The proteomic skin profile of moderate-to-severe atopic dermatitis patients shows an inflammatory signature

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Background: Moderate-to-severe atopic dermatitis (AD) is increasingly recognized as a systemic disease, largely due to proteomic blood studies. There are growing efforts to develop AD biomarkers using minimal tissues.

Objective: To characterize the AD skin proteomic signature and its relationship with the blood proteome and genomic skin profile in the same individuals.

Methods: We evaluated lesional and nonlesional biopsy samples and blood from 20 individuals with moderate-to-severe AD and 28 healthy individuals using Olink Proteomics (Uppsala, Sweden), using 10 μg/10 μL for skin and blood and RNA sequencing of the skin.

Results: The AD skin proteome demonstrated significant upregulation in lesional and even in nonlesional skin compared with controls in inflammatory markers (matrix metalloproteinase 12; T-helper cell [Th]2/interleukin [IL]-1 receptor-like 1[IL1RL1]/IL-33R, IL-13, chemokine [C-C motif] ligand [CCL] 17; Th1/C-X-C motif chemokine 10; Th17/Th22/PI3, CCL20, S100A12), and in cardiovascular-associated proteins (E-selectin, matrix metalloproteinases, platelet growth factor, myeloperoxidase, fatty acid binding protein 4, and vascular endothelial growth factor A; false discovery rate, <0.05). Skin proteins demonstrated much higher and significant upregulations (vs controls) compared with blood, suggesting a skin source for the inflammatory/cardiovascular profile. Gene and protein expressions were correlated (r = 0.410, $P < .001$), with commonly upregulated inflammatory and cardiovascular risk-associated products, suggesting protein translation in skin.

Limitations: Our analysis was limited to 354 proteins.

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Conclusions: The AD skin proteome shows an inflammatory and cardiovascular signature even in nonlesional skin, emphasizing the need for proactive treatment. Skin proteomics presents a sensitive option for biomarker monitoring. (J Am Acad Dermatol 2020;82:690-9.)

Key words: atherosclerosis; atopic dermatitis; biomarkers; blood; cardiovascular; inflammatory; Olink; proteomics; skin.

CAPSULE SUMMARY

- Our proteomic data extend the "inflammation map" of atopic dermatitis, which was created largely through gene expression profiling. It also suggests that risk proteins found in blood are primarily derived from skin.
- Early treatment of skin inflammation may lower systemic immune or cardiovascular risk mediators in blood, potentially modifying disease co-morbidities.

Atopic dermatitis (AD) is increasingly recognized as associated with a higher risk for cardiovascular comorbidities,[1-5] likely due to persistent systemic inflammation.[6-9] Current efforts to develop minimally invasive AD biomarkers are ongoing.[10-15] Proteomic studies in blood using the Olink Multiplex (Olink Proteomics, Uppsala, Sweden) assay (requiring only 10 μL serum) identified biomarkers reflecting AD severity and functional barrier measures,[10-12] highlighting systemic immune activation in adult[11] and even early-onset pediatric AD.[10]

Nevertheless, the molecular understanding of AD pathomechanisms is primarily based on skin studies. A comprehensive proteomic study in skin, with appropriate correlations with blood, is lacking. Only 2 cutaneous proteomic studies in AD have been performed, using tape-strips to evaluate a limited panel of inflammatory mediators in the stratum corneum in children with AD.[13,14] Furthermore, some hallmark AD biomarkers (ie, interleukin [IL]-4 and IL-13), typically highly elevated in whole-skin AD genomic signatures, were not detected or showed downregulation in these tape-strip studies.[14] Thus, a comprehensive proteomic profile of whole-skin biopsy samples in AD compared with controls is lacking.

This study is the first, to our knowledge, to characterize the proteomic profile of lesional and nonlesional skin of adult patients with moderate-to-severe AD, using a large panel of 354 markers measured with the high-throughput Olink platform.

METHODS

Patients

We enrolled 20 adults (9 women, 11 men) with moderate-to-severe AD. Patients were a mean age of 40.6 years, the mean SCORing Atopic Dermatitis (SCORAD) was 61.8, and the mean Eczema Area and Severity Index [EASI] score was 27.4. The healthy control group comprised 28 individuals (13 women, 15 men) who were a mean age of 37 years (Table I).

Patients did not use topical treatments (corticosteroids, calcineurin antagonists, or crisaborole) for ≥2 weeks, and phototherapy or systemic treatment, such as dupilumab or other investigational drugs, for ≥4 weeks before enrollment. There were no significant differences in demographics between patients and controls. Patients and controls did not report cardiovascular comorbidities, and the healthy controls had no atopic-related conditions. Patients had chronic AD since childhood. Severity scores included SCORAD and EASI.

Biopsy sample collection

Lesional, nonlesional AD, and healthy skin punch biopsies were obtained under Institutional Review Board-approved protocols. Nonlesional biopsies were obtained from the vicinity of the lesions, but ≥10 cm away from active lesions. Biopsied sites showed no evidence of infections. Most biopsy samples in patients and controls were taken from the extremities (because AD most commonly affects the folds) and only if not feasible, from the trunk.

Skin and blood protein quantification

Skin biopsy samples were embedded in Tissue-Tek Optimal Cutting Temperature compound (Sakura Finetek USA, Torrance, CA), ground, and centrifuged. Then, 10 μg protein was used for Olink Proseek multiplex ultrasensitive platform using 4 panels: inflammation, cardiovascular disease (CVD) II, CVD III, and neuroinflammation (354 established and exploratory markers), as reported.[10,11,16-19]

Abbreviations used:

AD:	atopic dermatitis
CCL:	chemokine (C-C motif) ligand
CVD:	cardiovascular disease
CXCL:	C-X-C motif chemokine ligand
DEP:	differentially expressed protein
EASI:	Eczema Area and Severity Index
FABP4:	fatty acid binding protein 4
FCH:	fold change
FDR:	false discovery rate
FLT3LG:	Fms-related tyrosine kinase 3 ligand
HMOX1:	heme oxygenase-1
IL:	interleukin
IL-1RL1:	IL-1 receptor-like 1/IL-33R
LOX-1:	lectin-type oxidized LDL receptor 1
LTBR:	lymphotoxin-beta-receptor
MCP-1:	monocyte chemoattractant protein-1
MPO:	myeloperoxidase
mRNA:	messenger RNA
OSM:	oncostatin M
PGF:	platelet growth factor
RETN:	resistin
RNAseq:	RNA sequencing
Th:	T-helper cell
SELE:	E-selectin
SCORAD:	SCORing Atopic Dermatitis
TNFSF10/TRAIL:	TNF-related apoptosis inducing ligand
VEGFA:	vascular endothelial growth factor A

Blood was collected from the same study participants, and 10 μL serum was analyzed using the same Olink panels, as described.[10,11,17-19]

Gene expression analysis using RNA sequencing

RNA was extracted from the same biopsy samples, and RNA sequencing (RNAseq) was performed using Illumina HiSeq4000 (Illumina, San Diego, CA), as described.[20-24]

Statistical analyses

Analyses were performed using R language (R-project.org) and Bioconductor Project packages (www.bioconductor.org), as described.[10,11,18,24] Protein and gene expression profiles were modeled by linear models using the R *limma* framework.[25] *P* values from moderated (paired) *t* tests were adjusted for multiple hypotheses using the Benjamini-Hochberg procedure to produce false discovery rates (FDRs). Proteins with fold changes (FCHs) >2.0 and FDRs <0.05 were considered differentially expressed.

A 21-protein subset linked with atherosclerosis signaling[10,11] was quantified using a gene-set variation analysis (GSVA z-score).[26] Correlations between messenger RNA (mRNA), protein, and severity scores in skin and blood were evaluated by Spearman correlations.

RESULTS

The proteomic AD skin profile shows increases in inflammatory and proatherogenic proteins

The study included 20 adults with moderate-to-severe AD and 28 healthy controls (Table I). The Olink high-throughput proteomic multiplex platform evaluated 354 proteins from the inflammation, CVD II, CVD III, and neuroimmunology panels,[10,11,18,27] in lesional and nonlesional AD and control skin. Of the 354 proteins, 161 were differentially expressed (FCH >2 and FDR <0.05) in lesional and 69 in nonlesional AD compared with control skin (Supplementary Table I, available on Mendeley at https://data.mendeley.com/datasets/388cpp5h5h/2). The top 50 of these 161 differentially expressed proteins (DEPs) in AD skin are presented in a heat map (Fig 1).

We observed elevations in protein markers of inflammatory cells, including dendritic cells (tumor necrosis factor-related apoptosis-inducing ligand [TNFSF10/TRAIL]) and T-cell/T-cell activation (interleukin-2 receptor alpha [IL-2RA], CD40) in both lesional and nonlesional AD (FDR <0.05). We also observed significant upregulation of proteins associated with general inflammation (matrix metalloproteinase [MMP] 12) and multiple immune axes, including T-helper cell (Th)2 (IL-13, IL-1 receptor-like 1 [IL1RL1]/IL-33R, chemokine [C-C motif] ligand [CCL] 13/CCL17), Th1 (C-X-C motif chemokine ligand [CXCL] 9/CXCL10), and Th17/Th22 (PI3, CCL20, S100A12) in nonlesional or lesional AD (FDR <0.05), or both, compared with controls.

The top significantly upregulated proteins in lesional or nonlesional AD skin, or both, compared with controls included multiple prognostic and therapeutic protein targets of atherosclerosis/cardiovascular risk (Fig 1, Supplementary Table I). These included potential biomarkers or therapeutic targets of atherosclerosis, such as E-selectin (SELE), matrix metalloproteinases (MMPs) (MMP1, MMP3, and MMP9), CCL19, CCL2, resistin (RETN), platelet growth factor (PGF), lectin-type oxidized low-density lipoprotein receptor 1 (LOX-1) (or OLR1), myeloperoxidase (MPO), and fatty acid binding protein 4 (FABP4) (FDR <0.05). We also found significant increases in innate cytokines and receptors (IL-6R, IL-8; FDR <0.05) that promote atherosclerosis.[28-30] Transforming growth factor (TGF)-β1, involved in cardiac hypertrophy, vascular remodeling, and renin-angiotensin regulation,[31]

Table I. Baseline patient demographics

Characteristics	Atopic dermatitis (n = 20)	Controls (n = 28)	*P* value
Age, y			
Mean ± SD	40.6 ± 15.5	37 ± 11.1	.39
Median (range)	37 (23-66)	38.5 (18-57)	
Sex, No.			
Female	9	13	.1
Male	11	15	
Clinical severity scores			
SCORAD			
Mean ± SD	61.8 ± 12.3	…	…
Median (range)	62 (40.9-78)	…	…
EASI			
Mean ± SD	27.4 ± 9.9	…	…
Median (range)	27.4 (8.7-42.4)	…	…
Immunoglobulin E, kU/L			
Mean ± SD	3572 ± 10,682	…	…
Median (range)	355 (12-48,390)	…	…
Patients with other atopic conditions, No.	8	…	…

EASI, Eczema Area and Severity Index; *No.*, number; *SCORAD*, SCORing of Atopic Dermatitis; *SD*, standard deviation.

the vascular endothelial growth factor A (VEGFA), which mediates angiogenesis, and heme oxygenase-1 (HMOX1), which protects against atherosclerosis,[32] and the proapoptotic and potential heart failure target, caspase-3 (CASP3), were upregulated in nonlesional or lesional skin, or both (FDR <0.05; Fig 1, Supplementary Table I).

Remarkably, the nonlesional skin of patients with AD demonstrated significant upregulation of inflammatory and atherosclerosis/cardiovascular risk-associated proteins compared with healthy skin (albeit smaller than in lesional skin), emphasizing the systemic nature of AD. This is emphasized by the gene set variation analysis of 21 atherosclerosis signaling-specific proteins, showing significant upregulation of this subset[10,11] in both lesional and nonlesional AD vs control skin ($P < .05$; Fig 2).

To ascertain whether patients with AD and other atopic conditions have a different and perhaps more inflammatory signature than patients with AD alone, we also performed a sensitivity analysis comparing the 2 groups. This sensitivity analysis did not show significant differences between the 2 groups (Supplementary Table II).

AD skin demonstrates much higher expressions than blood

We next investigated whether the skin or blood compartments (via blood flow to skin) are the source of the upregulated inflammatory and atherosclerosis/cardiovascular-related proteins in AD skin, evaluating the relationship between skin and blood proteomics.

Overall, the differences between lesional AD and healthy skin were much larger than those between AD and control blood, as visualized by the marked predominance of markers on the right side of the scatterplot (Fig 3). The FCHs in lesional skin were not only larger but also more significant than in blood. While 161 DEPs were found in lesional skin, only 1 DEP in blood fulfilled the FCH >2 and FDR <0.05 criteria, similar to prior reports.[10-12] With a looser criteria (FCH >1.3 and $P < .05$), not accounting for multiple hypotheses, 220 DEPs were detected in lesional skin and 20 DEPs in blood of AD vs controls (Supplementary Table I). Of the 20 blood DEPs, 17 overlapped with skin DEPs, suggesting that whereas skin proteomics captures most of the blood signature, this does not hold in the opposite direction. Among the 17 overlapping DEPs were proteins associated with Th2 (CCL13/CCL17), Th1 (CXCL9/CXCL10), and Th17 (PI3, CCL20) immune pathways ($P < .05$). MMP12 and IL-1RN, which binds IL-1R and inhibits IL-1α/β signaling (which has been associated with cardiac disease and is being targeted in clinical trials)[33] were also differentially upregulated in both skin and blood ($P < .05$).

To understand how protein expression in skin and blood correlate, we conducted Spearman correlation analyses (Supplementary Table III). Proteins with significant skin-blood correlations were primarily those linked with cardiovascular risk. Markers of tissue remodeling (MMP3; $r = 0.544$), Th1 (CXCL11; $r = 0.469$), Th2 (CCL13; $r = 0.472$), and lymphoid chemoattraction (CCL19; $r = 0.469$) demonstrated significant correlations between blood and lesional expressions; endothelial cell adhesion

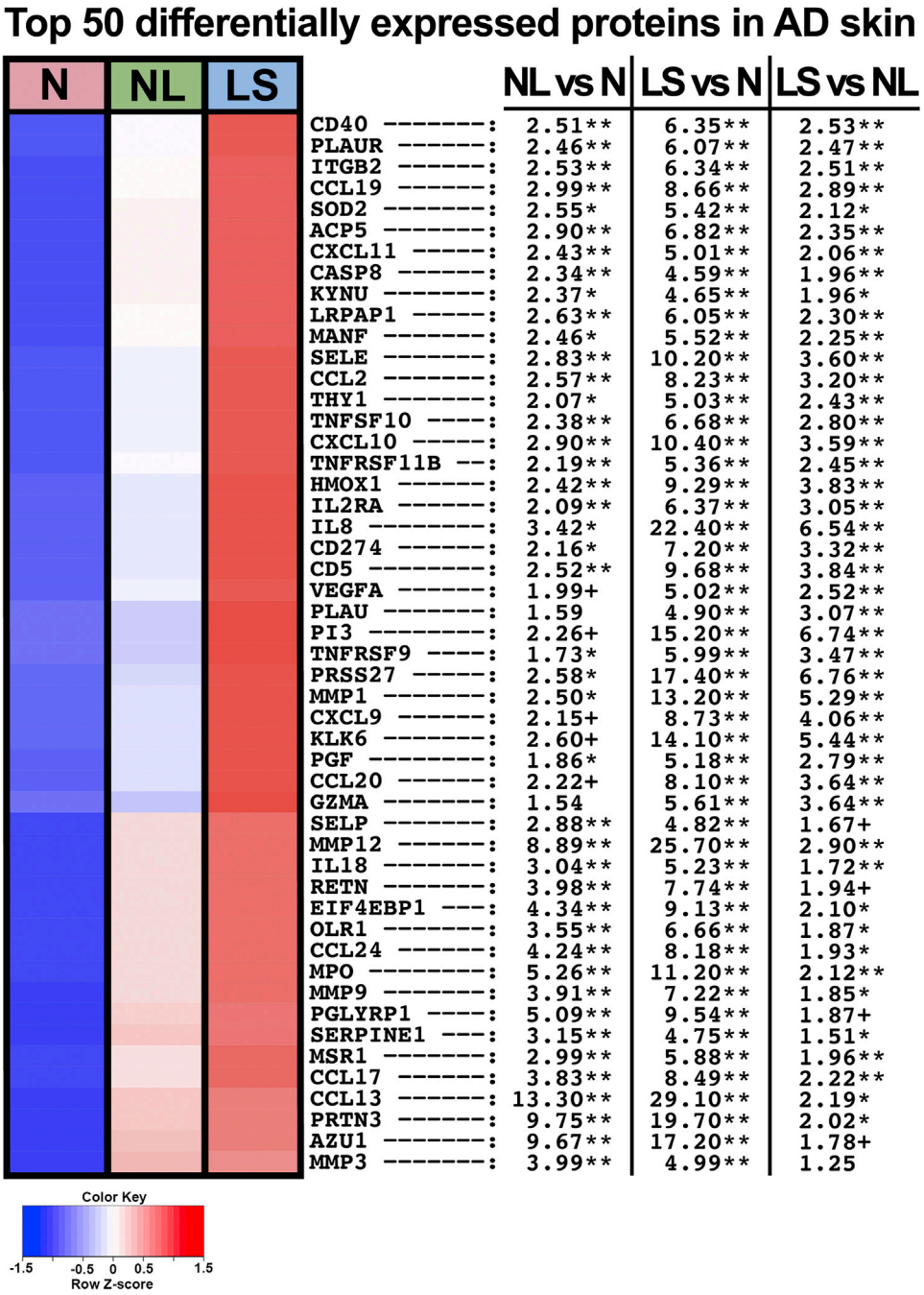

Fig 1. Heat map representing the top 50 of 161 differentially expressed proteins in atopic dermatitis (AD) lesional skin (*LS*) compared with nonlesional skin (*NL*) and normal (*N*) skin, using criteria of fold changes (FCH) of >2 and false discovery rate (FDR) of <0.05. Table shows upregulated markers and respective FCH in AD vs N skin and in LS AD vs NL AD skin. *FDR <0.05; **FDR <0.01.

(SELE; r = 0.618), T-cell activation (CD6, TREML2; r = 0.514, r = 0.547), and Th17-related (CCL20; r = 0.644) products were significantly correlated between nonlesional skin and blood ($P < .05$).

We also correlated protein expressions in skin with clinical severity (EASI and SCORAD) (Supplementary Table IV). In lesional, and particularly in nonlesional skin, we found significant correlations between expressions of cardiovascular-/atherosclerosis-related markers and disease severity, including IL-18[28,29] (r = 0.448, $P < .05$), angiotensin I converting enzyme 2, which catalyzes

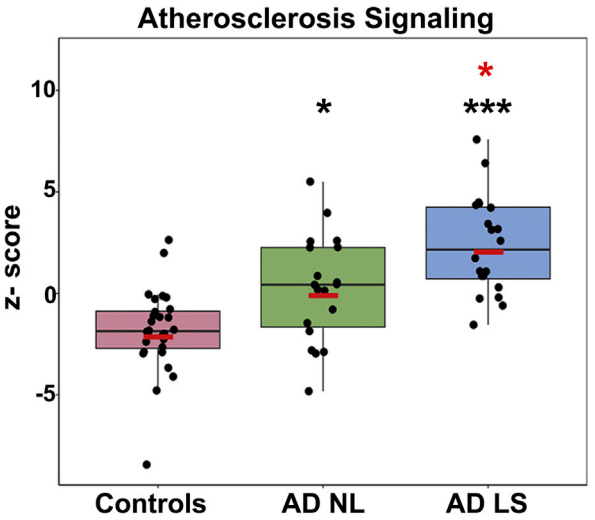

Fig 2. Atherosclerosis signaling pathway. A gene set variation analysis of 21 atherosclerosis signaling-specific proteins shows significant increases in *z*-scores of atherosclerosis proteins in atopic dermatitis (*AD*) nonlesional (*NL*) and lesional skin (*LS*) compared with normal skin. **P* < .05; ****P* < .001. *Black **: significance of comparison between AD skin and normal skin; *red **: significance of comparison between AD LS vs NL. The *black horizontal line* in the middle of each box indicates the median; the *red bar* indicates the mean; the *top and bottom borders* of the box mark the 75th and 25th percentiles, respectively, and the *vertical lines* mark minimum and maximum of the data.

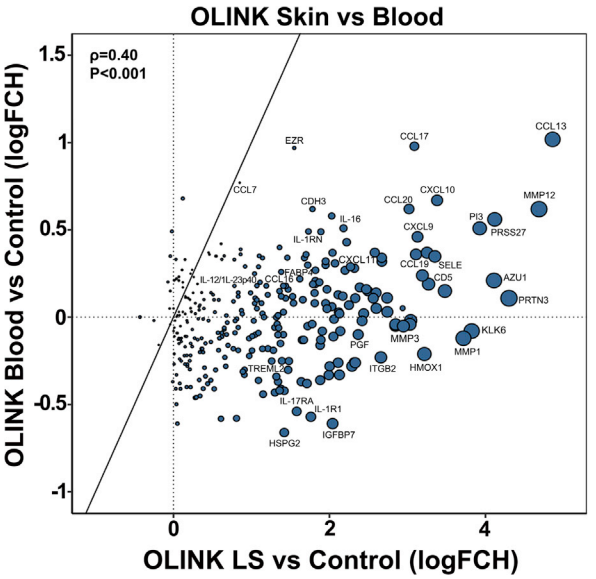

Fig 3. Olink proteomics (Olink Proteomics, Uppsala, Sweden) in blood (*y*-axis) compared with lesional skin (*x*-axis) from patients with moderate-to-severe atopic dermatitis (AD). Scatter plot depicts the $\log_2$ fold-change (*FCH*) proteomic differences in patients with AD compared with healthy controls. The size of the circles represents the absolute difference (in $\log_2$ FCH) between protein expressions of AD vs control participants in skin and blood.

cleavage of angiotensin and plays a role in regulation of cardiovascular and renal function (r = 0.537, $P < .05$) in lesional skin, and VEGFA (r = 0.519, $P < .05$) in nonlesional skin. In blood, FABP4 (r = 0.475), sortilin (SORT1) (r = 0.552), and HMOX1 (r = 0.602), which mediate lipid metabolism and are associated with atherosclerosis,[34-36] significantly correlated with EASI ($P < .05$).

Integrating protein and mRNA expression measures in AD skin

Given that AD pathomechanisms were largely studied using transcriptomics,[6,16,20,24,37,38] we assessed the relationship between the AD skin proteome and transcriptome gene signature using RNAseq on the same biopsy specimens. Because RNAseq covers many more genes, we limited our analysis to 339 markers, overlapping with Olink measures.

Overall, protein and mRNA differences between AD vs healthy skin significantly correlated in lesional (r = 0.410, $P < .001$; Fig 4) and nonlesional skin (data not shown). Genes with increased mRNA expressions frequently demonstrated concordant elevated protein levels, indicating local translation into protein. In lesional skin compared with controls, 83 of the 339 genes were differentially upregulated in RNAseq, whereas 160 of the 339 proteins were upregulated in Olink. Fifty-nine gene products were commonly upregulated (Supplementary Table I). The markers with parallel protein and mRNA increases in lesional or nonlesional skin included inflammatory (IL-13, oncostatin-M [OSM]) and cardiovascular risk/atherosclerosis measures such as IL-6, and MMPs, which also demonstrated significant protein and mRNA correlations (Supplementary Table V).

Several atherosclerosis-/cardiovascular-related markers showed much higher protein than mRNA expressions in lesional vs control skin (Fig 4). These include SOD2, SELE, MPO, FABP4, CCL2, HMOX1, the serine protease PRSS27, and proteinase 3, which has proapoptotic and proinflammatory activity and is associated with heart failure after myocardial infarction.[39] Some innate (IL-8), Th1 (CXCL9/CXCL10)-, and Th2 (CCL13)-related markers also demonstrated greater protein expressions. Conversely, higher mRNAs compared with protein expressions were seen in some products, including Fms-related tyrosine kinase 3 ligand (FLT3LG), which regulates dendritic cells,[40] IL-6, IL-10, IL-20, IL-12/23p40, S100A12, and CCL3.

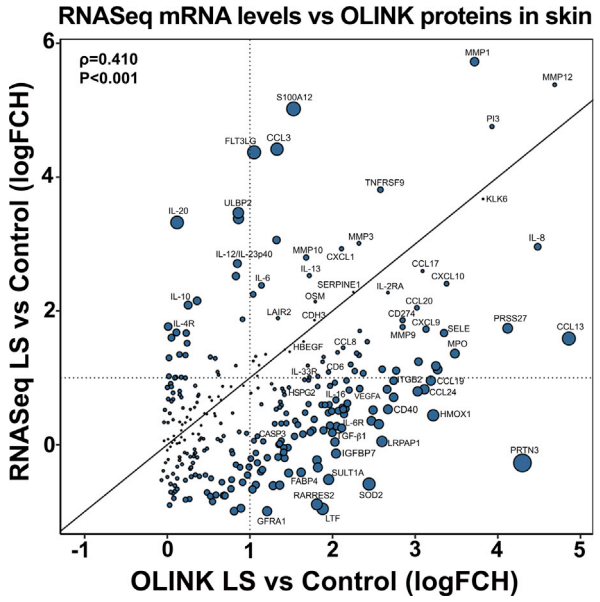

Fig 4. Olink proteomics (Olink Proteomics, Uppsala, Sweden) vs gene expression analysis using RNA sequencing (*RNASeq*) in lesional atopic dermatitis (AD) vs control skin. Scatter plot depicts $\log_2$ fold-changes (*FCH*) in the same patients with AD compared with their respective controls. The size of the circle represents the absolute difference (in $\log_2$ FCH) between AD and control participants in Olink and RNA-seq.

DISCUSSION

To our knowledge, this is the first evaluation of the proteomic signature of lesional and nonlesional skin in individuals with moderate-to-severe AD compared with healthy individuals. Two prior proteomic studies in tape-stripped skin from infants and children with AD evaluated limited panels of 27 and 28 inflammatory mediators, with detection rates of 19 of 27 and 13 of 28, respectively.[13,14] Furthermore, tape-stripping is limited to detection of biomarkers present in the outermost epidermis. In fact, key AD biomarkers (IL-4, IL-13), which are highly upregulated in transcriptomic analyses of AD whole-skin biopsy samples,[6,16,20,24,37,38] were not detected or showed lower protein expression in AD compared with control tape-strips.[13,14]

Congruent with gene expression (or transcriptome) skin studies that established AD pathomechanisms,[6,16,20,37,38] our proteomic AD skin profile captures the inflammatory milieu in AD skin, including significant elevations in markers related to Th2 (IL-13, CCL17), Th22 (S100A12), Th1 (CXCL10), and Th17 (CCL20) pathways.

Among the top upregulated proteins in both lesional and nonlesional AD, we found increases in multiple atherosclerosis-associated proteins,[10,11] including CCL2, CCL19, SELE, PGF, LOX-1/OLR1, FABP4, MPO, MMPs, RETN, CASP3, TGF-β1, and VEGFA. CCL2 or monocyte chemoattractant protein-1 (MCP-1) is released from macrophages and endothelial cells and overexpressed in atherosclerotic plaques.[41,42] The lymphoid-organizing chemokine, CCL19, has been shown to be upregulated in atherosclerotic plaques.[43,44] Levels of endothelial cell adhesion molecule, SELE, were associated with coronary artery disease,[45] PGF, a homolog of VEGF, has been linked to coronary artery disease prognosis,[30] and anti-PGF antibodies inhibited atherosclerotic plaques in mice.[46]

LOX-1/ORL1, is involved in critical steps of atherosclerosis.[47,48] Several anti-atherosclerotic drugs (statins, metformin, calcium channel blockers) inhibit LOX-1 signaling.[47,48] FABP4, a lipid metabolism mediator, is targeted in atherosclerosis and diabetes.[48,49]

MPO is produced by neutrophils, monocytes, and macrophages and catalyzes low-density lipoprotein modification. Elevated MPO levels are associated with coronary artery disease,[50] and plasma MPO is an early risk predictor of myocardial infarction.[51]

MMPs are implicated in vascular tissue remodeling[52] and atherosclerosis. RETN is associated with obesity, type 2 diabetes, and CVD.[53] Caspase-3 inhibition is investigated for treatment/prevention of heart failure.[54] TGF-β1 is involved in cardiac hypertrophy, and renin-angiotensin regulation.[31] TGF-β1 polymorphisms are correlated with hypertension,[55] and hypertensive patients have higher TGF-β1.[56] VEGFA, a mediator of angiogenesis, was also increased in AD.[57]

Several key atherosclerosis/cardiovascular risk-associated proteins in lesional or nonlesional skin were also significantly correlated with disease severity, including IL-18, VEGFA, and angiotensin I converting enzyme 2, which regulates cardiovascular and renal function.[58] The significant upregulations in inflammatory and atherosclerosis-/cardiovascular-associated proteins in nonlesional AD skin, together with the more significant correlations between severity and nonlesional compared with lesional protein expressions, emphasize the systemic nature of AD.

To investigate the primary origin of these inflammatory and atherosclerosis/cardiovascular risk-associated proteins (whether they originate from the skin and then leak to blood or initiate in blood and are distributed to skin through the blood supply), we evaluated the relationship between lesional skin and blood proteomics. Our findings suggest that the skin is likely the source of these upregulated proteins, because their protein expressions in AD lesions were significantly larger compared with

blood levels. Further, the skin proteomic signature captured many more markers than the blood profile, consistent with prior serum reports.[21,22] The limited number of correlations between blood and skin proteomics also implies that the elevated skin proteins are not simply deposited by the blood supply. Instead, it is more likely that the protein is leaking from skin into blood.

We also evaluated the differences between mRNA and protein expressions in lesional AD. Although the transcriptomic AD profile has been extensively studied,[6,16,20,21,37,38,59,60] whether the mRNA is ultimately translated into protein has not been investigated. Overall, mRNA and protein expressions were significantly correlated, indicating local translation of protein in the skin. This again suggests skin as the primary source for the upregulated proteins. Many protein and gene products were differentially upregulated in lesional skin in both the Olink and RNAseq platforms. These included atherosclerosis-related proteins such as IL-6, MMPs, SELE, OSM, and the IL-33 receptor, ST2/IL1RL1. OSM was suggested as a biomarker of atherosclerosis, with elevated OSM serum levels significantly correlated with coronary stenosis.[61,62] The IL-33/ST2 pathway plays a dual role in type 2 inflammation and in CVD.[63] Clinical trials for IL-33 (NCT03533751) and OSMR (NCT03754309) showed preliminary efficacy in adults with moderate-to-severe AD[64,65] and may also benefit patients at risk for cardiovascular comorbidities.

Several atherosclerosis/cardiovascular markers showed much higher protein rather than mRNA expression, including SELE, MPO, FABP4, HMOX1, and proteinase 3, which is associated with heart failure.[39] This could be attributed to accumulation of protein due to a longer half-life or an amplifying effect leading to increased translation. Some gene products also showed higher mRNA rather than protein expression, including FLT3LG, IL-6, IL-10, IL-20, IL-12/23p40, S100A12, and CCL3. These proteins may be less stable than the mRNAs and thus degrade quickly or are being consumed through binding and uptake by receptors. In this case, a lack of protein would not necessarily imply lack of function; they could be mediating strong signaling through receptor binding, which subsequently results in decreased levels.

One limitation of our study is that our proteomic analysis was limited to 354 markers. Nevertheless, to our knowledge, this is the first proteomic study in biopsy specimens and the largest in skin. Another limitation is that blood mRNA is not available; however, the proteomic studies in blood support the skin as the primary source of inflammatory proteins.

CONCLUSION

Overall, this study identified the adult AD proteomic signature in skin biopsy specimens.[14] Future studies should attempt to also validate this method in children and in longitudinal studies. The increased atherosclerosis/cardiovascular risk-associated proteins in AD lesional and even in the "visibly normal-appearing" (nonlesional) skin emphasize the importance of proactive treatment. Our data suggest that the skin rather than blood is the primary source for the inflammatory and atherosclerosis/cardiovascular signature. The proteomic skin profile can be valuable for future studies requiring close biomarker monitoring. This is particularly important considering that the Olink platform requires as little as 10 μg per tissue, which can be easily acquired through as little as a 1-mm punch biopsy (or 10 μL serum).

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