Proteomic Profiling of Central Centrifugal Cicatricial Alopecia Reveals Role of Humoral Immune Response Pathway and Metabolic Dysregulation

Aditi Gadre[1,2], Taylor Dyson[1,3], Jaroslaw Jedrych[1], Grant Anhalt[1], Angel S. Byrd[1,4] and Crystal Aguh[1]

Proteomic profiling on other primary cicatricial alopecias, such as frontal fibrosing alopecia and lichen planopilaris, have suggested a T helper 1–mediated inflammatory pathway, but in central centrifugal cicatricial alopecia (CCCA), the protein expression patterns are unknown. In this study, we sought to characterize protein expression patterns in CCCA to identify biomarkers of disease activity that will identify potential therapeutic avenues for treatment. Scalp protein quantification was performed to understand protein expression patterns in affected versus unaffected scalps in CCCA. A total of 5444 proteins were identified, of which 148 proteins were found to be differentially expressed in CCCA-affected scalp, with upregulation of adaptive immune pathways (*IGHG3*, $P = .034$; *IGHG4*, $P = .01$; *IGG1*, $P = .026$) and markers of fibrosis (*ITGA1*, $P = .016$; *SFRP2*, $P = .045$; *TPM2*, $P = .029$; *SLMAP*, $P = .016$) and downregulation of metabolic proteins (*ALOX15B*, $P = .003$; *FADS2*, $P = .006$; *ELOVL5*, $P = .007$; *FA2H*, $P = .017$; *FAR2*, $P = .011$; *SC5D*, $P < .001$). Our analysis revealed, to our knowledge, previously unknown humoral immune canonical pathways, notably IgG, implicated in CCCA and additionally confirmed aberrant lipid metabolism pathways implicated in diabetes mellitus, suggesting unique mechanisms of disease in patients with CCCA.

Keywords: Ethnic skin, Fibrosis, Hair loss, Scarring alopecia, Transcriptomics

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INTRODUCTION

Central centrifugal cicatricial alopecia (CCCA) is the most common form of cicatricial alopecia in Black women (Ogunleye et al, 2014). Previous studies have identified an upregulation in mRNA transcripts responsible for profibrotic processes, including those responsible for myofibrocyte migration, the cell type of interest in many disorders of fibrosis (Aguh et al, 2018; Wynn, 2007). In addition, genes regulating lipid metabolism and biosynthesis of fatty acids have been implicated in the disease (Aguh et al, 2018). Increasingly, data have suggested an association between insulin resistance and CCCA, with a small case series suggesting possible benefit of compounded metformin in patients with CCCA (Araoye et al, 2020; Roche et al, 2022).

CCCA is considered to be a primary lymphocytic cicatricial alopecia, similar to diseases such as lichen planopilaris and frontal fibrosing alopecia (FFA) (Flamm et al, 2020; Lawson et al, 2021). In the latter conditions, research suggests a T helper 1–mediated disease process, resulting in widespread destruction of follicular stem cells (Harries et al, 2013). In particular, proteomic profiling of FFA suggests a prominent role of a T helper 1/Jak–mediated pathway of disease involvement as well as upregulated fibrotic transcripts (Dubin et al, 2022). However, clinical distinctions in presentation suggest that despite these conditions eventuating a similar end-stage scarring phenotype, the mechanism of disease differs. Whereas in both lichen planopilaris and FFA, clinical erythema, pruritus, and scalp burning are suggestive of disease progression, similar symptoms are often minimal or absent in patients with CCCA, rendering disease monitoring more difficult in these patients (Ogunleye et al, 2014; Svigos et al, 2021). In CCCA, inflammation is variable, often subclinical, and when present, it is more reliably detected on histology, although characterization of inflammation in CCCA suggests a CD4+–mediated pathway (Flamm et al, 2020). Further characterization of the immune pathway is necessary to identify targeted therapies that may be of benefit to affected patients (Flamm et al, 2020; Ogunleye et al, 2014; Tan et al, 2019).

Proteomic profiling of disease is a helpful analytic tool used to understand intricate disease processes and identify

[1]*Department of Dermatology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA;* [2]*School of Medicine, Georgetown University, Washington, District of Columbia, USA;* [3]*Eastern Virginia Medical School, Norfolk, Virginia, USA; and* [4]*Department of Dermatology, College of Medicine, Howard University, Washington, District of Columbia, USA*

Correspondence: Crystal Aguh, Department of Dermatology, The Johns Hopkins University School of Medicine, 601 North Caroline Street, 8th Floor Dermatology, Suite 8065, Baltimore, Maryland 21231, USA. E-mail: Cagi1@jhmi.edu

Abbreviations: CCCA, central centrifugal cicatricial alopecia; FFA, frontal fibrosing alopecia

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disease biomarkers because mRNA expression patterns may not translate directly to protein expression levels in tissue. In this study, we sought to characterize protein expression patterns in CCCA to identify biomarkers of disease activity that will reveal potential therapeutic avenues for treatment.

RESULTS

Five treatment-naïve patients with clinical and histologically confirmed CCCA were enrolled in this study (Table 1). Samples were collected from affected scalp vertex, with samples from unaffected occipital scalp serving as intrascalp controls. Through proteomic analysis, a total of 5444 proteins were identified, of which 148 proteins were found to be differentially expressed in CCCA compared with those in healthy tissue (Figure 1).

Upregulated protein transcripts of CCCA scalp profile depict robust Ig production

Fifty-one differentially expressed proteins were upregulated in CCCA-affected tissue (fold change > 1.25; $P < .05$), of which 24 proteins were associated with immune response (Figure 2). The 3 most common canonical pathways identified through Gene Set Enrichment Analysis were the adaptive immune response, positive regulation of B-cell activation, and Ig production ($P < .001$). Notably, 3 of the Ig chains belonged to the IgG family (*IGHG3*, $P = .034$; *IGHG4*, $P = .01$; *IGG1*, $P = .026$).

Markers of fibrosis encoded by *ITGA1* ($P = .016$) (Ramos et al, 2012), *SFRP2* ($P = .045$) (Lin et al, 2016), *TPM2* ($P = .029$) (Bradbury et al, 2021), and *SLMAP* ($P = .016$) (Mlynarova et al, 2019) genes were also noted to be upregulated in the affected scalp, consistent with profibrotic canonical pathways observed in microarray data for CCCA (Aguh et al, 2018).

The CCCA proteome indicates metabolic dysfunction

Ninety-seven differentially expressed proteins were downregulated in CCCA-affected scalp (fold change > 1.25; $P < .05$), of which 39 proteins were associated with lipid metabolism (Figure 3). Genes responsible for these proteins include *ALOX15B* ($P = .003$), *FADS2* ($P = .006$), *ELOVL5* ($P = .007$), *FA2H* ($P = .017$), *FAR2* ($P = .011$), and *SC5D* ($P < .001$). Gene Set Enrichment Analysis further supported lipogenic involvement, with common pathways including metabolism, steroid biosynthesis, and fatty acid metabolism ($P < .001$). Furthermore, the peroxisome proliferator–activated receptor gene *PPAR* and AMP-activated protein kinase gene *AMPK* signaling pathways displayed overlap with CCCA ($P < .001$) (Table 2). Both pathways have been implicated in the development and treatment of metabolic syndromes such as diabetes (Entezari et al, 2022; Jay and Ren, 2007).

DISCUSSION

Our analysis revealed statistically significant increases in expression of proteins involved in immune canonical pathways, notably IgG. To our knowledge, CCCA has not previously been associated with the humoral immune pathway. A prior study investigating the immune profile of patients with FFA did not reveal this pattern, suggesting a possible unique mechanism of disease in patients with CCCA (Dubin et al, 2022).

Significance of Ig predominant inflammatory response

Overall, the inflammatory role of IgG in CCCA remains poorly understood, requiring further evaluation. Unlike scalp erythema seen in lichen planopilaris, active inflammation in CCCA is often subtle and often requires trichoscopy to detect evidence of inflammation, when present (Ogunleye et al, 2014). However, CCCA is unique among many of the primary lymphocytic cicatricial alopecias because it has been noted to have CD4+ predominant T-cell infiltrate (Flamm et al, 2020), unlike lichen planopilaris and FFA, which demonstrate a CD8+ predominant T-cell infiltrate (Ma et al, 2017). The presence of IgG-positive inflammatory response delineates B lymphocyte dysregulation, suggesting more nuanced immunobiology than previously considered.

Similar IgG-predominant inflammatory dysregulation has been reported in hidradenitis suppurativa (Byrd et al, 2019; Gudjonsson et al, 2020). However, in contrast to hidradenitis suppurativa, CCCA is a pauci-inflammatory condition, especially at advanced stages. Because B cells are known to activate T helper 2 skewing in fibroinflammatory conditions (Della-Torre et al, 2020), studies investigating the interaction between B and T lymphocytes may prove critical for understanding the pathophysiology of CCCA.

Metabolic dysregulation

The metabolic dysfunction seen among downregulated proteins in affected tissue was also consistent with results from microarray studies, supporting an association between mRNA and protein expression (Aguh et al, 2018). Of the 6 conserved lipogenic genes between the proteomics and microarray datasets (Aguh et al, 2018), *FAR2* and *FA2H* have been associated with alopecia in mouse models (Maier et al, 2011; Sundberg et al, 2018). *FAR2* encodes a protein important for the reduction of fatty acids to fatty alcohols during the first step of biosynthesis (Rittié et al, 2016). *Far2*-knockout mice consistently displayed follicular dystrophy and sebaceous gland abnormalities, exhibiting a phenotype

Table 1. Participant Demographics

Participant	Age, y	Gender	Race	CCCA Severity	Lesional Biopsy Site	Nonlesional Biopsy Site
1	47	Female	Black	Severe	Vertex scalp	Lateral occipital scalp
2	54	Female	Black	Severe	Vertex scalp	Lateral occipital scalp
3	51	Female	Black	Severe	Vertex scalp	Lateral occipital scalp
4	51	Female	Black	Severe	Vertex scalp	Lateral occipital scalp
5	57	Female	Black	Severe	Vertex scalp	Lateral occipital scalp

Abbreviation: CCCA, central centrifugal cicatricial alopecia.

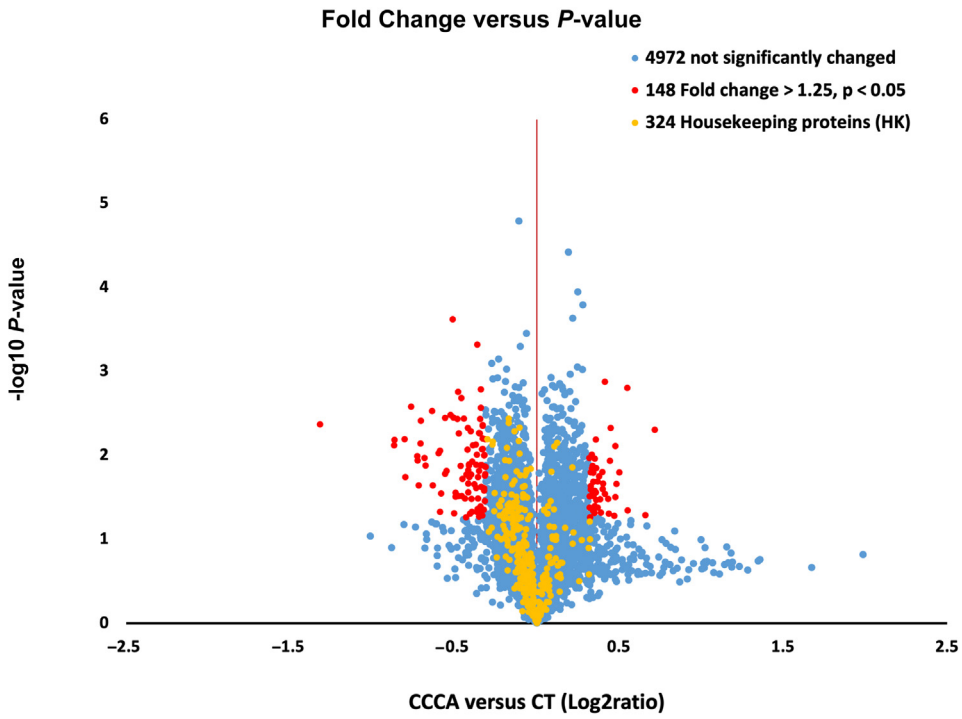

Figure 1. Volcano plot demonstrating the fold change of protein abundance between lesional and nonlesional tissue in patients with CCCA. Data points represented in blue are 4972 proteins without significant changes (fold change < 1.25), and data points represented in red are 148 proteins with significant changes (fold change > 1.25, $P < .05$). Yellow data points represent 324 HK proteins that were unchanged. CCCA, central centrifugal cicatricial alopecia; HK, housekeeping.

analogous to patchy cicatricial alopecia (Sundberg et al, 2018). In addition, lipidomic analysis of the skin surface in these mice demonstrated widespread alterations in the levels of wax esters, diacylglycerols, and other lipid components (Sundberg et al, 2018). Similarly, mice with loss of *Fa2h* demonstrate cycling alopecia between the telogen and early anagen phase as well as altered composition of sebum (Maier et al, 2011). *FA2H* is thought to be responsible for catalyzing the hydroxylation of sphingolipids in keratinocytes (Maier et al, 2011). These processes are of concern because dysregulated cholesterol biosynthesis is postulated to lead to an overabundance of sterol intermediates, which may serve as an early inciting inflammatory event in primary cicatricial alopecias (Panicker et al, 2012).

The overlap of downregulated proteins with peroxisome proliferator–activated receptor gene *PPAR* and AMP-activated protein kinase gene *AMPK* signaling canonical pathways also strengthened associations between CCCA and metabolic syndromes. Both induction of peroxisome proliferator–activated receptor gene *PPAR* and of AMP-activated protein kinase gene *AMPK* inhibit insulin resistance and promote glucose uptake and metabolism (Entezari et al, 2022; Jay and Ren, 2007). Importantly, recent studies cite a higher incidence of type 2 diabetes in patients with CCCA, highlighting a need for improved characterization of the role of lipid biosynthesis (Roche et al, 2022).

In conclusion, the unique deposition of IgG detected through proteomics reveals insights into the disease process of CCCA. In addition, the aberrant lipid metabolism profile detected suggests metabolic dysregulation. Given the potential role of humoral immunity in CCCA, further research must be conducted to determine Ig involvement in follicular scarring and antigenic epitopes, with an emphasis on understanding circulating tissue-specific antibodies. Although our study is limited by its small sample size at a single institution, the identified proteome may guide further insights into the intricate immunobiology of this condition and advance future therapeutic measures.

MATERIALS AND METHODS

Patient characteristics and sample collection

The study protocol was approved by the Johns Hopkins Hospital Institutional Review Board (number NA_00013177). Informed, written consent was obtained from all patients with approval from the Johns Hopkins Ethics Board. A total of 5 Black, female, treatment-naïve patients with clinical and histologically confirmed CCCA were enrolled in this study. Samples were collected from affected scalp vertex, with samples from unaffected occipital scalp unlikely to develop disease serving as intrascalp controls.

Scalp protein quantification

Fresh tissue samples were immediately submerged in a 2 ml tube containing RNAlater (Qiagen) stabilization reagent. Tissue was stored at 4 °C for 24 hours and transferred afterward to a freezer for long-term storage at −80 °C. Protein lysates were prepared using a BCA Protein Assay Kit. This process was followed by digestion with trypsin, and a tandem mass tag (TMT-11plex) set was utilized to label peptides. Fractionation of labeled peptides was conducted by reversed-phase Ultra-High-Performance Liquid Chromatography. Finally, liquid chromatography with tandem mass spectrometry analysis was executed using the Thermo Scientific Orbitrap Exploris 240 Mass Spectrometer and the Thermo Dionex UltiMate 3000 RSLCnano System.

Statistical analyses

A Student's *t*-test was used to compare lesional with nonlesional tissue protein expression. Analyses were conducted utilizing a pooled technique, comparing the average of results from lesional samples with the average of results from all nonlesional samples. Proteins with

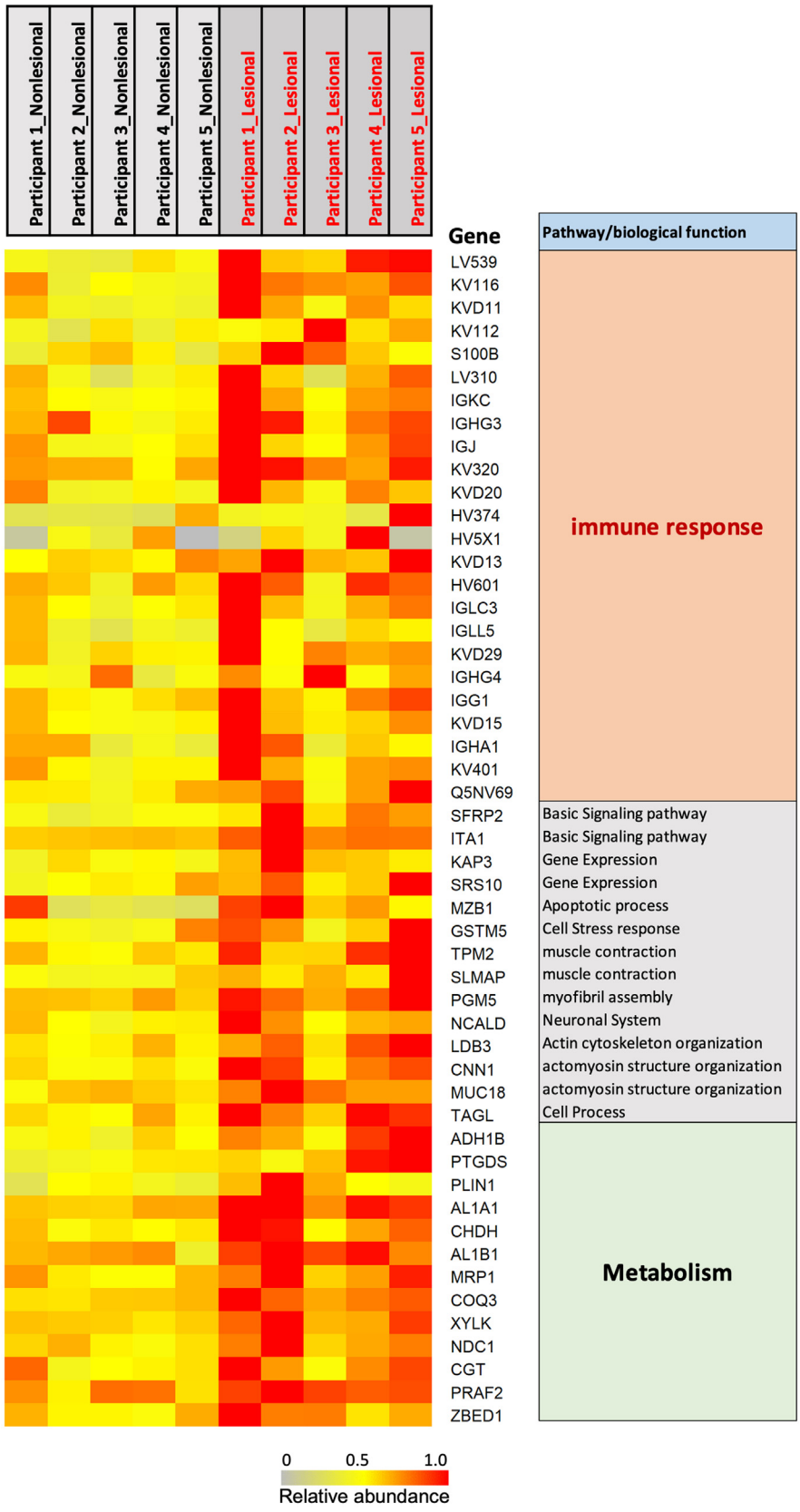

Figure 2. Heat map of DEP among lesional scalp tissue in CCCA compared with those in nonlesional, healthy scalp tissue. Red and orange boxes correspond with increased relative protein abundance, whereas yellow and green boxes represent less relative protein abundance (cutoff: fold change > 1.25, $P < .05$). Of 51 DEPs, 24 proteins were associated with immune response, with notable implications of genes responsible for encoding the IgG family (*IGHG3*, *IGHG4*, *IGG1*). In addition, protein markers of fibrosis were present, associated with genes *ITGA1*, *SFRP2*, *TPM2*, and *SLMAP*. CCCA, central centrifugal cicatricial alopecia; DEP, differentially expressed upregulated protein.

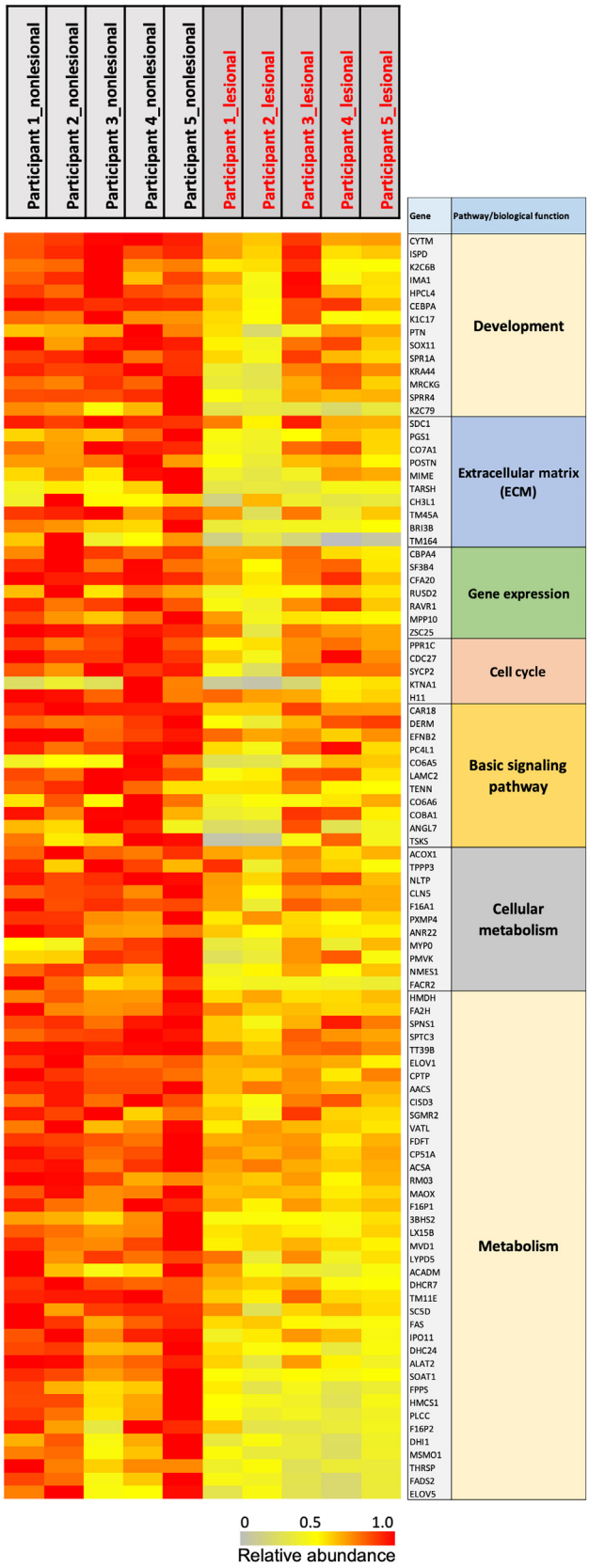

Figure 3. Heat map of DEPs among lesional scalp tissue in CCCA compared with those in nonlesional, healthy scalp tissue. Red and orange boxes correspond with increased relative protein abundance (fold change > 1.25, $P < .05$), whereas yellow and green boxes represent less relative protein abundance. Of 97 DEPs, 39 proteins were associated with lipid metabolism.

Table 2. Genes Implicated in Canonical Pathway Overlapping with Downregulated Proteins

Canonical Pathway (*P*-Value)	Genes
PPAR signaling (<.001)	*HMGCS1*
	ACADM
	ACOX1
	FADS2
	ME1
	SCP2
AMPK signaling (<.001)	*HMGCR*
	FASN
	FBP1
	FBP2

Abbreviations: AMPK, AMP-activated protein kinase; CCCA, central centrifugal cicatricial alopecia; PPAR, peroxisome proliferator–activated receptor.

Analysis of canonical pathways through the DAVID (Database for Annotation, Visualization, and Integrated Discovery) revealed involvement of specific genes responsible for downregulated protein expression among lesional tissue in CCCA. These genes are associated with signaling pathways, *PPAR* and *AMPK*, which are implicated in metabolic conditions.

fold changes >1.25 and a $P < .05$ were considered differentially expressed. The false discovery rate was set at <0.01 through Proteome Discoverer 2.4 software (Thermo Fisher Scientific), with the presented *P*-values adjusted accordingly. Gene Set Enrichment Analysis was performed through the Gene Ontology database, Kyoto Encyclopedia of Genes and Genomes database, and Reactome pathway database from the UniprotKB online tool and the DAVID (Database for Annotation, Visualization, and Integrated Discovery) to understand canonical pathways with $P < .05$ and false discovery rate < 0.05. Special attention was given to gene products that had been implicated in primary cicatricial alopecias.

DATA AVAILABILITY STATEMENT

Datasets related to this article can be found at Aguh and Crystal (2023), "CCCA Proteomics Data," Mendeley Data, V1, https://doi.org/10.17632/ttk7f9b69m.1

ORCIDs

Aditi Gadre: http://orcid.org/0000-0002-5008-9563
Taylor Dyson: http://orcid.org/0000-0002-8447-497X
Jaroslaw Jedrych: http://orcid.org/0000-0003-2385-7085
Grant Anhalt: http://orcid.org/0009-0005-3959-5683
Angel S. Byrd: http://orcid.org/0000-0001-5358-4247
Crystal Aguh: http://orcid.org/0000-0002-3590-9682

CONFLICT OF INTEREST

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Notable genes responsible for encoding these proteins include *ALOX15B*, *FADS2*, *ELOVL5*, *FA2H*, *FAR2*, and *SC5D*. CCCA, central centrifugal cicatricial alopecia; DEP, differentially expressed downregulated protein.

AUTHOR CONTRIBUTIONS

Conceptualization: CA; Formal Analysis: AG, CA; Investigation: AG, TD, JJ, GA, CA; Writing – Review and Editing: AG, TD, JJ, GA, ASB, CA

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