. The synthesis of sperm cell membranes is dependent on lipids. In men with infertility having abnormal lipid metabolism, reduced levels of phospholipids and elevated levels of free fatty acids have been observed. Mutation in the sphingomyelin synthase gene cause spermatogenesis defects in mice [21]. In summary, the pathogenesis of infertility in men is closely related

to various metabolic pathways, including the tricarboxylic acid cycle, glycolysis, lipid metabolism, and fatty acid biosynthesis. Collectively, these pathways regulate spermatogenesis, motility, and energetics by affecting the energy supply, cell membrane structure, and spermatozoa function.

A growing body of evidence has indicated that infertility is a metabolic disorder, with polycystic ovary syndrome (PCOS) being the most prevalent form of infertility in women. PCOS is characterized by anovulation and endocrine and metabolic disorders [22]. A prospective randomized controlled trial of patients with PCOS identified six plasma metabolites, including glutamic acid, aspartic acid, 1-methylnicotinamide, acetylcarnitine, glycerophosphocholine, and oleamide, which may serve as potential biomarkers for PCOS [23]. As identified in this study, the metabolites associated with infertility in women were predominantly lipid metabolites. Lipids play pivotal roles in human reproduction. Blood lipids can influence women's reproductive health by regulating the production of steroid hormones and may even have adverse effects on pregnant women and fetuses [24]. The composition of lipids in the follicular fluid influences the functionality of granulosa cells, which in turn affects oocyte development and maturation [25]. An MR study by Jansen et al. showed that elevated triglyceride, total cholesterol, and low-density lipoprotein cholesterol levels were associated with an increased risk of infertility in women [26]. The results of our study indicated that glycerate may be a risk

254 factor for infertility in women. Glycerates are conventionally classified into triglycerides,
255 1,2- or 1,3-glycerol diesters and 1- or 2-glycerol monoesters according to the number and
256 position of the acyl groups. Of these, triglycerides represent the most significant and
257 prevalent constituents; thus, we hypothesized that the primary cause of infertility in
258 women because of glycerate is the action of triglycerides. Furthermore, our findings
259 indicated a positive causal relation between bilirubin levels and infertility in women. One
260 study examined serum total bilirubin, conjugated bilirubin, and free bilirubin levels in
261 infertile and normal fertile women. The results indicated that all three parameters were
262 elevated in the infertile group compared with those in the control group. The implication
263 of these results is that elevated bilirubin levels may contribute to poor pregnancy
264 outcomes [27]. Robert B. Hood et al. identified nine metabolites, including bilirubin,
265 through the metabolomic analysis of follicular fluid from 125 infertile women undergoing
266 IVF [28]. This is consistent with our findings.

267 Studies of women's metabolic pathways have found that infertility in women is associated
268 with bile acid biosynthesis. A previous study identified that serum metabolic pathways in
269 infertile women include fatty acid metabolism, whereas metabolic pathways in follicular
270 fluid include bile acid biosynthesis [29], which is comparable to our findings. Smith et al.
271 identified bile acids and all enzymes involved in the bile acid synthesis pathway in human
272 follicular fluid [30]. These findings suggest that the bile acid synthesis pathway is

273 essential for follicular growth, development, and maturation. However, further
274 investigations are required to elucidate the underlying mechanisms of infertility. A
275 metabolomic analysis of follicular fluid in women with evidence of infertility because of
276 decreased ovarian reserve function revealed that the metabolic pathways involved
277 included pantothenate and CoA biosynthesis and glutathione metabolism [29], consistent
278 with our findings. The biosynthesis of pantothenate and CoA plays a role in the
279 metabolism of sugars, proteins, and fats and exhibits antioxidant effects. These processes
280 influence oocyte development and maturation.

281 This study had several strengths. It represents the most comprehensive investigation to
282 date of the potential causal relation between plasma metabolites and infertility. Second,
283 this study employed five distinct statistical methods for MR to enhance the robustness
284 and credibility of the findings.

285 The metabolic markers and pathways identified in this study have the following clinical
286 translational potential. For instance, the metabolites can be utilised as non-invasive
287 biomarkers for the screening and stratified diagnosis of infertility. The associated
288 metabolic pathways (such as lipid and glucose metabolism) provide new perspectives for
289 developing targeted therapeutic strategies. Furthermore, the monitoring of metabolic
290 processes in high-risk populations (like patients with a family history of infertility or
291 metabolic syndrome) in combination with lifestyle interventions has been shown to

reduce the risk of infertility. However, it should be noted that the study is subject to several limitations. First, the lack of detailed participant clinical characteristics in the database limits the in-depth interpretation of the causal relation between metabolites and infertility, as different etiologies can lead to significant differences in metabolite profiles. Failure to fully consider these clinical features may have affected the generalizability and clinical application of the findings. Future studies should incorporate more detailed clinical information combined with multicenter data to further validate and optimize the reliability of metabolites as infertility biomarkers. Second, the GWAS database employed in this study predominantly comprises data from European populations. Consequently, the generalizability of these results to other racial and geographical groups remains unclear. Third, although the present study revealed significant associations between some serum metabolites and infertility using MR, whether these metabolites directly contribute to infertility or whether their changes are a result of the disease remains uncertain. Some metabolites may be involved in pathological processes by affecting sperm or egg energy metabolism, oxidative stress, or cell membrane function, whereas others may simply reflect changes in the metabolic environment. Future studies combining functional experiments (including gene editing or metabolic interventions) and longitudinal cohort studies are needed to clarify the causal mechanisms.

Conclusion

The fundamental objective of metabolomics is the identification of specific biomarkers. In the present study, MR analysis was used to identify plasma metabolite risk factors and protective factors associated with infertility. The analysis involved 17 risk factors and 10 protective factors for infertility in women and 22 risk factors and 30 protective factors for infertility in men. Certain metabolites play a common role in infertility in both women and men. For instance, pyridoxal was positively associated with infertility in both women and men, whereas galacturonic acid and glycolic acid were identified as common risk factors. Through metabolic pathway analysis, we identified the pivotal metabolic pathways associated with infertility. These findings have yielded significant candidate molecules and established a theoretical foundation for future in-depth research on the molecular mechanisms underlying infertility and the development of targeted intervention strategies.

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Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Publicly available datasets were analyzed in this study. Further inquiries can be directed to the corresponding authors.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

J.H.S. and J.M.Z. conceived and presented the ideas. J.H.S. and T.T. processed the data and wrote the manuscript. J.H.S., T.T., and J.M.Z. participated in data acquisition and interpretation. All authors have read and approved the final manuscript.

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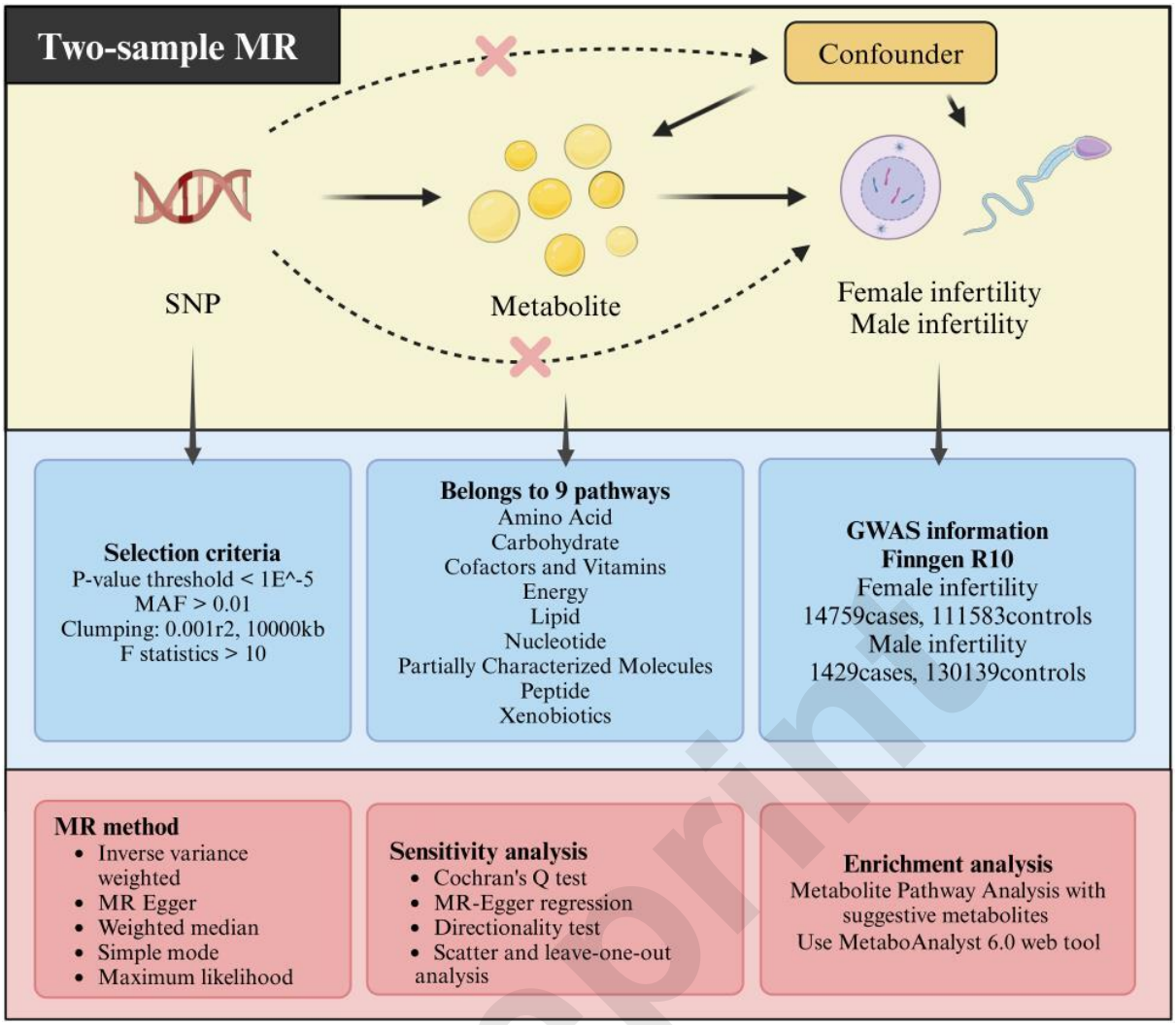
Figure legends

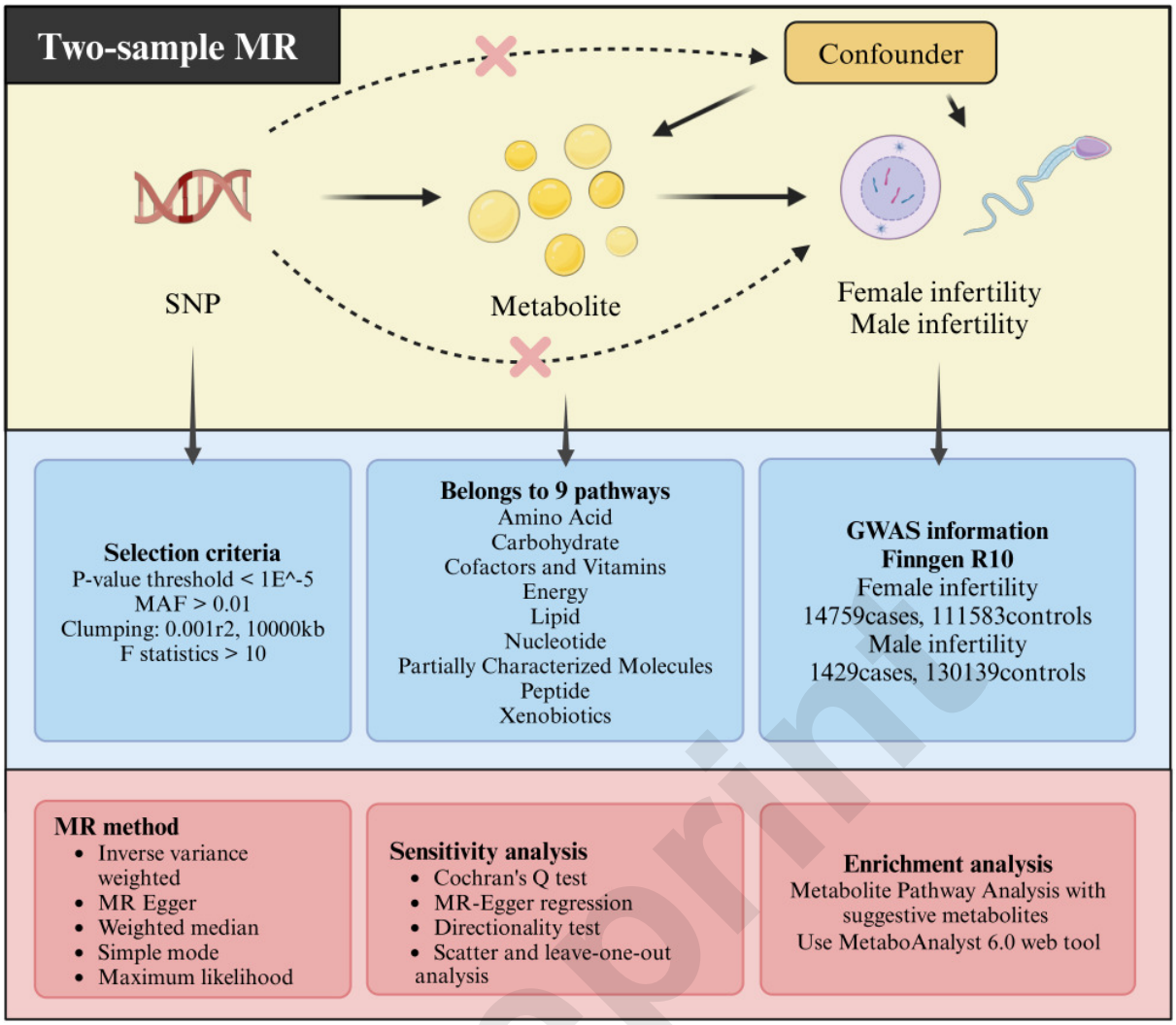
Figure 1 Flowchart of the study design in our Mendelian randomization (MR) analysis

Figure 2 Forest plot of Mendelian randomization (MR) analysis between plasma metabolites and infertility in women (IVW, inverse variance weighted; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval)

Figure 3 Forest plot of Mendelian randomization (MR) analysis between plasma metabolites and infertility in men (IVW, inverse variance weighted; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval)

Figure 4 Enriched significant metabolic pathways of infertility. (A) Significant metabolic pathways involved in infertility in women. (B) Significant enrichment of metabolic pathways in men with infertility


Two-sample MR
Confounder
SNP
Metabolite
Female infertility
Male infertility
Selection criteria
P-value threshold < 1E^-5
MAF > 0.01
Clumping: 0.001r2, 10000kb
F statistics > 10
Belongs to 9 pathways
Amino Acid
Carbohydrate
Cofactors and Vitamins
Energy
Lipid
Nucleotide
Partially Characterized Molecules
Peptide
Xenobiotics
GWAS information
Finngen R10
Female infertility
14759cases, 111583controls
Male infertility
1429cases, 130139controls
MR method
Inverse variance weighted
MR Egger
Weighted median
Simple mode
Maximum likelihood
Sensitivity analysis
Cochran's Q test
MR-Egger regression
Directionality test
Scatter and leave-one-out analysis
Enrichment analysis
Metabolite Pathway Analysis with suggestive metabolites
Use MetaboAnalyst 6.0 web tool


Two-sample MR
Confounder
SNP
Metabolite
Female infertility
Male infertility
Selection criteria
P-value threshold < 1E^-5
MAF > 0.01
Clumping: 0.001r2, 10000kb
F statistics > 10
Belongs to 9 pathways
Amino Acid
Carbohydrate
Cofactors and Vitamins
Energy
Lipid
Nucleotide
Partially Characterized Molecules
Peptide
Xenobiotics
GWAS information
Finngen R10
Female infertility
14759cases, 111583controls
Male infertility
1429cases, 130139controls
MR method
Inverse variance weighted
MR Egger
Weighted median
Simple mode
Maximum likelihood
Sensitivity analysis
Cochran's Q test
MR-Egger regression
Directionality test
Scatter and leave-one-out analysis
Enrichment analysis
Metabolite Pathway Analysis with suggestive metabolites
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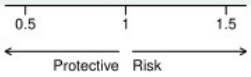
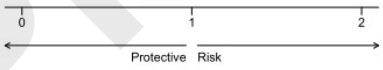
Exposure	SNPs	OR (95% CI)	Pval	Q_pval_IVW	Egger_intercept_pval
(16 or 17)−methylstearate (a19:0 or i19:0)	13	1.125 (1.007 to 1.258)	3.780e−02	0.909	0.833
7−methylxanthine	14	1.187 (1.076 to 1.311)	6.550e−04	0.475	0.985
Bilirubin (E,Z or Z,E)	17	1.059 (1.011 to 1.109)	1.497e−02	0.125	0.758
Deoxycholic acid glucuronide	21	1.068 (1.003 to 1.139)	4.161e−02	0.146	0.053
Eicosenedioate (C20:1−DC)	28	1.070 (1.019 to 1.124)	6.307e−03	0.556	0.446
Galactonate	6	1.192 (1.069 to 1.329)	1.617e−03	0.509	0.929
Glycerate	23	1.072 (1.002 to 1.148)	4.514e−02	0.607	0.864
Hexadecenedioate (C16:1−DC)	19	1.060 (1.013 to 1.110)	1.188e−02	0.298	0.498
Hydantoin−5−propionate	9	1.139 (1.027 to 1.264)	1.382e−02	0.453	0.501
Isovalerate (i5:0)	15	1.125 (1.034 to 1.225)	6.194e−03	0.542	0.361
Metabolonic lactone sulfate	23	1.067 (1.026 to 1.109)	1.065e−03	0.158	0.082
N−acetylserine	20	1.099 (1.008 to 1.198)	3.136e−02	0.938	0.720
Octadecadienedioate (C18:2−DC)	26	1.086 (1.041 to 1.134)	1.660e−04	0.917	0.434
Octadecenedioate (C18:1−DC)	14	1.082 (1.024 to 1.144)	5.410e−03	0.825	0.209
Octadecenedioylcarnitine (C18:1−DC)	13	1.065 (1.015 to 1.117)	1.090e−02	0.583	0.057
Pyridoxal	26	1.096 (1.027 to 1.170)	5.753e−03	0.817	0.436
Sphingomyelin (d18:0/18:0, d19:0/17:0)	21	1.112 (1.037 to 1.193)	3.016e−03	0.808	0.568
2,2'−Methylenebis(6−tert−butyl−p−cresol)	14	0.910 (0.837 to 0.989)	2.644e−02	0.780	0.368
Adenosine 5'−diphosphate (ADP)	22	0.935 (0.880 to 0.994)	3.076e−02	0.277	0.600
Glutarate (C5−DC)	15	0.917 (0.842 to 1.000)	4.918e−02	0.906	0.427
Glycocholate	29	0.923 (0.862 to 0.987)	1.964e−02	0.084	0.664
Glycohyocholate	12	0.869 (0.772 to 0.979)	2.078e−02	0.065	0.824
Glycoursodeoxycholate	19	0.892 (0.827 to 0.963)	3.236e−03	0.702	0.476
N−formylphenylalanine	27	0.935 (0.876 to 0.998)	4.209e−02	0.383	0.543
N−palmitoyl−heptadecasphingosine (d17:1/16:0)	20	0.937 (0.878 to 1.000)	4.857e−02	0.956	0.407
Oleate/vaccenate (18:1)	13	0.865 (0.784 to 0.954)	3.667e−03	0.761	0.328
Sphingomyelin (d18:2/23:1)	18	0.894 (0.819 to 0.975)	1.163e−02	0.430	0.661



Exposure	SNPs	OR (95% CI)	Pval	Q_pval_IVW	Egger_intercept_pval
1-oleoyl-GPI (18:1)	22	1.429 (1.151 to 1.773)	1.204e-03	0.433	0.875
11beta-hydroxyandrosterone glucuronide	21	1.382 (1.134 to 1.683)	1.315e-03	0.497	0.650
2'-deoxyuridine	17	1.228 (1.010 to 1.495)	3.980e-02	0.935	0.333
2-linoleoylglycerol (18:2)	19	1.324 (1.078 to 1.625)	7.488e-03	0.763	0.434
4-vinylguaiacol sulfate	17	1.408 (1.110 to 1.788)	4.875e-03	0.320	0.734
Arachidonoylcholine	16	1.291 (1.037 to 1.607)	2.227e-02	0.682	0.880
Argininate	21	1.308 (1.069 to 1.601)	9.204e-03	0.486	0.265
Carnitine C18:2	18	1.210 (1.013 to 1.445)	3.589e-02	0.620	0.426
Creatinine	17	1.343 (1.013 to 1.781)	4.026e-02	0.254	0.074
Galactonate	6	1.403 (1.022 to 1.928)	3.648e-02	0.411	0.624
Glycerate	24	1.255 (1.019 to 1.544)	3.237e-02	0.291	0.992
Glyco-beta-muricholate	18	1.167 (1.033 to 1.318)	1.296e-02	0.808	0.241
Hippurate	14	1.374 (1.039 to 1.819)	2.604e-02	0.426	0.944
Laurate (12:0)	14	1.378 (1.035 to 1.834)	2.792e-02	0.947	0.878
Margaroylcarnitine (C17)	21	1.273 (1.013 to 1.600)	3.823e-02	0.711	0.217
Pyridoxal	26	1.257 (1.042 to 1.518)	1.711e-02	0.954	0.827
Pyridoxate	13	1.408 (1.027 to 1.930)	3.365e-02	0.411	0.353
Sulfate of piperine metabolite C16H19NO3 (2)	20	1.313 (1.047 to 1.647)	1.823e-02	0.991	0.862
Sulfate of piperine metabolite C18H21NO3 (1)	18	1.292 (1.027 to 1.626)	2.852e-02	0.973	0.668
Tauro-beta-muricholate	19	1.178 (1.051 to 1.319)	4.757e-03	0.268	0.498
Trans-4-hydroxyproline	18	1.372 (1.095 to 1.718)	5.919e-03	0.965	0.639
Umbelliferone sulfate	16	1.302 (1.066 to 1.590)	9.645e-03	0.187	0.312
1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (p-16:0/18:2)	19	0.742 (0.608 to 0.904)	3.155e-03	0.480	0.143
1-(1-enyl-palmitoyl)-GPE (p-16:0)	18	0.773 (0.611 to 0.978)	3.162e-02	0.992	0.583
2-hydroxyphenylacetate	19	0.816 (0.676 to 0.985)	3.406e-02	0.898	0.243
3-methoxycatechol sulfate (2)	12	0.689 (0.484 to 0.981)	3.886e-02	0.092	0.347
4-guanidinobutanoate	16	0.852 (0.737 to 0.986)	3.190e-02	0.841	0.700
5-acetylamino-6-formylamino-3-methyluracil	16	0.898 (0.806 to 1.000)	4.973e-02	0.515	0.970
5alpha-androstan-3alpha,17beta-diol monosulfate (1)	12	0.902 (0.826 to 0.986)	2.369e-02	0.924	0.348
5alpha-androstan-3beta,17beta-diol monosulfate (2)	20	0.852 (0.767 to 0.947)	2.944e-03	0.676	0.947
Androstenediol (3beta,17beta) monosulfate (2)	21	0.803 (0.657 to 0.981)	3.167e-02	0.600	0.129
Androsterone sulfate	27	0.903 (0.835 to 0.976)	9.953e-03	0.876	0.602
Behenoyl dihydrosphingomyelin (d18:0/22:0)	29	0.758 (0.609 to 0.945)	1.358e-02	0.005	0.940
Behenoylcarnitine (C22)	20	0.776 (0.627 to 0.961)	2.006e-02	0.240	0.826
Bilirubin degradation product, C16H18N2O5 (1)	15	0.877 (0.777 to 0.989)	3.202e-02	0.928	0.851
Bilirubin degradation product, C16H18N2O5 (2)	19	0.863 (0.756 to 0.985)	2.946e-02	0.236	0.345
Carnitine C14	17	0.704 (0.543 to 0.914)	8.338e-03	0.275	0.062
Decanoylcarnitine (C10)	18	0.834 (0.704 to 0.986)	3.412e-02	0.937	0.678
Epiandrosterone sulfate	19	0.891 (0.813 to 0.976)	1.317e-02	0.795	0.708
Gamma-glutamylglutamine	24	0.833 (0.696 to 0.997)	4.681e-02	0.900	0.364
Glycerophosphorylcholine (GPC)	24	0.819 (0.673 to 0.996)	4.545e-02	0.446	0.953
Glycodeoxycholate	14	0.742 (0.574 to 0.960)	2.293e-02	0.549	0.806
Glycolithocholate	16	0.763 (0.596 to 0.977)	3.225e-02	0.378	0.739
N-acetylalliin	22	0.811 (0.689 to 0.955)	1.204e-02	0.814	0.449
N-lactoyl tyrosine	20	0.805 (0.658 to 0.985)	3.539e-02	0.623	0.689
Sphingomyelin (d18:0/18:0, d19:0/17:0)	22	0.783 (0.638 to 0.960)	1.857e-02	0.380	0.092
Sphingomyelin (d18:0/20:0, d16:0/22:0)	32	0.841 (0.719 to 0.982)	2.874e-02	0.083	0.816
Sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	24	0.795 (0.650 to 0.973)	2.639e-02	0.895	0.299
Sphingomyelin (d18:1/18:1, d18:2/18:0)	22	0.776 (0.633 to 0.951)	1.461e-02	0.743	0.711
Sphingomyelin (d18:1/20:2, d18:2/20:1, d16:1/22:2)	19	0.798 (0.649 to 0.981)	3.259e-02	0.938	0.828
Sphingomyelin (d18:1/24:1, d18:2/24:0)	14	0.659 (0.498 to 0.873)	3.617e-03	0.507	0.475
Stearoyl sphingomyelin (d18:1/18:0)	26	0.838 (0.711 to 0.987)	3.481e-02	0.998	0.839



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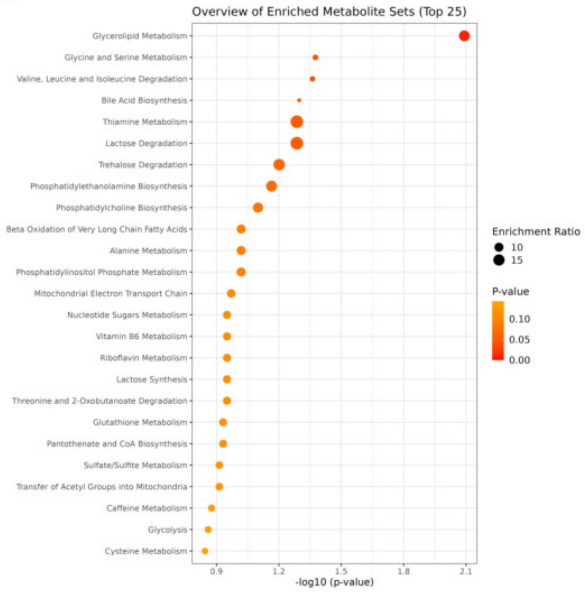
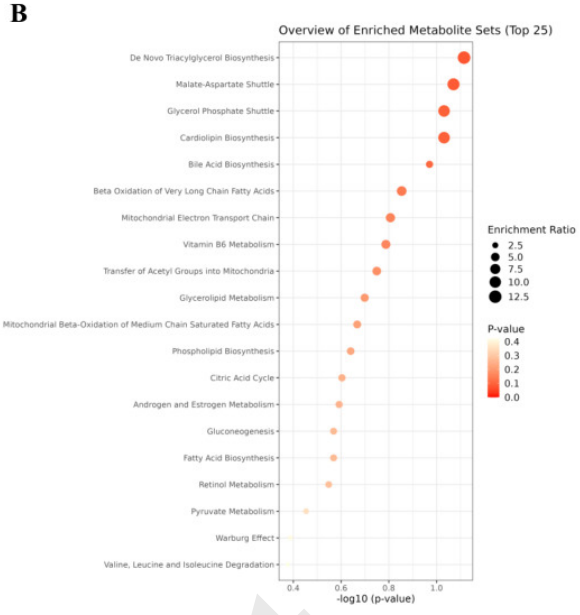

Overview of Enriched Metabolite Sets (Top 25)
Glycerolipid Metabolism
Glycine and Serine Metabolism
Valine, Leucine and Isoleucine Degradation
Bile Acid Biosynthesis
Thiamine Metabolism
Lactose Degradation
Trehalose Degradation
Phosphatidylethanolamine Biosynthesis
Phosphatidylcholine Biosynthesis
Beta Oxidation of Very Long Chain Fatty Acids
Alanine Metabolism
Phosphatidylinositol Phosphate Metabolism
Mitochondrial Electron Transport Chain
Nucleotide Sugars Metabolism
Vitamin B6 Metabolism
Riboflavin Metabolism
Lactose Synthesis
Threonine and 2-Oxobutanoate Degradation
Glutathione Metabolism
Pantothenate and CoA Biosynthesis
Sulfate/Sulfite Metabolism
Transfer of Acetyl Groups into Mitochondria
Caffeine Metabolism
Glycolysis
Cysteine Metabolism
0.9
1.2
1.5
1.8
2.1
-log10 (p-value)
Enrichment Ratio
10
15
P-value
0.10
0.05
0.00

B


Overview of Enriched Metabolite Sets (Top 25)
De Novo Triacylglycerol Biosynthesis
Malate-Aspartate Shuttle
Glycerol Phosphate Shuttle
Cardiolipin Biosynthesis
Bile Acid Biosynthesis
Beta Oxidation of Very Long Chain Fatty Acids
Mitochondrial Electron Transport Chain
Vitamin B6 Metabolism
Transfer of Acetyl Groups into Mitochondria
Glycerolipid Metabolism
Mitochondrial Beta-Oxidation of Medium Chain Saturated Fatty Acids
Phospholipid Biosynthesis
Citric Acid Cycle
Androgen and Estrogen Metabolism
Gluconeogenesis
Fatty Acid Biosynthesis
Retinol Metabolism
Pyruvate Metabolism
Warburg Effect
Valine, Leucine and Isoleucine Degradation
0.4
0.6
0.8
1.0
-log10 (p-value)
Enrichment Ratio
2.5
5.0
7.5
10.0
12.5
P-value
0.4
0.3
0.2
0.1
0.0