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ORIGINAL RESEARCH

Causal Relationship Between Blood Metabolomics and Female Pattern Hair Loss: A Bidirectional Mendelian Randomization Study

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Background: Metabolic disorders have been hypothesized to be associated with female-pattern hair loss. However, ambiguity persists regarding the causality and directionality of the relationship between blood metabolites and female hair loss patterns.

Methods: To evaluate the causal relationship between 1400 blood metabolites and female pattern hair loss, we conducted a bidirectional Mendelian randomization analysis using publicly available summary data from genome-wide association studies. The primary analyses employed the inverse variance weighted method supplemented by the weighted median, MR-Egger, and weighted mode approaches. To control for multiple testing, the false discovery rate method was applied to adjust P values. The leave-one-out method was employed for the sensitivity analysis. Heterogeneity was evaluated using Cochran's Q value, whereas horizontal pleiotropy was assessed using MR-Egger intercept and MR-PRESSO. Additionally, metabolic pathway analysis was performed for the metabolites that demonstrated significant correlations. We further performed colocalization analysis to delve into the underlying causality.

Results: After rigorous selection, 23 metabolites and 4 metabolic ratios were associated with female-pattern hair loss. There were no noticeable outliers, horizontal pleiotropy, or heterogeneity. Metabolic pathway analysis identified one significant pathway: fructose/mannose metabolism ($P < 0.05$). In the reverse analysis, dimethylglycine was identified as overlapping with the forward analysis results, thereby removing it from the final analysis.

Conclusion: Through integration of genomic and metabolomic data, we identified blood metabolites that may be associated with the development of female pattern hair loss. Our findings provide novel insights into the pathogenic mechanisms of this condition. These findings have significant implications for early diagnosis, preventive measures, and treatment.

Keywords: metabolites, Mendelian randomization, female pattern hair loss, fructose and mannose metabolism

Introduction

Female pattern hair loss (FPHL), also known as androgenetic alopecia (AGA), is the most common type of non-scarring alopecia with rising prevalence and is characterized by progressive hair follicle (HFs) miniaturization with a subsequent decrease in hair density.

To date, the pathogenesis of FPHL is multifactorial and has not been completely elucidated. Recently, an increasing number of studies have underscored the robust correlation between AGA and human blood metabolites. A meta-analysis of AGA investigated the levels of serum total cholesterol, TG, and LDL cholesterol, indicating the importance of abnormal lipid profiles in AGA pathogenesis.[1] Furthermore, contemporary multiple-omics research has revealed an intricate map of the primary and secondary metabolic pathways by which AGA impedes hair growth. For instance, untargeted metabolomic analysis revealed that carbohydrate, amino acid, and lipid metabolisms were significantly altered in alopecia. Transcriptomic results showed that genes involved in arachidonic acid metabolism, glutathione metabolism,

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and glycolysis gluconeogenesis were significantly different in alopecia compared with the control group.[2] Moreover, metabolic syndrome (MetS), characterized by insulin resistance, obesity, dyslipidemia, and abnormal vascular endothelial cell function, may be a key mechanism underlying AGA, suggesting a crucial role of extensive metabolic disorders in AGA onset.[3] However, similar studies showed obvious limitations in data timeliness, case sample size, blood metabolite categories, and potential confounders, which led to inaccuracies in the causal analysis of exposure and outcomes.[4,5] Therefore, the disturbance of metabolites in AGA pathophysiology in recent years remains incomplete, although a substantial number of studies have explored their association. In addition, no studies have investigated alterations in human blood metabolites targeted at FPHL.

Mendelian randomization (MR) is an outstanding epidemiological method that uses genetic variations as Instrumental Variables (IVs) to explore causal relationships between exposure factors and outcomes. AGA is a disease with a genetic predisposition accompanied by substantial alterations in metabolic determinants. The integration of MR, genomics, and metabolomics provides deep insight into the pivotal mechanisms underlying AGA, thereby discovering risk indicators and new treatment targets. MR can circumvent confounding biases due to random generation of genetic variants during maternal conception. Genome-wide association studies (GWAS), the foundation of MR, are used to identify genetic variations related to specific characteristics or diseases by comparing the whole genome of a considerable number of cases and control individuals. Reliable gene variations identified by GWAS were leveraged as instrumental variables of MR to explore the causal links between exposure and outcome variables. Bidirectional MR, the optimization of conventional MR, enables a more comprehensive understanding of the mutual causal interconnections between exposure factors and outcomes by simultaneously conducting forward and backward examinations. Bidirectional MR can eliminate potential reverse causal relationships and accurately reveal the intricate interactional mechanisms between genetics and metabolomics.

In this study, we performed a bidirectional MR analysis to investigate the reciprocal causal relationship between blood metabolites and FPHL using data from two GWAS. While GWAS can reveal numerous genetic associations, distinguishing causal variants from mere correlations poses a considerable challenge. Colocalization analysis provides evidence to ascertain whether the same causal variant influences multiple traits. Therefore, we employed a GWAS-GWAS colocalization analysis to identify and reduce false positive results. Our analysis identified several metabolites associated with FPHL and evaluated pathway enrichment, contributing to a comprehensive understanding of the biological processes and molecular mechanisms involved. The findings of our study enhance the understanding of the metabolic mechanisms underlying the onset and progression of FPHL, identify reliable risk indicators, and support the development of novel preventive and therapeutic strategies for this condition.

Materials and Methods

Study Design

Bidirectional Mendelian randomization (MR) analysis was conducted using publicly available genome-wide association studies (GWAS) summary data. Written informed consent was obtained from all participants in separate studies authorized by the ethics committees of the Institutional Review Boards. The human data used in this study were obtained through legitimate methods from publicly available databases, and all data were anonymized acquired. According to Article 32, Items 1 and 2, of the "Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects" issued on February 18, 2023, in China, this study is exempt from ethical approval.

To identify the single nucleotide polymorphisms (SNPs) that could serve as Instrumental Variables (IVs) to validate the causal relationships between exposure factors and outcomes, we employed inverse-variance weighted (IVW) as the main MR analysis approach in this study. The overall study design is illustrated in the flowchart (Figure 1). The blue line indicates the forward MR analysis, considering metabolites as exposures and FPHL as outcomes, revealing the potential effect of the genetically determined blood metabolome on FPHL onset. The red line represents reverse analysis, considering FPHL as the exposure and blood metabolites as the outcomes, probing the impact of FPHL pathogenesis on blood metabolites. To obtain more reliable results, we supplemented our analysis with the weighted median, MR-Egger, and weighted-mode approaches. A sensitivity analysis was conducted using the leave-one-out method.

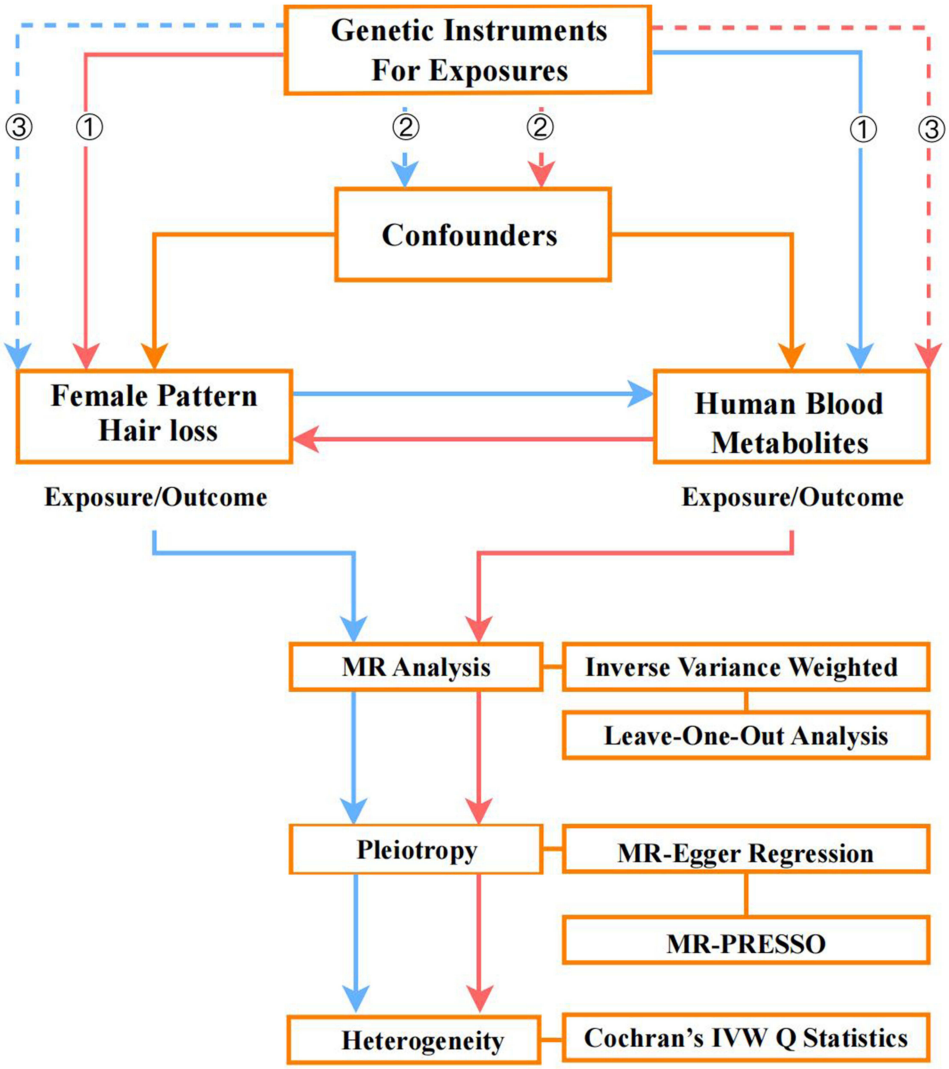

Figure 1 Overview of bidirectional Mendelian randomization.
Abbreviations: MR, Mendelian Randomization; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; IVW, Inverse Variance Weighting.

Heterogeneity was evaluated using Cochran's Q statistic and horizontal pleiotropy was assessed using the MR-Egger intercept and MR-PRESSO methods. Finally, pathway analysis of casually related metabolites was performed using Metaboanalyst 6.0 website.

Data Sources

The summary-level GWAS data of 1091 blood metabolites and 309 metabolite ratios utilized in this study were obtained from the Canadian Longitudinal Study of Aging (CLSA) cohort conducted by Chen et al, recruiting 8299 unrelated individuals.[6] Metabolites have been identified across eight pathways: lipids, amino acids, xenobiotics, nucleotides, cofactors, vitamins, carbohydrates, peptides, and energy metabolism.

GWAS data for FPHL were acquired from the latest released Finn Gen Biobank Analysis Round 11 in 2024, with a total sample size of 453,733 participants encompassing 21,311,942 SNPs (https://www.finngen.fi/en). This dataset was retrieved from a global project of the Finnish consortium, in which women diagnosed with FPHL had a mean age of 50.2 years. To the best of our knowledge, two-sample MR analysis utilizes GWAS datasets in nonoverlapping cohorts for exposure and outcome.

Selection of Instrumental Variables (IVs)

Strict criteria for SNPs selection contribute to accurate causal relationship inference between exposures and outcomes. In the forward analysis, we set a clumping window of >10000kb, linkage disequilibrium analysis (LD) $R^2 < 0.001$, and genome-wide significance threshold of $P < 5\times10^{-6}$ for the selection of strongly associated SNPs. In the backward analysis, we adopted a threshold of 10000 kb, $R^2 < 0.001$, and $P < 5\times10^{-6}$ to identify SNPs that were significantly correlated with FPHL. The F-statistic was used to measure SNPs' strength of the SNPs in estimating the causal relationships. SNPs with an F-statistic < 10 represent weak IVs and were excluded from our study to guarantee the accuracy of genetic variation.[7,8] In addition, metabolites relevant to fewer than 3 SNPs were eliminated. Finally, the SNPs that conformed to these requirements were selected as IVs.

Two-Sample MR Analysis

The causal associations between blood metabolites and FPHL were examined by two-sample MR analysis using selected IVs. The MR analysis was performed utilizing R (version 4.3.1) and the R package "Two Sample MR" (version 0.5.7).[9] All analyses were consistent with current recommendations for MR.[10] In both forward and reverse analyses, we adopted the Inverse Variance Weighted (IVW, $P < 0.05$) method as the primary approach to investigate the causal relationship between metabolites and FPHL.[11] The IVs should meet three assumptions: 1) they must be strongly correlated with exposure, 2) they must influence the outcome only through the exposure pathway, and 3) they must remain unaffected by confounders to reduce bias caused by linkage disequilibrium. When IVs conform to the aforementioned assumptions, IVW provides the most precise estimates of the potential causal effects. In addition, to control the false positive rate caused by multiple comparisons, False Discovery Rates (FDR) correction were applied to adjust p-values in the IVW results, ultimately producing corrected FDR values. A significance threshold of FDR < 0.05 was utilized to obtain prime candidates. In particular, the formula we used for FDR correction is:

$$\mathrm{FDR} = \mathrm{P\,value} \times \frac{\mathrm{Rank_{max}}}{\mathrm{P_{rank}}}$$

However, complementary sensitivity analysis methods should be used to obtain more accurate results if some of the IVs violate these assumptions. Therefore, we employed sensitivity analyses, such as the MR-Egger,[12] Weighted Median (WM),[13] and weighted mode methods. In addition, we conducted a leave-one-out analysis to detect whether an individual SNP caused excessive deviations in the robustness of the causal inference. Next, we performed Cochran's Q test using the IVW and MR-Egger methods to detect the potential heterogeneity ($P < 0.05$). Moreover, we implemented the MR-Egger intercept and MR-PRESSO[14] to evaluate pleiotropy, which assessed the possibility of exposure affecting outcomes through unexpected pathways, with $P < 0.05$, representing the existence of pleiotropy. In addition, we excluded metabolites that exhibited different directions and magnitudes in the IVW, MR-Egger, the Weighted Median, and weighted mode methods to ensure accuracy.

Reverse MR Analysis

The causal effects of FPHL on the metabolites were repeated in opposite directions via reverse MR analysis. We implemented IVW ($P < 0.05$) as the main method to identify the potential impact of FPHL on selected blood metabolites and excluded overlapping metabolites.

Colocalization Analysis

To ascertain whether the associations between the identified blood metabolites and FPHL were influenced by a shared causal variant, we employed the R package "coloc". This analysis involved investigating regional loci within a 50 KB range above and below the lead SNP in the exposure data, thereby reducing the likelihood of reinforcing spurious associations between the two phenotypes. The "coloc" package utilizes a Bayesian framework to calculate posterior probabilities for five mutually exclusive hypotheses concerning the sharing of causal variants between the two traits: H0 (neither trait has a causal variant), H1 or H2 (a causal variant affects only one trait), H3 (two distinct causal variants, one

for each trait), and H4 (a single causal variant shared between both traits). A posterior probability exceeding 80% for H4, under various prior and window conditions, was considered strong evidence of colocalization.

Metabolic Pathway Analysis

To unveil the biological mechanisms, we conducted metabolic pathway analysis involving selected metabolites robustly associated with FPHL (IVW, $P < 0.05$) using Metaboanalyst 6.0 (https://www.metaboanalyst.ca/). MetaboAnalyst 6.0, an online platform designed for metabolomic data analysis, provides a visualized analysis process.

Results

Preliminary Analysis

Single-nucleotide polymorphisms (SNPs) of 1091 blood metabolites and 309 metabolite ratios were ultimately included in this analysis, varying from 3 to 51. All SNPs associated with metabolites had F-statistics greater than 10, eliminating the potential effects of weak IVs (Supplementary Table S1). The causal associations of these metabolites and metabolic ratios with FPHL were substantiated using inverse variance weighting (IVW) as the main method. Fifty metabolites were identified to have a significant causal association with FPHL (IVW method, $P < 0.05$) (Supplementary Table S2). The most significant metabolite was 3-hydroxyhexanoate ($P = 8.77E-05$), followed by citrulline to ornithine ratio ($P = 0.000959$) and cysteine-glutathione disulfide ($P = 0.001682$). Subsequently, of the 50 analyzed metabolites, we further selected 26 metabolites and six metabolic ratios with FDR < 0.05 as prime candidates to screen false positives and obtain more accurate results (Supplementary Table S3). From these, we identified and excluded five metabolites that exhibited inconsistent directions and magnitudes across the four MR analysis methods: N-acetylisoleucine, Glycosyl ceramide (d18:2/24:1, d18:1/24:2), sphingadienine levels, Phosphate to 2'-deoxyuridine ratio, and Leucine to N-palmitoyl-sphingosine (d18:1 to 16:0) ratio (Supplementary Table S4). Finally, 23 metabolites and four metabolic ratios that conformed to the above-mentioned rigorous screening criteria were observed as strongly correlated candidates, as depicted in the forest plot (Figure 2) (Supplementary Table S5).

Our findings indicated that 14 metabolites were risk factors for FPHL: 3-hydroxyhexanoate, N2, n2-dimethylguanosine, citrulline to ornithine ratio, adenosine 5'-monophosphate (AMP) to phenylalanine ratio, Cholesterol, Cysteine-glutathione disulfide, 8-methoxykynurenate, Octadecanedioate, Lithocholate sulfate, Beta-citrylglutamate, N-acetyl-2-aminoadipate, and Octadecadienedioate (C18:2-DC), which can be classified into several categories such as amino acids, nucleotides, fatty acids, Cholesterol, Bile acids, and sulfocompounds. In contrast, (16or17)-methylstearate(a19:0 or i19:0), N6,N6-dimethyllysine, Dimethylglycine, Picolinate, Sphingomyelin (d18:1/19:0, d19:1/18:0), Citraconate/glutaconate, Ascorbic acid 3-sulfate, proline to trans-4-hydroxyproline ratio, EPA (20:5n3), alpha-ketobutyrate to 3-methyl-2-oxobutyrate ratio, and docosapentaenoate (n6 DPA; 22:5n6) were protective factors. The detailed traits of the metabolites are listed in Supplementary Table S5. Leave-one-out analysis was carried out, successively excluding each SNP, to determine whether the results were disproportionately influenced by individual SNPs. In addition, we performed Cochran's Q test using the IVW and MR-Egger methods to detect potential heterogeneity Supplementary Table S6, and utilized the MR-Egger intercept and MR-PRESSO to evaluate possible pleiotropy Supplementary Table S7. Our results indicate that none of the determined metabolites showed underlying heterogeneity or horizontal pleiotropy, suggesting robustness in causal relationship inference. Moreover, MR leave-one-out sensitivity analysis was conducted to ensure the stability and consistency of each metabolite. Overall, these findings collectively elucidate the reliability of causal relationships between metabolites and the risk of FPHL. Therefore, further investigations are warranted. These 27 serum metabolites warrant further investigation to shed light on the novel pathogenesis mechanisms and etiology of FPHL.

Reverse Causation Between Metabolites and FPHL

To obtain comprehensive and reliable results, we explored the genetic relationships between FPHL and its metabolites to conduct reverse MR analysis, employing the IVW method as the primary estimation approach. We identified 43 metabolites that were significantly associated with FPHL (IVW; $P < 0.05$) (Supplementary Table S8). Among these

Exposure	Method	nSNP	OR (95% CI)	P value
3-hydroxyhexanoate levels	IVW	9	4.825 (2.198 , 10.594)	<0.001
N2,n2-dimethylguanosine levels	IVW	5	4.164 (1.300 , 13.342)	0.016
Citrulline to ornithine ratio	IVW	13	3.376 (1.640 , 6.952)	<0.001
Adenosine 5'-monophosphate (AMP) to phenylalanine ratio	IVW	9	3.037 (1.278 , 7.215)	0.012
Cholesterol levels	IVW	8	2.659 (1.097 , 6.445)	0.030
X-12100 levels	IVW	11	2.304 (1.239 , 4.283)	0.008
Cysteine-glutathione disulfide levels	IVW	13	2.087 (1.319 , 3.304)	0.002
8-methoxykynurenate levels	IVW	11	1.848 (1.007 , 3.392)	0.048
Octadecanedioate levels	IVW	18	1.825 (1.133 , 2.940)	0.013
Lithocholate sulfate (1) levels	IVW	15	1.825 (1.105 , 3.013)	0.019
Beta-citrylglutamate levels	IVW	8	1.583 (1.089 , 2.300)	0.016
N-acetyl-2-aminoadipate levels	IVW	14	1.531 (1.003 , 2.338)	0.048
Octadecadienedioate (C18:2-DC) levels	IVW	15	1.501 (1.039 , 2.168)	0.030
X-21467 levels	IVW	20	1.356 (1.023 , 1.799)	0.034
(16 or 17)-methylstearate (a19:0 or i19:0) levels	IVW	11	0.681 (0.505 , 0.919)	0.012
N6,N6-dimethyllysine levels	IVW	11	0.669 (0.503 , 0.890)	0.006
Dimethylglycine levels	IVW	14	0.598 (0.396 , 0.905)	0.015
Picolinate levels	IVW	14	0.544 (0.332 , 0.892)	0.016
Sphingomyelin (d18:1/19:0, d19:1/18:0) levels	IVW	13	0.535 (0.332 , 0.860)	0.010
X-11483 levels	IVW	14	0.503 (0.283 , 0.892)	0.019
Citraconate/glutaconate levels	IVW	15	0.495 (0.259 , 0.944)	0.033
Ascorbic acid 3-sulfate levels	IVW	11	0.486 (0.253 , 0.931)	0.030
Proline to trans-4-hydroxyproline ratio	IVW	8	0.448 (0.205 , 0.981)	0.045
Eicosapentaenoate (EPA; 20:5n3) levels	IVW	10	0.393 (0.215 , 0.719)	0.002
Alpha-ketobutyrate to 3-methyl-2-oxobutyrate ratio	IVW	6	0.333 (0.123 , 0.904)	0.031
X-22776 levels	IVW	9	0.315 (0.136 , 0.732)	0.007
Docosapentaenoate (n6 DPA; 22:5n6) levels	IVW	7	0.271 (0.110 , 0.670)	0.005

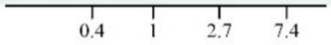

Figure 2 Forest plot of 27 significantly related metabolites.
Abbreviations: IVW, inverse-variance weighted; SNP, single-nucleotide polymorphism; CI, confidence interval; OR, odds ratio.

metabolites, Indoleacetylglutamine showed the strongest association with FPHL (P = 0.00083868), followed by 1-(1-enyl-palmitoyl)-2-oleoyl-gpc (p-16:0/18:1) (P = 0.001867447) and Carotene diol (P = 0.002757193), involving in Amino acid, Glycerophospholipids, and Carotenoid metabolism. However, one of these 43 metabolites, dimethylglycine, overlapped with the forward analysis results; therefore, it was removed from our final analysis, as reported in Supplementary Table S8.

Colocalization Analysis

27 metabolites with significant MR associations with FPHL were performed colocalization analysis. The results are detailed in Supplementary Table S9. The analysis suggests that the associations between FPHL and these metabolites are not due to shared causal variant loci.

Metabolic Pathway Analysis

Metabolic pathway analysis of the metabolites that showed a robust causal correlation with FPHL in this study (IVW, P < 0.05) revealed one metabolic pathway with considerable significance (P < 0.05): fructose and mannose metabolism. Notably, the overlapping metabolite with bidirectional effects, dimethylglycine, was not included in pathway analysis. As shown in Figure 3, the colors in the metabolic pathway analysis bubble diagram varied from light yellow to red, indicating that the metabolites were in the data with incremental levels of significance.

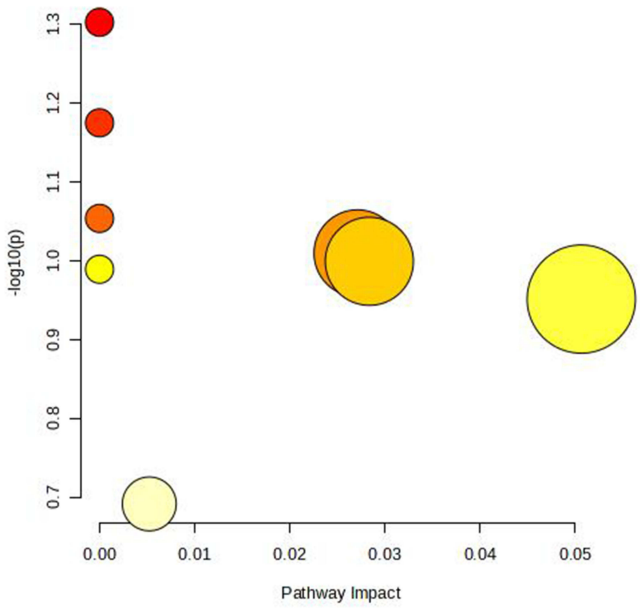

Figure 3 Metabolic pathway analysis bubble diagram.

Discussion

Here, we performed a bidirectional Mendelian randomization (MR) analysis to evaluate the intricate links between 1091 blood metabolites and 309 metabolite ratios, and the onset of female pattern hair loss (FPHL), utilizing two expansive GWAS summary datasets. We identified 50 blood metabolites that were causally related to FPHL (IVW, $P < 0.05$). After False Discovery Rate (FDR) validation and multiple rigorous sensitivity tests, 27 of them ultimately remained strongly associated with candidate metabolites. 3-hydroxyhexanoate, Cysteine-glutathione disulfide (CSSG), and citrulline-to-ornithine ratio were considered the top three significant risk factors for FPHL. Eicosapentaenoate (EPA;20:5n3), docosapentaenoate (n6 DPA; 22:5n6), and N6,N6-dimethyllysine are the most potent protective factors. Furthermore, GWAS-GWAS colocalization analysis provided robust evidence for causal relationships that are independent of overlapping SNPs. Our findings provide vital insights into the etiology and molecular mechanism of FPHL by providing robust evidence of causal nexus inference and prevention and treatment strategies by targeting these determined blood metabolites.

FPHL is a public health concern that needs to be specifically noticed because of its negative impact on patients' social confidence, in addition to its protracted disease duration. Several studies have demonstrated a possible association between FPHL and typical metabolic conditions such as cardiovascular disease,[15] insulin resistance (IR),[16] polycystic ovarian syndrome (PCOS),[17–19] and metabolic syndrome (MS),[16,20] indicating the involvement of metabolic disorders in the onset of FPHL. Although previous studies have elucidated the effects of dysregulation of lipid, organic acid, porphyrin, and polyol metabolism on hair loss, their contribution remains constrained by limited data timeliness and extensiveness.[4,5] Moreover, none of these studies specifically targeted at causal relationship inference between blood metabolites and FPHL. Therefore, we conducted a bidirectional MR study to elucidate the pivotal metabolic mechanisms of FPHL, paving the way for early screening and treatment strategies.

Among the three identified protective factors, eicosapentaenoate (EPA; 20:5n3) is an Omega-3 long-chain polyunsaturated fatty acid (LC-PUFA) that offers protective benefits against conditions such as atherosclerosis, obesity, and inflammation.[21] An in vitro study showed that eicosapentaenoate could induce anagen entry and hair growth by promoting hair follicle dermal papilla cells (DPCs) proliferation.[22] Docosapentaenoate (n6 DPA; 22:5n6) is an Omega-6 LC-PUFA. Ryu et al suggested that Omega-6 treatment might alleviate testosterone-induced signaling and induce DPCs growth.[23] Moreover, women with FPHL may have an increased risk of cardiovascular or metabolic disorders.[16,17,20] PUFA can reduce the level of blood lipids and exhibit anti-inflammatory properties, which may benefit

patients in terms of both hair growth and metabolic homeostasis.[24] Unfortunately, there is limited research on N6, N6-dimethyllysine, and its effects on hair loss, which requires further exploration.

While Among the top three risk-associated factors, 3-hydroxyhexanoate appears to be an intermediate product of fatty acid and glucose metabolism. A previous study found that targeting fatty acid metabolism could regulate bulge niche formation, hair follicle stem cell (HFSCs) activity, and hair growth, consistent with our hypothesis of fatty acid involvement in the mechanism of FPHL.[25] CSSG is a mixed disulfide formed upon the oxidation of GSH and serves as an indicator of oxidative stress levels.[26] Elevation of intracellular oxidative stress inhibits the function of HF morphogenesis-related proteins, thereby suppressing hair growth.[27] However, the association of citrulline to ornithine ratio with FPHL indicated significant abnormalities in serum amino acid metabolism (urea cycle and nitrogen balance) and energy metabolism, warranting further exploration of its impact on hair growth. In addition to the known metabolites, we also identified four unnamed metabolites associated with FPHL (X-12100, X-21467, X-22776, and X-11483), which function and link with FPHL and remain to be studied, allowing for a new understanding of preventive and therapeutic measures.

Our metabolic pathway analysis demonstrated that the fructose/mannose metabolic pathway is significantly associated with FPHL. Mannose, a crucial hexose for glycoprotein synthesis, has consistently demonstrated a significant association between high blood glucose levels and hair loss in multiple studies. A cross-sectional study investigating the association between sugar-sweetened beverage (SSB) consumption and male pattern hair loss (MPHL) highlighted the intertwined links between serum glucose metabolism and alopecia, similar to our findings.[28] Furthermore, previous studies have shown that a lack of glucose in outer root sheath (ORS) keratinocytes is a potential cause of MPHL.[29] The utilization of glucose in the polyol pathway reduces the amount of glucose that can be reused by keratinocytes in ORS, which may lead to hair loss. In addition, chronic dysregulation of glucose and the consequent lipid metabolism may induce IR. Evidence has shown a strikingly increased risk of IR-associated disorders, such as obesity and dyslipidemia, with an early onset of alopecia.[30,31] Estrogen can be inversely converted into testosterone by 17.20-lyase and 17-hydroxylase under IR, and patients with FPHL exhibit higher susceptibility to PCOS and a myriad of intricate metabolic dysregulation.[18,31] In addition, IR leads to local androgen production from cholesterol, enhancing the local conversion of testosterone to dihydrotestosterone (DHT), which contributes to the progression of FPHL.[32] Increased circulating insulin levels due to IR may cause vasoconstriction and impaired nutrient intake in the scalp hair follicles, resulting in aggravated microvascular insufficiency and subsequent hair loss.[33]

However, our study had several limitations. First, only a limited number of genome-wide significant SNPs were available for MR analysis. Therefore, we employed a relatively relaxed significance threshold for IVs selection. Second, although our study provides convincing evidence of the associations between metabolites and FPHL risk, rigorous in vivo and in vitro validation is required to elucidate the underlying mechanisms. Third, the GWAS dataset utilized in this study was of European and Canadian ancestry, which may constrain the universality of our findings in a broader human background.

Conclusion

Mendelian randomization (MR) analysis revealed several metabolites that showed robust causal connections with FPHL, highlighting the involvement of fatty acids, amino acid metabolism, and oxidative stress in FPHL risk. Notably, Fructose/mannose metabolism is considered a promising risk-increasing molecular mechanism for FPHL. These results deepen our understanding of the metabolite-driven mechanisms of FPHL, opening novel perspectives for early diagnosis, preventive measures, and treatment regimens.

Abbreviations

FPHL, Female pattern hair loss; AGA, Androgenetic alopecia; HFs:Hair follicles; MetS, Metabolic syndrome; MR, Mendelian randomization; IVs, Instrumental Variables; GWAS, Genome-wide association study; SNPs, Single nucleotide polymorphisms; IVW, Inverse-variance weighted; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; CLSA, Canadian Longitudinal Study of Aging; LD, linkage disequilibrium; WM, Weighted Median; FDR, False Discovery Rates; CI, Confidence interval; OR, Odds ratio; AMP, Adenosine 5'-monophosphate; CSSG, Cysteine-

glutathione disulfide; IR, Insulin resistance; PCOS, Polycystic ovarian syndrome; MS, Metabolic syndrome; DPCs, Dermal papilla cells; HFSCs, Hair follicle stem cells; SSB, Sugar-sweetened beverage; MPHL, Male pattern hair loss; ORS, Outer root sheath; DHT, Dihydrotestosterone.

Data Sharing Statement

The datasets mentioned in this manuscript were downloaded online. Detailed information is available upon reasonable request from the corresponding author (Miao Jiang, e-mail address: jiang_miao2014@126.com). Publicly available datasets were used for this study. GWAS summary data for FPHL can be accessed using Finn Gen Biobank R11 (https://www.finngen.fi/en). GWAS summary data for the metabolites were sourced from the Catalog GWAS database. (https://www.ebi.ac.uk/gwas/studies/GCST90043616).

Ethics Approval and Consent to Participate

The human data used in this study were obtained through legitimate methods from publicly available databases, and all data were anonymized acquired. According to Article 32, Items 1 and 2, of the "Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects" issued on February 18, 2023, in China, this study is exempt from ethical approval.

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Disclosure

The authors declare no conflicts of interest in this work.

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