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Transcriptomic evidence for atopic dermatitis as a systemic disease in NC/Nga mice

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Abstract

Background In the current study, we evaluated whether atopic dermatitis (AD) affects the entire body rather than being limited to skin barrier damage and inflammation. We hypothesized that medium-term exposure of distant organs to systemic inflammatory cytokines in sub-chronic inflammatory skin diseases has detrimental effects on distant tissues.

Results Our findings demonstrated the dysregulation of genes and pathways associated with inflammation and the skin barrier, as well as genes and pathways involved in muscle development that respond to chemicals or stress in muscle tissues, all of which were reversed by hydrocortisone (Hc) administration. The expression of *Ces1d* showed significant differences during disease onset and after treatment in both skin and skeletal muscle, suggesting that *Ces1d* is likely responsible for the alleviation of subchronic AD.

Conclusions Using NC/Nga mice with AD-like symptoms, we compared the transcriptomes of the skeletal muscle (a tissue that is relatively distant from the skin) with those of the skin (the lesion induction site) before and after disease induction, after which Hc was administered. Although further study is needed to better understand the effects of Ces1d on AD, skeletal muscle was associated with AD pathogenesis, and AD-like symptoms appeared to affect the body in a systemic manner. Given the importance of evidence-based medicine and the development of precision medicine, our findings provide insights into the mechanisms of AD onset and progression.

Keywords Atopic dermatitis, Carboxylesterase activity, Muscle damage, Skin-barrier dysfunction, Systemic disease

Background

Atopic dermatitis (AD) is a multifactorial heterogeneous skin disease resulting from a complex interaction between genetic and environmental factors [1]. AD is part of the "atopic complex" or "atopic syndrome," characterized by systemic, organ-specific inflammatory responses [2]. Recent studies have demonstrated that AD is mediated by a T helper (Th) 1/Th2 lymphocyte imbalance and increased serum immunoglobulin E (IgE), Th22, and Th17 levels as an immune response to allergens [1].

Recent evidence has demonstrated that activation of the interleukin (IL)-23/Th1 cytokine pathway is related to the barrier function of the epidermis [3]. In addition,

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recent advances in "-omics" research have provided novel insights into the genes involved in the pathogenesis of skin barrier damage, including those associated with immune responses and lipid composition, as well as the mechanisms that lead to the expression of common AD phenotypes [4]. To test the hypothesis that AD is a multisystem systemic disease, most studies have evaluated the various tissues and blood in which the lesion appears [5, 6].

Physiological dysfunction of the skin barrier appears to be caused by defects in the stratum corneum and tight junctions [7]. The skin, the largest organ in the body in terms of weight and surface area, mediates communication with the external environment, separates the external and internal environments of the body, and covers and protects skeletal muscle [8]. Pruritus is a prominent symptom among patients with AD, and chronic itching is closely linked to stress and anxiety [1]. Conversely, stress and anxiety not only exacerbate pruritus but also influence patient behavior (scratching) and lead to muscle tension [9]. Persistent muscle tension can trigger various bodily reactions over prolonged periods, potentially causing stress-related disorders [10]. The skeletal muscle is a large endocrine organ that plays important roles in metabolism and systemic homeostasis [11]. Investigating the muscle physiology of patients with AD could provide insights to enhance our understanding of the disease; however, research on this aspect of AD remains limited [12].

We previously confirmed that NC/Nga mice exhibiting AD-like symptoms had increased serum IgE and IL-12 levels and increased expression of *Il4*, *Il6*, *Il10*, and *Il13* at the lesion site, which was reversed by hydrocortisone (Hc) treatment [13]. In addition, transcriptome profiling of skeletal muscle (gluteus medius muscle) tissue distant from the skin lesion confirmed an association between mitochondrial function, inflammation, and mitochondria-related genes [14]. Thus, these findings suggest that sub-chronic inflammation in AD is caused by skin barrier–environment interactions involving immune responses. Therefore, in this study, we expanded these observations by testing the hypothesis that AD ultimately develops into a systemic disease.

Patients with AD typically develop several skin lesions, characterized by severe itching, erythematous papules, scaly papules, thickened skin, skin marking, and fibrous papules, which often warrant aggressive therapeutic intervention with emollients [15]. Hc is a commonly prescribed topical glucocorticoid (GC) drug used for the management of AD. Topical GCs exhibit various anti-inflammatory properties and are transported to the nucleus via interactions with GC receptors, which trigger changes in gene expression and protein translation by affecting the activator protein-1 and nuclear factor kappa B pathways [16]. Here, we aimed to gain insights into the whole-body effects of AD by comparing the entire transcriptome of treated skin and skeletal muscle before and after disease induction using NC/Nga mice with moderate-to-severe AD-like symptoms.

Methods

NC/Nga mice

Female NC/Nga mice (7 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). All animal experiments were approved by the Institutional Animal Care and Use Committee of Chung-Ang University (approval No. 201800012) and were performed in accordance with current animal testing regulations. Animals were randomly assigned to three experimental groups: control group (no treatment), NC/Nga group (house dust mite ointment [HDM] only), and treatment group (NC/Nga + 0.1% Hc) (n = 4 mice per group). Mice were housed under conventional conditions for AD modeling [13].

AD induction

AD was induced as previously described [17]. Briefly, the dorsal skin of mice was epilated and treated with 150 µL of 4% sodium dodecyl sulfate solution (Sigma-Aldrich, St. Louis, MO, USA), after which 100 mg HDM (*Dermatophagoides farinae* extracts; Biostir, Inc., Osaka, Japan) was applied. Induction was performed twice a week for 8 weeks. For the treatment group, 0.1% (w/w) Hc cream was applied topically to the lesion five times a week from 4 weeks after the first induction. For the control group, only epilation was performed. All mice were euthanized the day after the last AD induction under anesthesia (Supplementary Figure S1). The anesthetic solution was composed of alfaxalone (20 mg/kg, Alfaxan®, JUROX Pty Limited, NSW, Australia) and xylazine hydrochloride (10 mg/kg, Rompun® inj., BAYER KOREA Ltd., Ansan, Korea) and intraperitoneally injected 10 ml/kg of body weight [18]. After confirming the absence of the flexion reflex, mice were euthanized by collecting a total of over 1 mL of blood from the abdominal aorta. We checked for cardiac arrest at the end of euthanasia to comply with current animal testing regulations.

RNA isolation

Total RNA was extracted from the dorsal skin tissues (four samples/group) and gluteal muscles (three samples/group) of mice using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

RNA-sequencing

The expression of all annotated mouse mRNAs in the Ensembl database was quantified using the *Sailfish* pipeline with default settings [19]. Differentially expressed

genes (DEGs) were identified using the "edgeR" [20] package with default settings (NC/Nga vs. control, Hc-treated vs. NC/Nga). Gene ontology (GO) [21, 22] analyses were performed using the *DAVID* tool [23].

Microarray

All data were summarized and normalized using the robust multi-average (RMA) method using the Affymetrix® Power Tools command-line suite. The results of the gene-level RMA analysis were exported for subsequent DEG analysis. The expression profiles of the experimental groups were compared using Affymetrix Mouse 2.0ST data. The statistical significance of the expression data was determined using the local-pooled-error test, in which the null hypothesis states that there was no significant difference in the |fold change (*FC*)| values among the groups. The false discovery rate (*FDR)* was determined by adjusting the *P*-value using the Benjamini–Hochberg method. Gene enrichment and functional annotation analyses for significant probe lists were performed using the GO databases. All statistical analyses and visualizations of DEGs were conducted using R (version 3.0.2; www.r-project.org) [24].

Human public array-based data

Our experiments were described previously [25]. In this study, three human cohorts with AD from the Gene Expression Omnibus database [26] were investigated, including the German cohort (DE), based on the Illumina HumanHT-12 V3.0 expression beadchip (GSE60709) [27]; the Swedish cohort (SE), based on the Affymetrix Human Genome U133A Array (GSE6012) [28]; and the USA1 cohort (US), based on the Affymetrix Human Genome U133 Plus 2.0 Array (GSE120721) [29]. Additionally, the USA2 cohort (GSE107361) was used as a validation dataset for the target gene [30]. For a gene with multiple probes/probe sets, the geometric mean of all probes/probe sets mapped to the gene was used to measure the expression levels. The significance analysis of the microarray [31] algorithm was used to compare the $\log_2$-transformed gene expression fold changes ($\log_2 FC$) between lesional AD and control samples. The *FDR* was controlled using the *q*-value method [32].

Comparison of the transcriptional contribution to AD

The Pearson correlation test was used to assess the predictive association between DEGs across each tissue disease group, tissue type, and mouse–human skin groups [33]. The statistical methods used in each experiment are mentioned in the Results section.

Results

Skin and muscle tissues of NC/Nga mice with AD-like symptoms exhibit dysregulated genes and pathways, which are reversed by HC treatment

The skin and muscle transcriptomes of the NC/Nga mice contained 21,831 and 30,922 genes, respectively. Overall, 20,531 genes were shared between the two transcriptomes (Fig. 1). Comparing the transcriptome pattern of the NC/Nga skin samples with that of the control skin, we identified 216 upregulated and 115 downregulated genes with an $FDR < 5\%$ and $|FC| > 2$ (Fig. 1A). Furthermore, the transcriptome pattern of the Hc-treated group exhibited 383 upregulated and 87 downregulated genes compared to the NC/Nga samples (Fig. 1A). Interestingly, the $\log_2 FC$ between the NC/Nga and control mice was negatively correlated with the $\log_2 FC$ between treated and untreated NC/Nga mice (Pearson correlation test: $r = -0.629$ and $P < 10^{-10}$) (Fig. 1B).

No DEGs were detected in muscle samples using the same significance criteria ($FDR < 5\%$ and $|FC| > 2$). However, we identified 22 upregulated and 11 downregulated genes with $P < 0.05$ and $|FC| \geq 1.5$ when comparing NC/Nga muscles to the control muscles (Fig. 1C). When these DEG criteria ($P < 0.05$ and $|FC| \geq 1.5$) were used to examine the effects of Hc treatment, we identified 24 upregulated and 26 downregulated genes compared to the NC/Nga group (Fig. 1C). Furthermore, the $\log_2 FC$ between muscles from NC/Nga and control mice was negatively associated with the $\log_2 FC$ between muscles from Hc-treated and untreated NC/Nga mice (Pearson correlation test: $r = -0.320$ and $P < 2.2 \times 10^{-16}$) (Fig. 1D).

GO analysis revealed that the genes upregulated in NC/Nga mouse skin relative to controls were significantly associated with GO biological process (GOBP) terms related to AD pathology, including "keratinocyte differentiation," "keratinization," "inflammatory response," and "epidermis development" (Fig. 2A). In contrast, downregulated genes were enriched in these GOBP terms in the Hc-treated NC/Nga mouse skin compared to untreated NC/Nga mouse skin (Fig. 2B), confirming that the DEGs in NC/Nga mice were effectively restored to normal levels after Hc treatment.

Compared to control muscle samples, NC/Nga mice exhibited upregulation of terms related to muscle development and adaptation and downregulation of responses to chemicals or stress (Fig. 2C). Importantly, topical Hc treatment reversed some changes in the GO terms affected by AD and downregulated the response to steroids (Fig. 2D).

Ces1d may be involved in the alleviation of sub-chronic AD by inducing a physiological response to stress or chemicals

No linear correlation was identified between the DEGs of skin and muscle in the NC/Nga and control groups

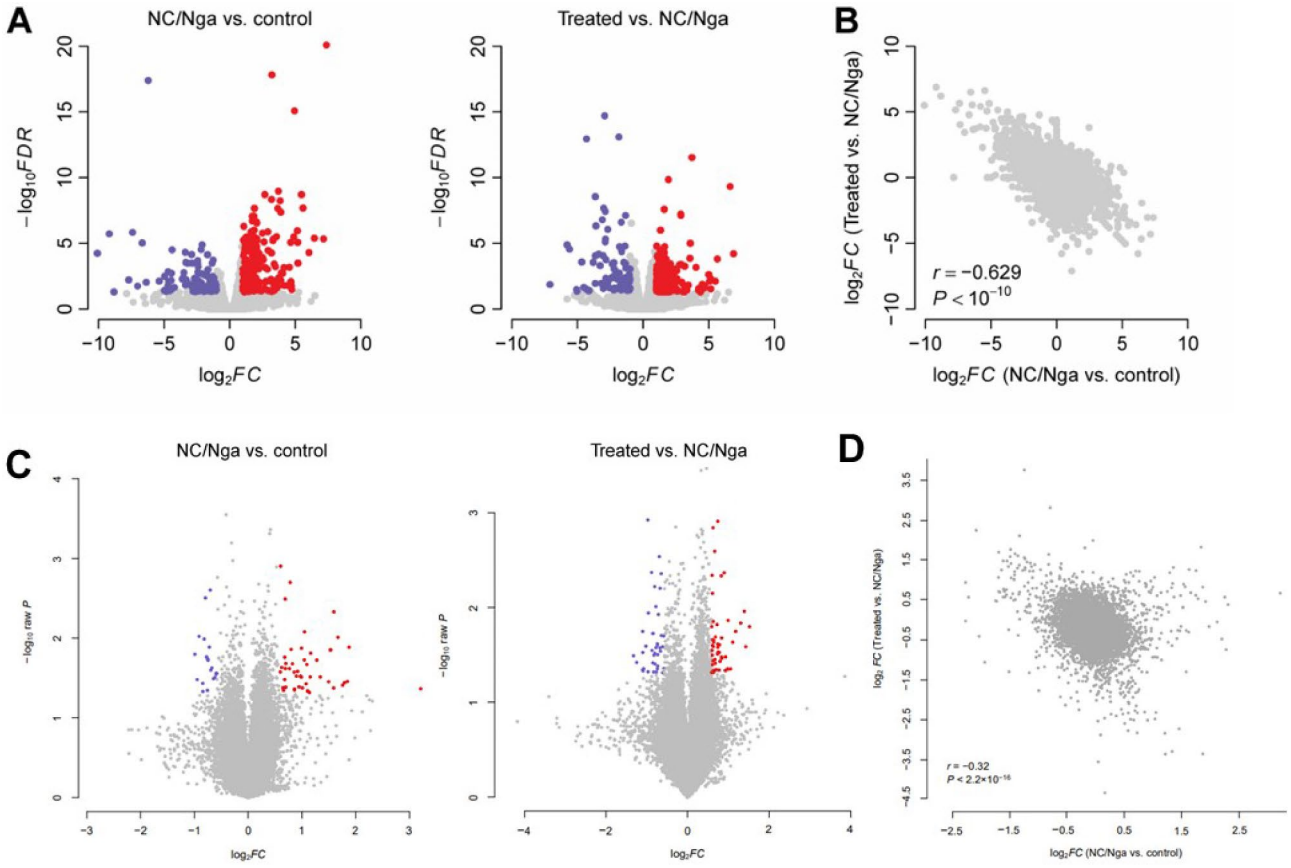

Fig. 1 Differentially expressed genes (DEGs) in the NC/Nga murine model. Volcano plots of DEGs detected in the skin (**A**) and muscle (**C**). The red and blue dots represent the upregulated and downregulated genes, respectively. Left panel: Upregulated and downregulated genes in NC/Nga mice compared to controls. Right panel: Upregulated and downregulated genes in hydrocortisone (Hc)-treated NC/Nga mice compared to untreated NC/Nga mice. Correlation of $\log_2$-transformed fold change ($\log_2FC$) between NC/Nga and control mice (*x*-axis) and between Hc-treated NC/Nga and untreated NC/Nga mice (*y*-axis) in the skin (**B**) and muscle (**D**). Each dot represents a different gene. The $\log_2FC$ between NC/Nga and control mice was negatively correlated with the $\log_2FC$ between Hc-treated NC/Nga mice and untreated NC/Nga mice, suggesting that the dysregulated genes in NC/Nga mice were largely recovered after Hc treatment. DEG: Differentially expressed gene, Hc: Hydrocortisone, $\log_2$FC: Logarithm base 2-transformed gene expression fold change

(Fig. 3B). However, *Egln3* was upregulated in the skin and muscle during disease (Fig. 3A; Supplementary Figure S2 and S4). Additionally, there was no linear correlation between the DEGs of the skin and muscle in the Hc-treated and NC/Nga groups (Fig. 3D). *Ces1d* and *Marc1* were upregulated in the Hc-treated groups compared to the NC/Nga groups (Fig. 3C; Supplementary Figure S3 and S5). Particularly, when AD was induced the skin and muscle tissues exhibited no significant changes and decreased expression of *Ces1d*, respectively, whereas the Hc-treated groups exhibited *Ces1d* upregulation (NC/Nga skin vs. control: $\log_2FC=-1.375$, $FDR=0.271$; Hc-treated skin vs. NC/Nga: $\log_2FC=1.854$, $FDR=3.108\times10^{-3}$; NC/Nga muscle vs. control: $\log_2FC=-0.841$, $P=3.701\times10^{-2}$; Hc-treated muscle vs. NC/Nga: $\log_2FC=0.977$, $P=4.454\times10^{-2}$) (Fig. 3A and C; Supplementary Figure S2–S5). The GOBP terms related to *Ces1d* included "positive regulation of transcription from the RNA polymerase II promoter in response to cold," "cytosolic lipolysis," and "response to toxic substances."

We also detected variations in cutaneous CES1 transcriptomes between the control and lesional groups within the four GEO datasets (Supplementary Figure S6). Generally, there was a trend for the downregulation of CES1 in the lesional samples.

NC/Nga and contact hypersensitivity (CHS) models share common transcriptomic features among the three murine AD models

We expanded our previous investigation of the transcriptomic patterns of two murine AD models, comprising CHS and skin-scratching stimulation (SSS) [25, 34], to include the NC/Nga model. First, we investigated the overlapping dysregulated genes among the three murine models, which shared 42 upregulated and 19 downregulated genes (Fig. 4A). Overall, 159 upregulated and 41 downregulated genes overlapped between the NC/Nga

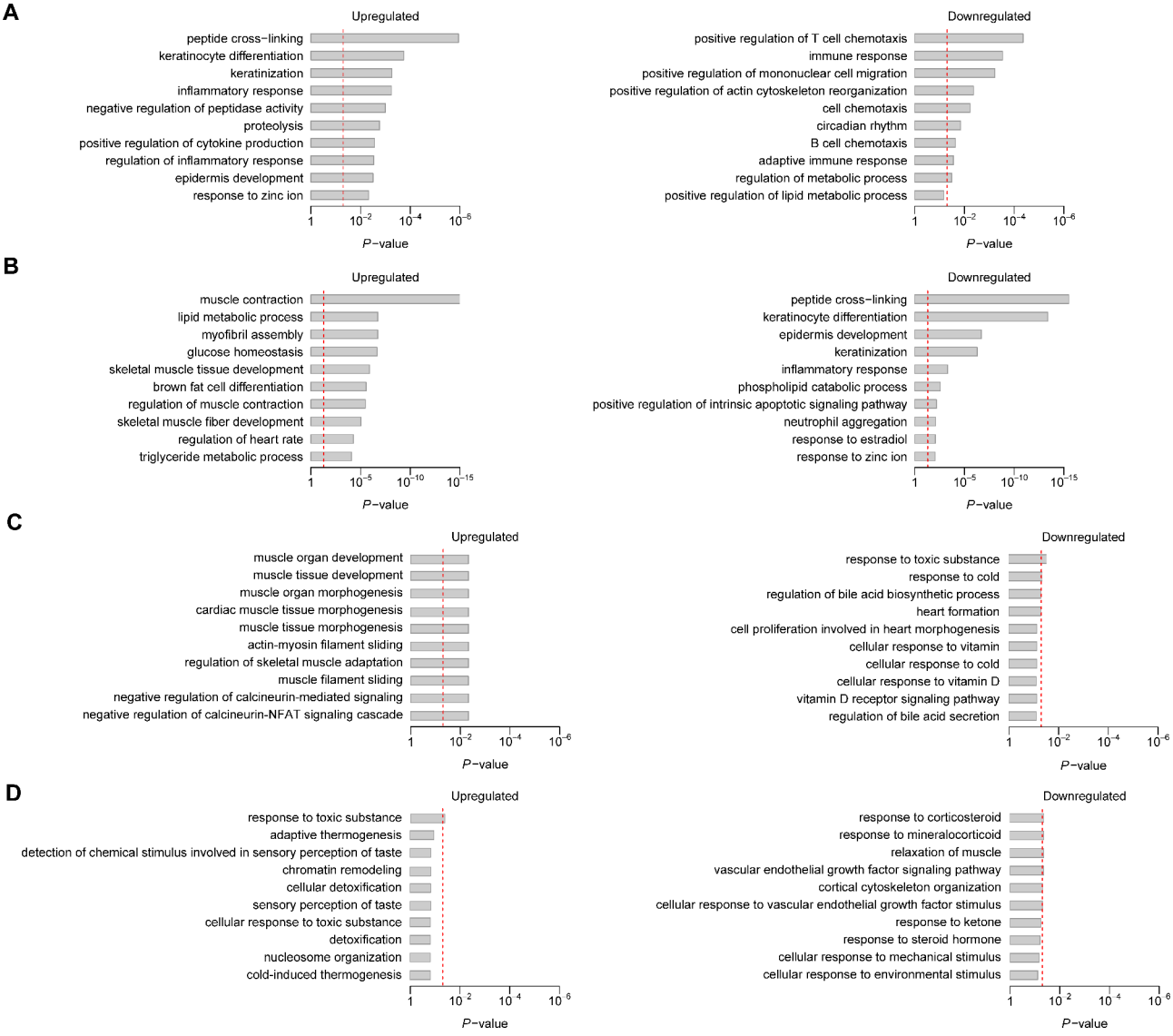

Fig. 2 Gene ontology (GO) analysis of differentially expressed genes (DEGs) in the NC/Nga murine model. Gene Ontology biological process (GOBP) terms associated with DEGs in the skin (**A**) and muscle (**C**) of NC/Nga mice compared to controls. GOBP terms associated with DEGs in hydrocortisone (Hc)-treated NC/Nga mice compared to untreated NC/Nga mice in the skin (**B**) and muscle (**D**). The red dashed lines indicate a *P*-value of 0.05. DEG: Differentially expressed gene, GO: Gene Ontology, GOBP: Gene Ontology biological process, Hc: Hydrocortisone, $\log_2$FC: Logarithm base 2-transformed gene expression fold change

and CHS models, whereas only 54 upregulated and 37 downregulated genes overlapped between the NC/Nga and SSS models (Fig. 4A).

Next, we examined the correlation of gene expression in the murine models. The $\log_2 FC$ of the NC/Nga model was positively correlated with that of the CHS model (Pearson correlation test: $r=0.355$ and $P<10^{-10}$) (Fig. 4B). A weaker positive correlation was also observed between the NC/Nga and SSS models (Pearson correlation test: $r=0.140$ and $P<10^{-10}$) (Fig. 4B).

We also investigated to what extent the transcriptomic profile of the three murine models mimicked the transcriptome of human patients with AD. The gene expression between control and AD-lesioned skin samples was calculated for each cohort. Our findings indicated that the $\log_2 FC$ of the human cohorts with AD was positively correlated with the $\log_2 FC$ obtained from the three murine models. Thus, NC/Nga mice, characterized by high transcriptomic homology with the human AD transcriptome, are expected to have translational relevance for the American endotype (Fig. 5 and Supplementary Figure S6).

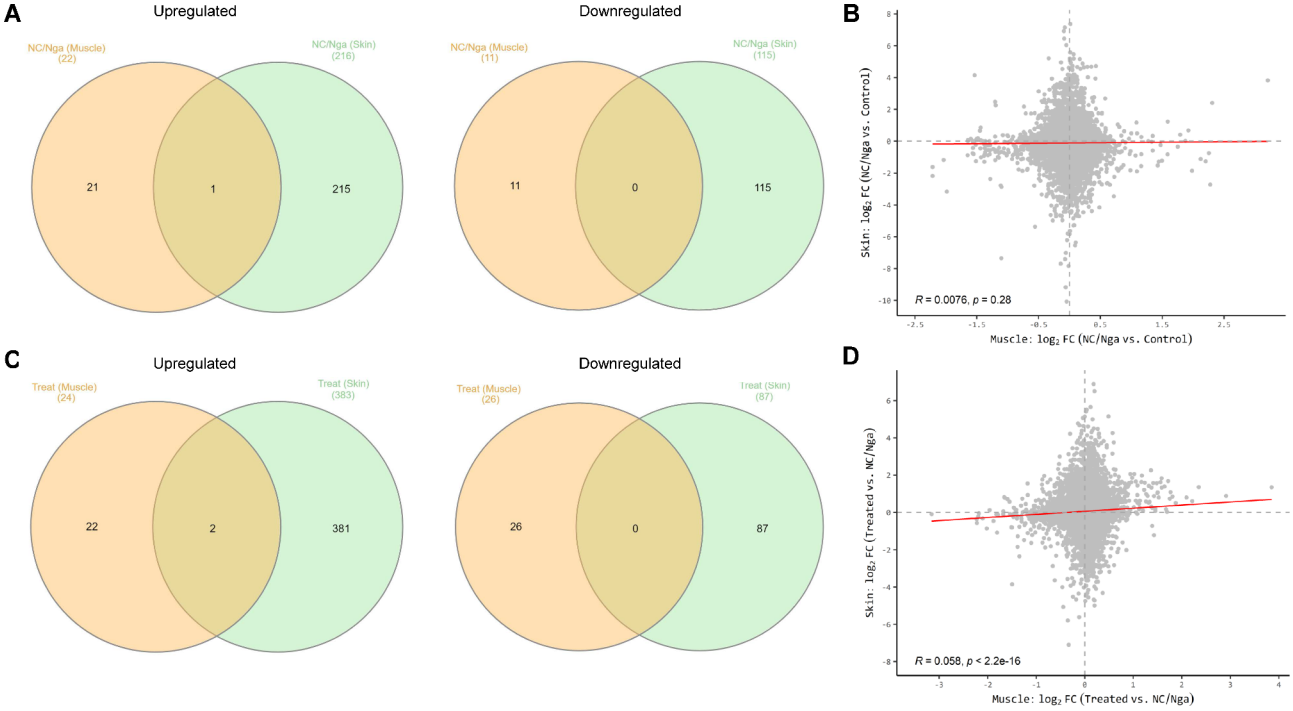

Fig. 3 Comparison between skin and muscle samples in the NC/Nga model. (**A**) Venn diagrams showing the overlapped dysregulated genes between skin and muscle samples in NC/Nga mice compared to controls. (**B**) Correlation of the gene $\log_2$-transformed fold change ($\log_2 FC$) between NC/Nga muscle (*x*-axis) and skin (*y*-axis). Each dot represents a different gene. (**C**) Venn diagrams showing the overlapped dysregulated genes between skin and muscle samples in Hc-treated NC/Nga mice compared to untreated NC/Nga mice. (**D**) Correlation of $\log_2 FC$ between NC/Nga muscle (*x*-axis) and skin (*y*-axis). Each dot represents a different gene. DEG: Differentially expressed gene, Hc: Hydrocortisone, $\log_2$FC: Logarithm base 2-transformed gene expression fold change

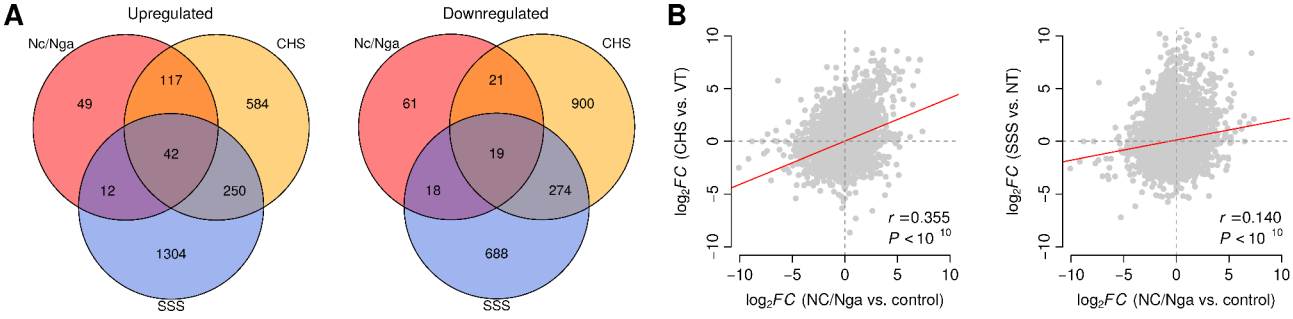

Fig. 4 Comparison between NC/Nga, contact hypersensitivity (CHS), and skin-scratching stimulation (SSS) murine models. (**A**) Venn diagrams showing the overlapping dysregulated genes between the NC/Nga, CHS, and SSS murine models. (**B**) Correlation of $\log_2$-transformed fold change ($\log_2 FC$) between NC/Nga mice (*x*-axis) and CHS/SSS mice (*y*-axis). Each dot represents a different gene. CHS: Contact hypersensitivity, $\log_2$FC: Logarithm base 2-transformed gene expression fold change, SSS: Skin-scratching stimulation

Discussion

The detrimental effects of AD extend beyond the damaged skin and influence the body systemically [5, 6]. We sought to profile the impact of prolonged exposure to systemic inflammatory cytokines on distant organs from the damaged skin through a comprehensive transcriptome analysis (Fig. 1). Specifically, circulating IL-12 levels and Hc-induced *Ces1d* expression in both the skin and distant muscle of the subjects were identified as important markers for AD's systemic effects, providing a strong foundation for understanding the disease's broader implications.

Cytokines provide growth, differentiation, inflammatory, and immunosuppressive signals, which promote immune responses. Prolonged inflammatory stimulus may lead to excessive production of cytokines, which then enter the plasma, affecting cells that are distant from the original site of inflammation in an endocrine manner [35]. IL-12 A, a proinflammatory cytokine that promotes T cell proliferation and cytotoxic activity, drives the immune system toward a Th1-type response

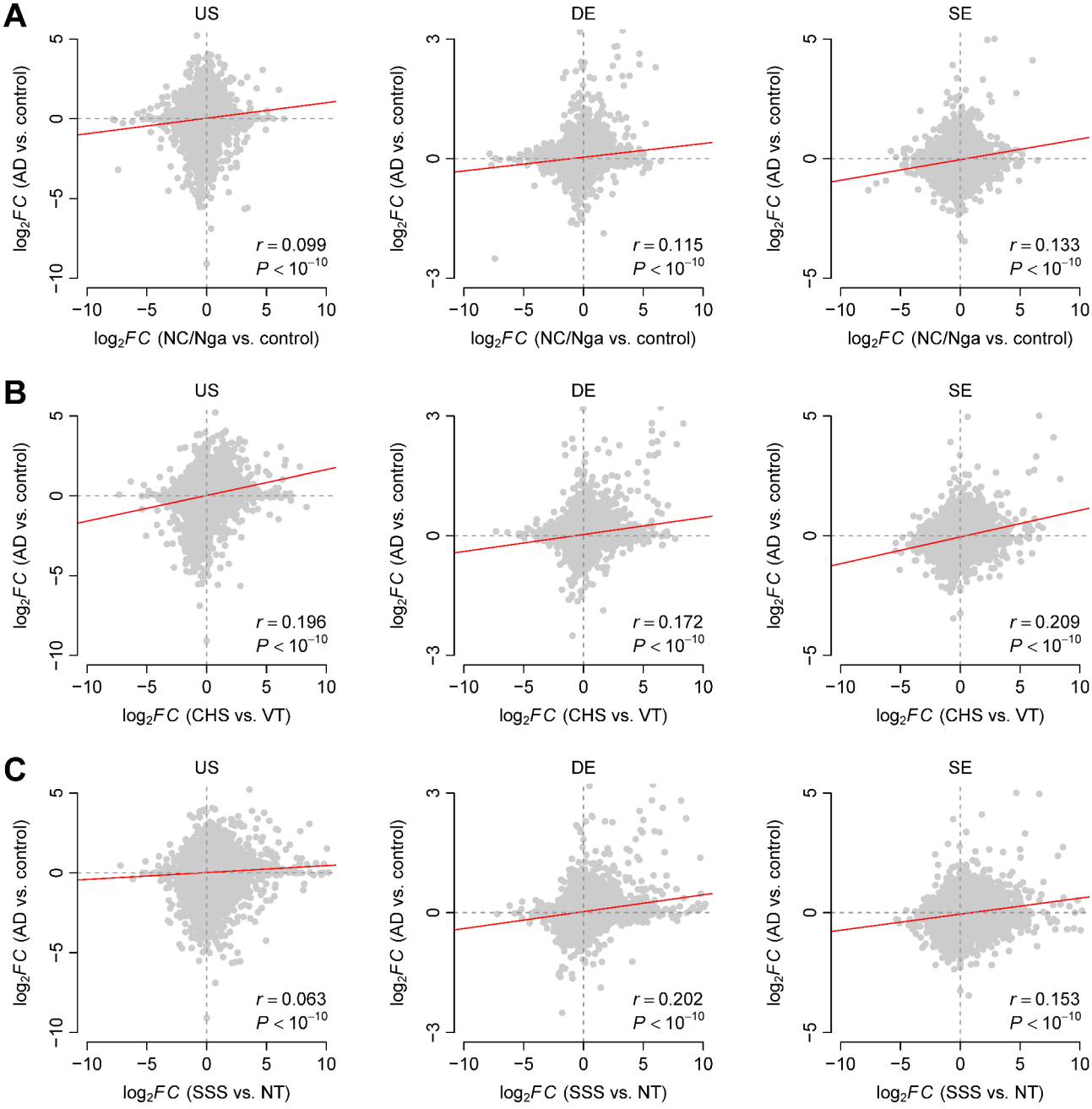

Fig. 5 Gene level comparison between murine models and human cohorts of patients with atopic dermatitis **(AD).** For each given gene, a positive/negative $\log_2$-transformed fold change ($\log_2 FC$) suggests up-/downregulation in murine models or patients with AD. (**A**) Comparison between the NC/Nga model and human cohorts. (**B**) Comparison between the CHS model and human cohorts. (**C**) Comparison between the SSS model and human cohorts. AD: Atopic dermatitis, CHS: Contact hypersensitivity, DE: German cohort, $\log_2$FC: Logarithm base 2-transformed gene expression fold change, SE: Swedish cohort, SSS: Skin-scratching stimulation, US: USA cohort

and prevents Th2-type immunity [36]. Recent studies have reported that high levels of IL-12 are produced and secreted in the chronic phase of AD [37]. Furthermore, IL-12 may be involved in myogenic differentiation because endogenous and exogenous IL-12 have been shown to strengthen myogenic differentiation in C2C12 mouse skeletal muscle cells [38]. Sarcopenia and aging were negatively correlated with the expression of the IL-12 gene and/or the secretion of serum IL-12 [39]. In this study, NC/Nga mice with AD-like symptoms produced IL-12, which was secreted into the blood [in line with the results published by Choi et al. [13]]. In addition to conventional methods such as immune assay, and histological staining, we employed GO term analysis to

confirm that our animal model accurately reflects the characteristics of atopic dermatitis (Fig. 2). We also noted an increase in the expression of genes related to organ development (*Tnni1*, *Myl6b*, *Myh7*, *Rcan1*, *Ankrd1*) in the diseased muscle. We speculate that the production of high levels of IL-12 is likely to be involved in gene pathways related to tissue repair following skin damage, and that promoting muscle development could be a compensatory effect of skin damage. On the other hand, downregulation of *Ass1* [40], *Ccnd1* [41], and *Hdac8* [42] in Hc-treated muscles likely results from the resolution of excessive inflammatory responses, characterized by reduced T-cell activation and suppressed cell proliferation (Fig. 3).

Ces1d was the only gene consistently altered in both muscle and skin, making it a key candidate for understanding systemic effects. Also known as Ces3, it encodes carboxylesterase 1 (CES1), which is a functional ortholog of the human CES1 protein [43, 44]. Skin can bioactivate sensitizing chemicals even though it possesses a metabolic capability that is 10–20% less than that of the liver [45]. Carboxylesterase is involved in the detoxification and metabolism of (pre)drugs and environmental toxicants, the hydrolysis of numerous endogenous esters (cholesteryl esters and triacylglycerols), and contributes to crucial physiological and pathological processes, such as lipid and cholesterol metabolism [46]. Many studies have linked the impaired skin barrier function of patients with AD to changes in the expression of genes involved in the biosynthesis and metabolic pathways of lipids that constitute the epidermal permeability barrier [47]. However, to the best of our knowledge, the association between CES1 and AD remains unexplored. Most studies have suggested that skin lesions in patients with AD have normal and/or high cholesterol levels and low total ceramide levels compared to healthy controls [48, 49]. High cholesterol levels in peripheral tissues are eliminated by reverse cholesterol transport [50]. CES1 in human macrophages catalyzes the hydrolysis of cholesterol to facilitate free cholesterol efflux, promote cholesterol excretion, and mediate reverse cholesterol transport [51]. However, AD skin may be unable to remove excessive cholesterol due to downregulated Ces1d, suggesting that normal cholesterol levels cannot be obtained. Indeed, in a previous study, patients with cirrhosis exhibited increased secretion of proinflammatory cytokines and decreased CES1-mediated hydrolysis capacity [52]. Moreover, in human hepatocytes, dexamethasone (a type of corticosteroid) moderately induced the expression of CES1 [51, 53]. CES1has also been shown to hydrolyze the endocannabinoid 2-arachidonoylglycerol and its cyclooxygenase-derived prostaglandin glyceryl esters (PG-Gs) in human THP-1 monocytes/macrophages [51]. PGD2-G is hydrolyzed by CES1 in mouse macrophages and is then converted to PGD2, which affects tumor necrosis factor-alpha-related inflammatory responses. In contrast, blocking the degradation of PGD2-G by CES1 further attenuates the IL-6-related inflammatory response in human monocytes. However, the instability of PG-Gs in enzymatic hydrolysis limits the comprehensive characterization of their effects in vivo [54]. Although the exact mechanism underlying this phenomenon remains to be elucidated, our results suggest that *Ces1d* expression was decreased in the skin and muscle of NC/Nga mice and was associated with the expression of various cytokine genes after AD induction. Moreover, Hc treatment restored the expression of Ces1d owing to the effects of proinflammatory cytokines. Topical administration of GCs is used in the treatment of skin diseases, with the aim to prevent systemic side effects. However, percutaneous absorption involves passing the drug through the skin layers, resulting in the drug entering the systemic circulation. The stratum corneum can store once-applied drugs and continuously penetrate them into the body. Less than 2% of Hc is absorbed into the systemic circulation when topically applied for more than 1 day [55]. A lower dose of Hc can produce systemic effects, particularly following repeated applications. Therefore, *Ces1d* is a biomarker of AD that links endotypes and phenotypes and likely participates in the alleviation of subchronic AD by triggering responses associated with stress and/or chemical exposure.

In this study, correlation tests in the fold change of gene expression were heavily used to provide a global picture of the relationship of the transcriptome between different murine models. For instance, even though the $\log_2 FC$ of the NC/Nga model was positively correlated with those of the CHS and SSS models, the correlation coefficient was much stronger for the CHS model than for the SSS model. This observation suggests a relatively higher similarity in the transcriptomic pattern between the NC/Nga and CHS models relative to the similarity between the NC/Nga and SSS models, which is further confirmed by the number of commonly dysregulated genes, as indicated by the Venn diagrams in Fig. 4. Correlation analyses in a mouse model of subchronic AD identified potential therapeutic targets based on transcriptomic data and provided insights into the systemic nature of this disease. However, the precise molecular mechanism of the genes involved in atopy requires further characterization.

Our study has several limitations that warrant discussion. First, we only evaluated a few human cohorts, and the inclusion of Asian cohorts is necessary for comprehensive analysis. Second, the assessments were limited to a single time point and we did not perform any functional studies. As a result, the gene expression changes we identified can only indirectly suggest physiological or pathological outcomes. Future studies that incorporate these

aspects will be necessary for strengthening the causal inferences drawn from these findings. Nonetheless, this study underscores the importance of comprehensively assessing the systemic effects of AD through transcriptome analysis of both the distant muscle and damaged skin of AD mice while also confirming the potential for extrapolation to human cohorts. Third, our study demonstrated the challenges in elucidating the causal relationship between skin barrier damage, inflammation, and systemic disease in AD, which were further exacerbated by the lack of an established animal model that incorporates all of the pathological facets of AD (Fig. 5). Thus, classification of disease subtypes with complex profiles, such as AD, is required. Because multiple genetic and environmental factors underlie AD, developing a single comprehensive animal model is unrealistic.

Conclusions

Our study confirmed that AD is not simply a skin condition but rather a disease that affects the entire body, establishing a basis for evidence-based disease management. Our results also provide important insights into disease heterogeneity. In addition to phenotype classification, endotype classification, including mechanistic aspects, will be a useful criterion for the development of effective treatment strategies for the symptoms of AD and its subtypes.

Abbreviations

AD	Atopic Dermatitis
CES1	Carboxylesterase 1
CHS	Contact Hypersensitivity
DE	German Cohort
DEG	Differentially Expressed Gene
FC	Fold change
FDR	False Discovery Rate
GC	Glucocorticoid
GO	Gene Ontology
GOBP	Gene Ontology Biological Process
Hc	Hydrocortisone
HDM	House Dust Mite Ointment
IgE	Immunoglobulin E
IL	Interleukin
log_2FC	Logarithm base 2-transformed gene expression fold change
MHC	Major Histocompatibility Complex
NF-κB	Nuclear Factor Kappa B
PG-G	Prostaglandin Glyceryl Ester
RMA	Robust Multi-Average
SE	Swedish Cohort
SSS	Skin-scratching Stimulation
Th	T helper
TNF-α	Tumor Necrosis Factor-alpha
US	USA cohort

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12865-024-00666-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

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Not applicable.

Author contributions

All authors read and approved the final manuscript.Y-W K and E-A K: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. J J, S J, D K, J S S, and S-Y L: Investigation, Writing – review & editing. I L, S-C J, and J-H Kim: Resources, Project administration, Writing – review & editing. T Z: Visualization, Formal analysis, Writing – review & editing. H B and J-H Ko: Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

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Data availability

The human cohort datasets related to this study can be found in the Gene Expression Omnibus database [https://www.ncbi.nlm.nih.gov/geo/, GSE60709, GSE6012, GSE120721, GSE107361]. Data pertaining to mice during the current study are available in the Gene Expression Omnibus database repository, [https://www.ncbi.nlm.nih.gov/geo/, GSE251989].

Declarations

Ethics approval and consent to participate

All animal experiments were approved by the Institutional Animal Care and Use Committee of Chung-Ang University (approval No. 201800012) and were performed in accordance with current animal testing regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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