Differential Metabolic Profile in Scalps of Male Androgenetic Alopecia Patients and Female Pattern Hair Loss Patients: A Focus on Citrate Synthase, Pyruvate Kinase M2, and Pyruvate Dehydrogenase

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Abstract

Male Androgenetic Alopecia (AGA) and Female Pattern Hair Loss (FPHL) are the most common hair loss disorders among worldwide populations. AGA is a common hair loss condition characterized by the progressive miniaturization of hair follicles with a specific pattern in individuals with a genetic predisposition. In AGA, there are known pathological including genetic and sex hormone factors and effective treatments. Unlike in FPHL, there are still unknown mechanism, pathological factors and sex hormone factors and effective treatments. This study was performed to better understand differences between AGA and FPHL via protein expression using proteomic technology to analysis of the qualitative and quantitative protein data. Seven AGA patients, eight FPHL patients, two normal males and two normal females were enrolled in this study. A punch biopsy was performed from the advancing border of the active disease at the vertex area for proteomic analysis by liquid chromatography-mass spectrometry (LC-MS). Obtained protein expression data eventually were evaluated using differential expression analysis. Subsequently, protein-protein interaction (PPI) network analysis with clustering was performed. The pathways associated with the major PPI clusters were related to central metabolic processes, including glycolysis and the tricarboxylic acid (TCA) cycle. The important proteins including Citrate Synthase, Pyruvate Kinase M2, and Pyruvate Dehydrogenase proteins were up- regulation. The up regulation of metabolic proteins in male with AGA suggested an adaptive response to the pathological state of hair follicles, potentially reflecting increased energy demands or altered metabolic states as the body attempts to sustain cellular functions despite hair loss. In contrast, a downregulation of metabolic proteins was observed in FPHL. This decline may contribute to an impaired ability of hair follicles to regenerate and maintain their normal growth cycles, leading to the characteristic thinning and loss associated with FPHL. The relationships between metabolic pathways and signaling mechanisms offer promising opportunities for future research focused on creating novel treatments for androgenetic alopecia and deepening our understanding of hair follicle biology.

Keywords**:** *Male androgenetic alopecia, Female pattern hair loss, Citrate synthase, Pyruvate kinase M2, Pyruvate dehydrogenase*

1. Introduction

Androgenetic alopecia (AGA) is a common hair loss condition characterized by a progressive miniaturization of hair follicles with a specific pattern in individuals with a genetic predisposition (Lolli et al., 2017; Rojhirunsakool, & Suchonwanit, 2018). The term "female pattern hair loss (FPHL)" is preferred over "female AGA" (Kaufman, 2002). While the influence of androgens and genetic factors in male AGA is generally acknowledged, their impact on female patients is less clear. Most affected women have normal androgen levels. Although hormonal and genetic factors are believed to play a significant role in FPHL, the

exact mechanism remains unknown. Unlike male AGA, FPHL can be triggered by various factors, including lifestyle and environmental factors that lead to oxidative stress and microinflammation in relevant cells (Su et al., 2013; Trüeb, 2015; Mahé et al., 2000; Yi et al, 2020; Ramos et al., 2016). FPHL can lead to psychological stress. Research has shown that skin conditions negatively affect mental health, causing not only aesthetic concerns but also feelings of vulnerability, loss of self-esteem , and changes in self-perception at any age (Williamson, & Finlay, 2001).

Despite the increasing prevalence of these common non-scarring forms of hair loss worldwide (Gan, & Sinclair, 2005; Pathomvanich et al., 2002), there is a lack of practical, widely accepted guidelines for treating FPHL. Early intervention is often not prioritized, and there are limited effective treatment options available (Iamsumang et al., 2020). Currently, the only FDA-approved medication for treating FPHL is topical minoxidil (Rogers et al., 2008; Suchonwanit et al., 2019c) and there are a few alternative treatments such as low-level laser therapy, fractional laser therapy, and hair transplantation (Suchonwanit et al., 2019a; 2019b). Many patients need to commit to lifelong medication to sustain the treatment's effectiveness. The side effect of Minoxidil is scalp irritation, which must be tolerated or discontinue the treatment. Additionally, the unclear pathogenesis contributes to unpredictable severity and prognosis. The application of proteomics-based biomedical research is crucial for enhancing our comprehension of disease processes and for identifying new biomarkers and therapeutic targets for FPHL and AGA. Nearly all biological activities hinge on proteins, any mis-regulation of protein function can significantly contribute to the development of various disorders (Chen et al., 2020). Recent advancements in MS-based proteomics have facilitated significant strides in comprehending cellular mechanisms, disease advancement, and the connection between genotype and phenotype (Gillet et al., 2016).

2. Objectives

To explore and compare the protein expression profiles in male androgenetic alopecia and female pattern hair loss through proteomic analysis, aiming to uncover novel biomarkers and distinct molecular pathways between these conditions.

3. Materials and Methods

Patients and Biopsy Samples

This study was approved by the Human Research Ethics Committee of Thammasat University (Medicine) MTU-EC-OO-6-085/64 (approval no. 151/2564). According to the ICH Good Clinical Practice guidelines, informed consent was obtained from all subjects for conducting the experiments. Patients with FPHL were recruited between December 2020 and February 2021. Trichoscopy (Dino-Lite, CA, USA) was performed to confirm FPHL and Male AGA diagnosis. The exclusion criteria are as follows: patients with hyper-androgenism, systemic diseases (diabetes mellitus, nutrition deficiency, autoimmune diseases, immunocompromised conditions, and cancer), and a history of treatment for the disease before the study (topical therapies within six months, systemic therapies within one year, and a history of hair restoration surgery). In FPHL, eight patients were enrolled. In male AGA, seven patients were enrolled. Each sex had two control participants with two age-matched, all presenting with a healthy scalp that shows no signs of hair loss, to minimize variability in protein expression due to age-related metabolic differences. Subsequently, a punch biopsy was performed. The 6 mm tissue biopsy was obtained from the advancing border of the active disease at the vertex area for proteomic analysis.

LC-MS/MS Analysis

Scalp samples, after removing hair were lysed in 100 µl of lysis buffer (8M Urea+ Protease inhibitor) using a mixer mill at 30 Hz for 2 minutes, followed by centrifugation at 13,000 rpm for 5 minutes at 4°C. The supernatant was collected and centrifuged again under the same conditions. After the second centrifugation, the supernatant was transferred to a new microtube, and its volume was adjusted to 100 µl with lysis buffer. Protein concentration was determined by BCA assay. For digestion, 100 µg of protein was mixed with 100 µl of 100 mM TEAB. The mixture was reduced with 10 µl of 100 mM DTT and incubated for 30 minutes at

37°C with shaking at 300 rpm. Following this, 40 µl of 100 mM IA was added, and the sample was incubated for 30 minutes at room temperature with shaking at 300 rpm, protected from light. Then, another 40 µl of 100 mM DTT was added, and the sample was incubated for at least 15 minutes at room temperature with shaking at 300 rpm. The concentration of 8M urea was diluted to 1M by adding 1520 µl of 100 mM TEAB. Trypsin was added at a ratio of 1:50 (trypsin to protein), and the sample was incubated overnight at 37°C with shaking at 300 rpm, not exceeding 16 hours. The reaction was stopped by adding 8.55 µl of 100% TFA and incubating for 15 minutes at room temperature. The sample was then centrifuged at 15,000 x g for 10 minutes and dried in a vacuum centrifuge.

For desalting, the dried peptides were reconstituted in 500 µl of 0.1% FA (200 µg/ml). A C18 StageTip column was prepared by packing C18 material into a pipette tip fitted on a collection tube. The sample was loaded onto the column, washed, and the peptides were eluted with 0.1% FA in 50% ACN. The eluted peptides were dried and stored at -20°C. The sample was prepared for LC-MS analysis by reconstituting in 30 µl of 0.1% FA and vortexed. It was centrifuged at 13,000 x g for 10 minutes at 4°C, and 20 µl of the supernatant was transferred to an MS vial for LC-MS/MS analysis. The peptides were analyzed using an LC-MS/MS system, and data were processed with Proteome Discoverer 2.1, searching against the Human Uniprot database.

Statistical Analysis

PCA was performed on the matrix of expression values using the R library PCA tools. DEPs between AGA and controls and FPHL and controls, were identified using the limma package in R and Protein intensity data were normalized using LOESS normalization. The significance threshold for differential gene expression was set at a p-value of 0.05 and an absolute Log2 fold change (|Log2FC|) of 1. It is essential to note that no additional adjustments for multiple testing were applied. This decision was made based on the exploratory nature of our study, aiming to identify potential patterns and hypotheses for future validation rather than to confirm definitive biomarkers at this stage.

The predicted functional and physical protein-protein interaction (PPI) networks were analyzed and visualized using the STRING database. The DEP lists were inputted, and interactions were assessed using a confidence cutoff of 0.4. MCL clustering was performed to visualize the large interactive clusters of proteins.

4. Results and Discussion

4.1 Results

Distinct protein profiles in pattern hair loss compared to controls

Principal Component Analysis (PCA) showed clear differences in protein expression between scalp samples from AGA (MD) and FPHL (FD) patients, compared to those from male (MC) and female (FC) normal controls, respectively. While there was some overlap, the protein expression profiles of both MD and FD patients were still significantly different from those of their control groups.

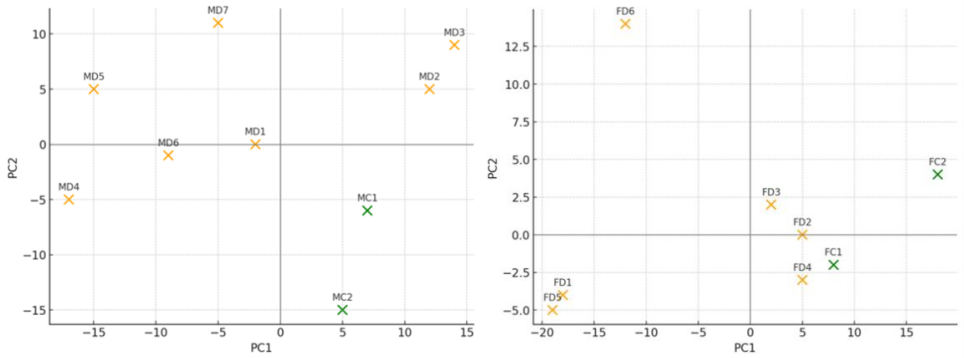
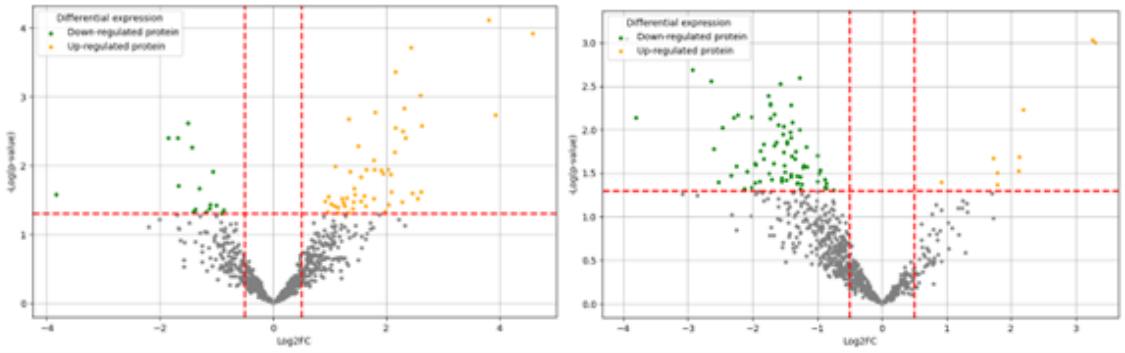


Figure 1 Principal Component Analysis (PCA). (a) PCA for MD and MC. (b) PCA for FD and FC *Differential expression analysis*

The differential expression analysis, this volcano plot highlights 68 differentially expressed proteins (DEPs) between MD and MC scalp samples.52 proteins are up-regulated in MD and 16 proteins are down-regulated in MC. In FPHL, the volcano plot highlights 84 differentially expressed proteins (DEPs) between FA and FN scalp samples. Among these, 9 proteins are up-regulated in FD and 75 proteins are down-regulated in FD.



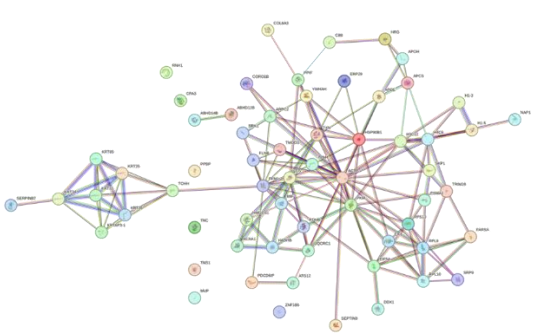
A. **B.**

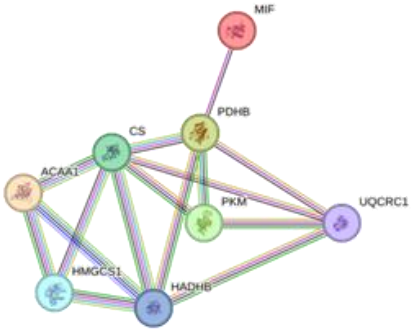
Figure 2A Volcano plot of differentially expressed proteins (DEPs) between the alopecic vertex scalp of male androgenetic alopecia (MD) patients and the corresponding scalp area of male normal controls (MC). The horizontal line indicates the threshold for significance (p-value < 0.05), and the vertical lines indicate the thresholds for fold change (|logFC| = 0.5). Orange points indicate proteins that are up-regulated in MD, and green points indicate proteins that are down-regulated in MD.

Figure 2B Volcano plot of differentially expressed proteins (DEPs) between the alopecic vertex scalp of female pattern hair loss (FD) patients and the corresponding scalp area of female normal controls (FC). Each point represents a protein. The horizontal line indicates the threshold for significance (p value < 0.05), and the vertical lines indicate the thresholds for fold change (|logFC| = 0.5). Orange points indicate proteins that are up-regulated in FD, and green points indicate proteins that are down-regulated in FC.

Protein-Protein Interaction (PPI) Network and Cluster Analysis

To explore the complexities of sex differences in pattern hair loss, our research employed a comprehensive approach to construct Protein-Protein Interaction (PPI) networks, as shown in Figure 4A (male) and Figure 4D (female). The PPI network analysis, guided by the Markov Clustering Algorithm (MCL), revealed strong connections among the DEPs, particularly within a metabolic-related sub-network in both males and females (Figures 4B and 4E). In MD, this cluster's mapping to KEGG pathways highlighted its involvement in the Citrate cycle, Metabolic pathways, and Fatty acid metabolism and degradation, as shown in Figure 4C. In females, the cluster was involved in Glycolysis/Gluconeogenesis, Carbon metabolism, Biosynthesis of amino acids, the Pentose phosphate pathway, and Fructose and Mannose metabolism, detailed in Figure 4F. The proteins in the MD metabolic-related cluster were predominantly up-regulated, whereas they were mainly down-regulated in FD. In MD, Citrate synthase is the most highly connected protein in the metabolic cluster highlighting its importance. Additionally, the cluster shows that CS interacts with the key enzymes in central metabolic pathway including PDHB and PKM2 (Weitzman, & Danson, 1976; Schormann et al., 2019; Koukourakis et al., 2005). In FD, among proteins in the metabolic cluster, TPI1 is the hub protein, which exhibits the most interaction with others.



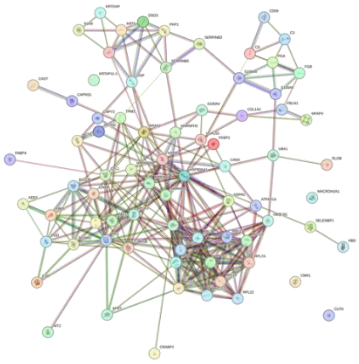


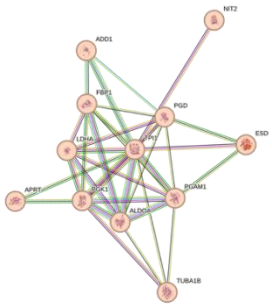
A **B**

KEGG Pathways

pathway	description	count in network	strength	signal	false discovery rate
hsa00280	Valine, leucine and isoleucine degradation	3 of 46	2.26	2.42	8.44e-05
hsa00020	Citrate cycle (TCA cycle)	2 of 28	2.3	1.41	0.0052
hsa01100	Metabolic pathways	7 of 1435	1.14	1.33	3.72e-06
hsa00071	Fatty acid degradation	2 of 42	2.13	1.26	0.0085
hsa01212	Fatty acid metabolism	2 of 54	2.02	1.17	0.0111

C





D E

KEGG Pathways

pathway	description	count in network	strength	signal	false discovery rate
hsa00010	Glycolysis / Gluconeogenesis	6 of 64	2.19	4.36	2.46e-10
hsa01200	Carbon metabolism	7 of 116	2.0	4.16	8.04e-11
hsa01230	Biosynthesis of amino acids	4 of 73	1.95	2.34	8.74e-06
hsa00030	Pentose phosphate pathway	3 of 29	2.23	2.14	5.69e-05
hsa00051	Fructose and mannose metabolism	3 of 32	2.19	2.1	6.25e-05

F

Figure 4 Protein-Protein Interaction (PPI) networks clustered by the Markov Clustering Algorithm (MCL) for AGA and FPHL. **(A)**: The network shows the interactions between differentially expressed proteins (DEPs) in MD vs MC. Each node represents a protein, and edges between nodes represent interactions between proteins. **(B)**: metabolic-related cluster includes Citrate synthase (CS), Pyruvate dehydrogenase (PDHB) and Pyruvate kinase (PKM). **(C)**: KEGG pathways show the function of each enzyme in the metabolic-related cluster in AGA. **(D)**: The network shows the interactions between differentially expressed proteins (DEPs) in FD vs FC. **(E)**: metabolic-related cluster includes TPI1, ALDOA, PGAM1, FBP1, and LDHA. **(F)**: KEGG pathways illustrate the function of each enzyme in the metabolic-related cluster in FPHL.

4.2 Discussion

This study revealed distinct protein expression profiles in MD and FD when compared to controls, highlighting significant metabolic adaptations within hair follicles in response to pathological conditions. In AGA, the predominance of up-regulated metabolic proteins suggests a cellular response to increase energy production and metabolic flux. Conversely, in FPHL, a notable downregulation of metabolic proteins was observed, suggesting a reduced metabolic capability, which may contribute to impaired maintenance and regeneration of hair follicles.

For AGA, there was a predominant up-regulation of metabolic proteins such as citrate synthase (CS), pyruvate dehydrogenase (PDHB), and pyruvate kinase M2 (PKM2). While the upregulation could potentially supply the energy necessary for sustaining hair growth, it may paradoxically exacerbate hair loss by promoting conditions that do not favor follicular stability and longevity. Understanding these metabolic shifts is crucial for developing targeted therapies that could modulate these pathways to balance follicular metabolism and counteract hair loss.

Citrate synthase (CS), Pyruvate kinase M2 (PKM2) and Pyruvate dehydrogenase E1 component beta (PDHB) are all crucial enzymes involved in cellular metabolism, particularly in the pathways of energy production and metabolism. CS is an enzyme in the citric acid cycle (Krebs cycle), catalyzing the condensation of acetyl-CoA and oxaloacetate to form citrate. This is a key step in the oxidation of carbohydrates, fats, and proteins for energy production (Morrison, 2021). PDHB is part of the pyruvate dehydrogenase complex, which converts pyruvate (from glycolysis) into acetyl-CoA. This step is critical for linking glycolysis with the citric acid cycle, allowing the flow of metabolites from one pathway to another (Harris et al., 2002). PKM2 is involved in glycolysis, catalyzing the conversion of phosphoenolpyruvate (PEP) to pyruvate (Harris et al., 2002). The pyruvate produced is then further processed by PDHB, linking glycolysis to the citric acid cycle. Together, these enzymes demonstrate a flow of energy conversion in the cell: glycolysis (via PKM2) generates pyruvate, which is then converted into acetyl-CoA by PDHB, allowing entry into the citric acid cycle (where CS operates) for further ATP production (Xiong et al., 2011). This interconnectedness underscores the importance of these enzymes in maintaining energy homeostasis. Under conditions of metabolic stress or altered cellular environments (such as in alopecia), the regulation of these enzymes can change. For example, the upregulation of PKM2 and PDHB could reflect an adaptive response to increased energy demands or stress, allowing cells to efficiently utilize available substrates for energy production.

A study from Ryu et al. (2022) revealed that PKM2 is crucial for the glycolytic metabolism necessary for HFSC activation during hair growth. The expression and activity of PKM2 were found to increase significantly during the anagen phase of hair growth in mice, particularly following depilation. The research indicates that PKM2 expression is regulated by the Wnt/β-catenin signaling pathway, which is known to be involved in hair follicle development and regeneration. Activation of this pathway enhances PKM2 levels, thereby promoting HFSC proliferation and hair growth. This research sheds light on the intricate interplay between metabolism and hair regeneration (Ryu et al., 2022). The increased expression of PKM2 could reflect an attempt to compensate for the Wnt/β-catenin dysfunction in AGA, activating glycolysis and promoting energy production to support HFSCs in an environment where follicle regeneration is impaired. Since glycolysis and cellular metabolism are tightly linked to cell proliferation, PKM2's increase could be part of an attempt by the follicles to maintain or restore hair growth. PKM2 is known to boost glycolysis and support the activation and proliferation of HFSCs through its interaction with critical signaling pathways such as Wnt/β-catenin, essential for HFSC function and hair follicle regeneration. However, the potential benefits of increased PKM2 activity may not fully counteract the negative effects caused by the enhanced activity of TCA cycle enzymes. The shift towards oxidative metabolism by TCA cycle enzymes, including PDHB and CS, may be more pronounced that the even increase in PKM2 may not be enough to counteract. Additionally, AGA is influenced by factors such as androgens, oxidative stress, inflammation, and apoptosis, which collectively contribute to hair follicle miniaturization and hair loss.

In 2022, Shi et al. (2022) investigated the role of citric acid in hair growth and found that the injection of citric acid in mice caused hair growth suppression and catagen entry promotion. Through their research,

they illuminate how alterations in citric acid metabolism pathways can profoundly impact the regulation of hair growth processes. These findings offer valuable insights into potential therapeutic targets for addressing hair loss conditions associated with citric acid metabolism dysregulation (Xiangguang et al., 2022). This might explain the early onset of catagen in AGA, leading to the typical thinning and hair loss observed in these patients. Thus, citric acid not only contributes to energy metabolism but also directly influences hair cycle dynamics, suggesting that alterations in citric acid metabolism, specifically an increase in citric acid or its metabolites, could disrupt normal hair growth processes by shifting the hair cycle toward catagen. Regarding an increased citrate synthase in AGA's hair follicles, it could lead to higher levels of citrate, which may promote shifts in the hair cycle, potentially leading to hair growth suppression or an earlier entry into catagen, resulting in hair thinning or shedding. Moreover, it can imply that altered energy metabolism in hair follicles is a contributing factor in AGA. The increased citric acid cycle activity might influence follicular cells' behavior, possibly triggering signaling pathways that favor catagen or limit the duration of the anagen phase.

Although there is still no previous evidence about PDHB on hair follicle metabolism or hair loss, there is a previous study that demonstrated that the inhibition of pyruvate oxidation serves as a significant stimulant of the hair cycle, particularly in models of alopecia (Flores et al., 2021). By enhancing PDHB activity, pyruvate oxidation is activated, hair growth is inhibited, resulting in hair loss. This inhibition appears to promote the activation of hair follicle stem cells (HFSCs), which are typically quiescent in conditions like AGA (Kidangazhiathmana, & Santhosh, 2022). The upregulation of PDHB suggests that increased pyruvate oxidation may lead to reduced availability of metabolic substrates necessary for hair follicle stem cell activation and proliferation.

Specifically, the upregulation of PDHB and CS leads to increased mitochondrial oxidation. This leads to a reduction in pyruvate availability, decreases the production of lactate-by-lactate dehydrogenase (LDH). Given that lactate plays a crucial role in activating hair follicle stem cells (HFSCs) and triggering the hair growth phase (anagen), diminished lactate levels could impede the hair cycle's progression in AGA patients (Flores et al., 2021).

This complex interplay of metabolic pathways within the hair follicle suggests that therapeutic strategies for AGA should address the balance between glycolysis and oxidative metabolism. Targeted modulation of these pathways, potentially using metabolic activators or inhibitors, could help restore a healthy balance of energy production and signaling molecule availability, potentially reversing the hair cycle dysregulation observed in AGA.

The contrasting expression profiles of proteins involved in metabolic pathways between AGA and FPHL provide critical insights into the underlying biological mechanisms that contribute to these conditions. In AGA, the up-regulation of metabolic proteins suggests an adaptive response to the pathological state of hair follicles, potentially reflecting increased energy demands or altered metabolic states as the body attempts to sustain cellular functions despite hair loss. This up-regulation may indicate a compensatory mechanism aimed at maintaining hair follicle viability and function, even in the face of ongoing miniaturization and loss (Kidangazhiathmana, & Santhosh, 2022).

Conversely, FPHL is characterized by a decrease in metabolic proteins, suggesting a reduced ability to metabolize effectively. This reduction may hinder hair follicles from sustaining their normal growth cycles, leading to the thinning commonly seen in this condition. Enzymes such as triosephosphate isomerase (TPI1), an extremely efficient metabolic enzyme that catalyzes the interconversion between dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (G3P) in glycolysis and gluconeogenesis, which is essential for ATP production, are particularly significant; diminished activity could result in inadequate energy for growth and regeneration, causing follicles to enter the telogen or catagen phases prematurely (Ationu, & Humphries, 1998). These findings add valuable insights by clarifying the metabolic changes associated with AGA and FPHL, highlighting the need for targeted therapies that can modulate these pathways. For example, restoring the balance between glycolysis and oxidative metabolism might improve hair follicle function and encourage hair regrowth. Furthermore, the small sample size (7 AGA, 8 FPHL) could limit the applicability of these results, emphasizing the necessity for additional studies with larger

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cohorts to confirm these findings. Additionally, our research did not include functional validation, such as knockdown or overexpression experiments, to verify the roles of the identified proteins in hair follicle metabolism and growth. Future studies that incorporate these methods could shed light on how these metabolic changes directly affect hair loss.

In summary, this study underscores the different expression of metabolic proteins in AGA and FPHL, offering insights into their respective pathogenesis. The upregulated proteins in AGA may act as potential biomarkers for diagnosis or treatment monitoring, while the downregulated proteins in FPHL could serve as therapeutic targets aimed at restoring normal hair follicle function. Future research into the interactions between metabolic pathways and signaling mechanisms could pave the way for innovative treatment strategies for both conditions.

5. Conclusion

The differential expression of key metabolic enzymes such as CS, PDHB and PKM2 in the scalp of AGA patients offers valuable insights into the altered metabolic landscape of hair follicles in this condition. The upregulation of these enzymes suggests an adaptive response to increased energy demands or cellular stress, as the body attempts to sustain hair follicle function despite the pathological processes driving hair loss. CS, PKM, and PDHB are central to cellular energy metabolism, with CS involved in the citric acid cycle, PKM in glycolysis, and PDHB in the conversion of pyruvate to acetyl-CoA, linking glycolysis to the citric acid cycle. Their coordinated activity underscores the importance of metabolic flexibility in maintaining cellular homeostasis. However, this upregulation may also reflect a compensatory mechanism that inadvertently contributes to hair follicle miniaturization by promoting early transitions into the catagen phase and disrupting normal hair cycle progression. Specifically, elevated CS activity could increase citrate levels, potentially favoring the catagen phase and impairing hair growth. Similarly, enhanced pyruvate oxidation through PDHB could limit the availability of key metabolic substrates for hair follicle stem cell activation, ultimately inhibiting hair growth. In contrast, the downregulation of certain metabolic proteins in FPHL indicates a different set of metabolic disruptions, likely involving impaired energy production and diminished cellular processes necessary for hair follicle health, such as cell proliferation and differentiation. This decline in metabolic activity could contribute to the characteristic thinning and loss of hair seen in FPHL, highlighting the complexity and variability in metabolic dysregulation across different forms of hair loss. Together, these findings suggest that while metabolic adaptation through upregulation of CS, PKM, and PDHB may initially help maintain follicular function under stress, these very changes might also inadvertently accelerate hair loss in AGA. Understanding these metabolic shifts could offer new strategies for targeting both the metabolic and signaling pathways involved in hair follicle regeneration, with potential implications for the treatment of AGA and other forms of hair loss.

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7. References

Ationu, A., & Humphries, A. (1998). The feasibility of replacement therapy for inherited disorder of glycolysis: triosephosphate isomerase deficiency. *International Journal of Molecular Medicine*, *2*(6), 701-705.https://doi.org/10.3892/ijmm.2.6.701

Chen, C., Hou, J., Tanner, J. J., & Cheng, J. (2020). Bioinformatics methods for mass spectrometry-based proteomics data analysis. *International journal of molecular sciences*, *21*(8), Article 2873. https://doi.org/10.3390/ijms21082873

Flores, A., Choi, S., Hsu, Y., & Lowry, W. E. (2021). Inhibition of pyruvate oxidation as a versatile stimulator of the hair cycle in models of alopecia. *Experimental Dermatology*, *30*(4), 448–456. https://doi.org/10.1111/exd.14307

Gan, D. C., & Sinclair, R. D. (2005). Prevalence of male and female pattern hair loss in Maryborough. *Journal of Investigative Dermatology Symposium Proceedings*, *10*(3), 184–189. https://doi.org/10.1111/j.1087-0024.2005.10102.x

Gillet, L. C., Leitner, A., & Aebersold, R. (2016). Mass spectrometry applied to bottom-up proteomics: entering the high-throughput era for hypothesis testing. *Annual review of analytical chemistry*, *9*(1), 449-472. https://doi.org/10.1146/annurev-anchem-071015-041535

Harris, R. A., Bowker-Kinley, M. M., Huang, B., & Wu, P. (2002). Regulation of the activity of the pyruvate dehydrogenase complex. *Advances in enzyme regulation*, *42*, 249-259. https://doi.org/10.1016/S0065-2571(01)00061-9

Iamsumang, W., Leerunyakul, K., & Suchonwanit, P. (2020). Finasteride and its potential for the treatment of female pattern hair loss: evidence to date. *Drug design, development and therapy*, *14*, 951-959. https://doi.org/10.2147/DDDT.S240615

Kaufman, K. D. (2002). Androgens and alopecia. *Molecular and cellular endocrinology*, *198*(1-2), 89-95. https://doi.org/10.1016/S0303-7207(02)00372-6

Kidangazhiathmana, A., & Santhosh, P. (2022). Pathogenesis of androgenetic alopecia. *Clinical Dermatology Review*, *6*(2), 69-74. https://doi.org/10.4103/cdr.cdr_29_21

Koukourakis, M. I., Giatromanolaki, A., Sivridis, E., Gatter, K. C., Harris, A. L., & Tumor and Angiogenesis Research Group. (2005). Pyruvate dehydrogenase and pyruvate dehydrogenase kinase expression in non small cell lung cancer and tumor-associated stroma. *Neoplasia*, *7*(1), 1-6. https://doi.org/10.1593/neo.04373

Lolli, F., Pallotti, F., Rossi, A., Fortuna, M. C., Caro, G., Lenzi, A., ... & Lombardo, F. (2017). Androgenetic alopecia: a review. *Endocrine*, *57*, 9-17. https://doi.org/10.1007/s12020-017-1280-y

Mahé, Y. F., Michelet, J. F., Billoni, N., Jarrousse, F., Buan, B., Commo, S., ... & Bernard, B. A. (2000). Androgenetic alopecia and microinflammation. *International journal of dermatology*, *39*(8). https://doi.org/10.1046/j.1365-4362.2000.00612.x

Morrison, H. (2020). *Citrate synthase*. In Elsevier eBooks (pp. 45–49).

Pathomvanich, D., Pongratananukul, S., Thienthaworn, P., & Manoshai, S. (2002). A random study of Asian male androgenetic alopecia in Bangkok, Thailand. *Dermatologic surgery*, *28*(9), 804-807. https://doi.org/10.1097/00042728-200209000-00005

Ramos, P. M., Brianezi, G., Martins, A. C. P., Da Silva, M. G., Marques, M. E. A., & Miot, H. A. (2016). Apoptosis in follicles of individuals with female pattern hair loss is associated with perifollicular microinflammation. *International Journal of Cosmetic Science*, *38*(6), 651-654. https://doi.org/10.1111/ics.12341

Rogers, N. E., & Avram, M. R. (2008). Medical treatments for male and female pattern hair loss. *Journal of the American Academy of Dermatology*, *59*(4), 547-566. https://doi.org/10.1016/j.jaad.2008.07.001

Rojhirunsakool, S., & Suchonwanit, P. (2017). Parietal scalp is another affected area in female pattern hair loss: an analysis of hair density and hair diameter. *Clinical, Cosmetic and Investigational Dermatology*, 7-12. https://doi.org/10.2147/CCID.S153768

Ryu, Y. C., Kim, Y. R., Park, J., Choi, S., Ryu, W. J., Kim, G. U., ... & Choi, K. Y. (2022). Pyruvate kinase M2 promotes hair regeneration by connecting metabolic and Wnt/β-catenin signaling. *Pharmaceutics*, *14*(12), Article 2774. https://doi.org/10.3390/pharmaceutics14122774

Schormann, N., Hayden, K. L., Lee, P., Banerjee, S., & Chattopadhyay, D. (2019). An overview of structure, function, and regulation of pyruvate kinases. *Protein Science*, *28*(10), 1771-1784. https://doi.org/10.1002/pro.3691

Shi, X., Chen, Y., Yang, K., Zhu, Y., Ma, Y., Liu, Q., ... & Wu, W. (2022). Disrupted citric acid metabolism inhibits hair growth. *The Journal of Dermatology*, *49*(10), 1037-1048. https://doi.org/10.1111/1346-8138.16509

Su, L. H., Chen, L. S., & Chen, H. H. (2013). Factors associated with female pattern hair loss and its prevalence in Taiwanese women: a community-based survey. *Journal of the American Academy of Dermatology*, *69*(2), e69-e77. https://doi.org/10.1016/j.jaad.2012.09.046

Suchonwanit, P., Chalermroj, N., & Khunkhet, S. (2019a). Low-level laser therapy for the treatment of androgenetic alopecia in Thai men and women: a 24-week, randomized, double-blind, sham device-controlled trial. *Lasers in medical science*, *34*, 1107-1114. https://doi.org/10.1007/s10103-018-02699-9

Suchonwanit, P., Rojhirunsakool, S., & Khunkhet, S. (2019b). A randomized, investigator-blinded, controlled, split-scalp study of the efficacy and safety of a 1550-nm fractional erbium-glass laser, used in combination with topical 5% minoxidil versus 5% minoxidil alone, for the treatment of androgenetic alopecia. *Lasers in medical science*, *34*, 1857-1864. https://doi.org/10.1007/s10103-019-02783-8

Suchonwanit, P., Thammarucha, S., & Leerunyakul, K. (2019c). Minoxidil and its use in hair disorders: a review. *Drug design, development and therapy*, 2777-2786. https://doi.org/10.2147/DDDT.S214907

Trüeb, R. M. (2015). The impact of oxidative stress on hair. *International journal of cosmetic science*, *37*(Suppl. 2), 25-30. https://doi.org/10.1111/ics.12286

Weitzman, P., & Danson, M. J. (1976). Citrate synthase. *Current Topics in Cellular Regulation*, *10*, 161–204. https://doi.org/10.1016/b978-0-12-152810-2.50011-5

Williamson, D., Gonzalez, M., & Finlay, A. Y. (2001). The effect of hair loss on quality of life. *Journal of the European Academy of Dermatology and Venereology*, *15*(2), 137-139. https://doi.org/10.1046/j.1468-3083.2001.00229.x

Xiong, Y., Lei, Q., Zhao, S., & Guan, K. (2011). Regulation of glycolysis and gluconeogenesis by acetylation of PKM and PEPCK. *Cold Spring Harbor Symposia on Quantitative Biology*, *76*, 285–289. https://doi.org/10.1101/sqb.2011.76.010942

Yi, Y., Li, X., Jia, J., Didier, D. N. G., Qiu, J., Fu, J., ... & Hu, Z. (2020). Effect of behavioral factors on severity of female pattern hair loss: an ordinal logistic regression analysis. *International Journal of Medical Sciences*, *17*(11), 1584. https://doi.org/10.7150/ijms.45979