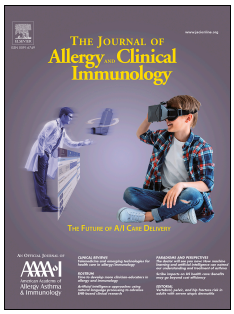
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The molecular features of normal and atopic dermatitis skin in infants, children, adolescents and adults

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The molecular features of normal and atopic dermatitis skin in infants, children, adolescents and adults

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Dermira, Galderma, Glenmark, Novartis, Pfizer, Vitae, Leo Pharma, Abbvie, Eli Lilly, Kyowa, Mitsubishi Tanabe, Asana Biosciences, and Promius. JGK has received research support (grants paid to his institution) and/or personal fees from Pfizer, Amgen, Janssen, Lilly, Merck, Novartis, Kadmon, Dermira, Boehringer, Innovaderm, Kyowa, BMS, Serono, BiogenIdec, Delenex, AbbVie, Sanofi, Baxter, Paraxel, Xenoport, and Kineta. ASP has received research support (grants paid to her institution) from AbbVie, Anaptysbio, Celgene, Eli Lilly, Galderma, Incyte, Leo, Janssen, Novartis, and Regeneron. She has been a consultant for Almirall, Amgen, Asana, Boehringer-Ingelheim, Castle Creek, Celgene, Dermavant, Dermira, Eli Lilly, Exicure, Forte, Galderma, Lenus, Leo, MEDA Corp, Meiji Seika, Novan, Novartis, Pfizer, Regeneron, Sanofi-Genzyme, and Sol Gel. The rest of the authors have no conflicts of interest to disclose.

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Abbreviations used:

AD: Atopic dermatitis

ADQ: Atopic dermatitis quickscore

DEG: Differentially expressed gene

EASI: Eczema area and severity index

FCH: Fold-change

FDR: False discovery rate

FLG: Filaggrin

GSVA: Gene set variation analysis

IL: Interleukin

LCE: Late cornified envelope

LOR: Loricrin

qRT-PCR: Quantitative reverse transcription polymerase chain reaction

SCORAD: SCORing of Atopic Dermatitis

TEWL: Transepidermal water loss

Th: T helper

y/o: Years old

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Abstract:

Background: Although atopic dermatitis (AD) often presents in infancy and persists into adulthood, comparative characterization of AD skin among different pediatric age-groups is lacking.

Objective: To define skin biopsy profiles of lesional and non-lesional AD across different age-groups (0-5 y/o infants with disease duration <6 months, 6-11 y/o children, 12-17 y/o adolescents, ≥18 y/o adults) versus age-appropriate controls.

Methods: We performed gene expression analyses by RNA-sequencing and real-time polymerase chain reaction (RT-PCR) and protein expression analysis using immunohistochemistry.

Results: Th2/Th22-skewing, including IL-13, CCL17/TARC, IL-22 and S100As characterized the common AD signature, with a global pathway-level enrichment across all ages. Nevertheless, specific cytokines varied widely. For example, IL-33, IL-1RL1/IL-33R and IL-9, often associated with early atopic sensitization, showed greatest up-regulations in infants. Th17 inflammation presented a two-peak curve, with highest increases in infants (including IL-17A and IL-17F), followed by adults. Th1 polarization was uniquely detected in adults, even when compared to adolescents, with significant up-regulation in adults of IFNγ and CXCL9/CXCL10/CXCL11. While all AD age-groups had barrier abnormalities, only adults had significant decreases in filaggrin expression (FLG). Despite the short duration of the disease, infant AD presented robust down-regulations of multiple barrier-related genes in both lesional and non-lesional skin. Clinical severity scores significantly correlated with Th2/Th22-related markers in all pediatric age-groups.

Conclusion: The shared signature of AD across ages is Th2/Th22-skewed, yet differential expression of specific Th2/Th22-related genes, other Th-pathways, and barrier-related genes portray heterogenetic, age-specific molecular fingerprints.

Clinical Implication:

As targeted treatments are developing for pediatric atopic dermatitis, molecular skin profiling reveals specific immune and barrier signatures in different age-groups, which may inform age-specific therapeutic choices.

Capsule Summary:

Skin profiling portrays the molecular abnormalities of different pediatric atopic dermatitis age-groups throughout maturation, with a shared Th2/Th22-skewing, relative increase of the Th17 pathway in infants and adults, and a Th1-polarization only in adult patients.

Key Words: Atopic dermatitis, maturation, pediatric, transcriptomics, Th2, Th22, epidermal barrier, maturation, normal skin.

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Introduction

Atopic Dermatitis (AD) is the most common inflammatory skin disease and its prevalence continues to increase worldwide.[1,2] Although AD often presents in infancy and persists into adulthood,[1] the evolution of the AD skin phenotype from infancy to adulthood has not yet been characterized. Since AD may be the window to the atopic march, understanding immune and barrier changes of AD across pediatric populations may provide insights into the initiation of other atopic comorbidities, such as food allergy, allergic rhinitis, and asthma.

Recently, we compared biopsied skin from infants and young children (less than 5 years of age) with AD within six months of disease initiation to skin from adults (≥18 years of age) with chronic AD.[3,4] The early disease profile has substantial differences compared with adult chronic AD. While both share robust Th2/Th22-skewing, infants have greater Th17 inflammation, lack Th1 polarization, and have different barrier abnormalities.[3,4]

While data from blood of AD patients in different age-groups were recently published,[5] skin of school-aged children and adolescents [ages 5-18 year-old (y/o)] with AD has not been investigated comprehensively. In blood, the immune fingerprint of AD was found to be age-specific, with the adult AD phenotype achieved only in adults.[5] As cutaneous analyses capture skin dysregulations more accurately and also allow assessment of barrier abnormalities, investigation of AD skin through development is imperative.

Current therapeutics for extensive AD are sparse[6-8] and therapeutic options available for pediatric AD patients are even more limited.[9,10] Narrow targeting agents for

AD are emerging,[11-13] including for pediatric patients.[14-16] As new therapies that target different type 2 immune pathway components or other pathways are developed, characterization of immune and barrier abnormalities in different pediatric AD age-groups may become more important in the decision-making about optimal choice of therapeutic agent. A better understanding of age-specific disease markers will also potentially contribute to identifying novel age-specific treatment strategies.

To discover age-specific biomarkers, AD skin of different age-groups (0-5y/o, 6-11y/o, 12-17y/o and ≥18y/o) was compared with age-appropriate controls. Molecular dysregulations were assessed by RNA-sequencing, real-time polymerase chain reaction (RT-PCR), and immunohistochemistry (IHC). Biomarker analyses showed different expression patterns of specific Th2-related cytokines and chemokines across the age spectrum, although Th2/Th22-skewing was shared among all pediatric groups and adults. Th1-related products progressively increased with age, including being significantly higher in adults versus adolescents. For Th17, despite global up-regulations at all ages, a two-curve peak of Th17-related polarization was captured, highest in infancy and less enriched in adulthood. These data portray the evolution of AD skin through maturation and improve our understanding of shared and age-specific disease mechanisms.

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Methods

Clinical characteristics

Forty-four patients with moderate-to-severe AD in different age-groups (17 infants with disease duration of less than six months, 0-5y/o, mean age 1.2yrs; 10 children, 6-11y/o, mean age 7.8yrs; 14 adolescents, 12-17y/o, mean age 14.8yrs; 13 adults ≥18y/o, mean age 45.4yrs) were enrolled after parents and patients 12 years and up signed Institutional Review Board-approved informed consents and assents (Table 1 and Table E1). The cohort across age-groups was composed primarily of European American patients. Sensitivity analyses on European American-only patients yielded similar results to analysis including subjects of all races (Table E7, Fig E15-E16). Lesional and non-lesional skin biopsies were obtained, and exclusion criteria included recent use of systemic immunosuppressive treatment and phototherapy (<4 weeks), topical steroids or immunomodulators (<1 week), or moisturizers (<12 hours) prior to this study.

Clinical severity was recorded using SCORing of AD (SCORAD), Eczema Area and Severity Index (EASI), and Atopic Dermatitis Quickscore (ADQ) in pediatric patients (≤18y/o), and SCORAD in adults (≥18y/o). IgE levels (IU/ml) were available for some AD patients. In pediatric participants, measures of epidermal barrier function were captured using transepidermal water loss (TEWL) of lesional and non-lesional arm skin (AquaFlux Model AF200; Biox, London, England).[17]

RNA-sequencing

RNA was extracted from skin biopsy samples using Qiagen miRNeasy Mini kit, as previously described.[18] Libraries were generated using TruSeq Stranded mRNA Library Prep kit (Illumina). mRNA was first extracted from 240ng of total RNA using oligo-dT magnetic beads and fragmented at high temperature. A cDNA library was then prepared by reverse transcription. Next generation sequencing was performed on Illumina Hiseq 4000 (Illumina INc.) with single-ended read 100 cycles. Our sequencing depth ranged between 35-45M reads and the reference genome used was hg19 (GRCh37). Image analysis and bases calling was conducted in real-time by the Illumina analysis pipeline.

Quantitative Real-Time Polymerase Chain Reaction

For RT-PCR, reverse transcription to complementary DNA (cDNA) was carried out using the High Capacity cDNA reverse transcription (Thermo fisher). Pre-amplification was performed on all samples. TaqMan Low Density Array (TLDA) cards (Thermo fisher) were used for quantitative RT-PCR (qRT-PCR). Primers are listed in Table E2. 100ng total RNA was used for PreAMP pool and TLDA. Eukaryotic 18S recombinant RNA (rRNA) was used as an endogenous control. Expression values were normalized to *Rplp0*.

Immunohistochemistry

Immunohistochemistry was performed on frozen skin sections as previously described[19,20] using antibodies as listed in Table E3 (also see online supplementary

materials). Epidermal thickness and cell counts were quantified using ImageJ V1.42 software (National Institutes of Health, Bethesda, Maryland).

Statistical Analyses

Statistical analyses were performed using statistical language R (www.R-project.org). qRT-PCR data was profiled using TLDA. Summaries of mean expression of all markers are summarized in a heatmap, where unsupervised clustering was performed using Euclidean distance and average agglomeration criteria. Threshold cycles [Ct] were normalized to *Rplp0* by negatively transforming the Ct values to −dCt. The undetected expression values were estimated for each gene as 20% of the minimum unlogged expression across all samples. Log_2-scale qRT-PCR expression data were modeled by a linear mixed effect model with biopsy type as a fixed effect and a random intercept for each patient. Means of each group were estimated using *lsmeans* and comparisons of interest were tested using contrast. For correlation studies, unsupervised hierarchical clustering of variables or samples/patients was performed using the correlation coefficient as a distance metric with the average agglomeration algorithm.

RNA-sequencing data was pre-processed using standard pipeline incorporating quality control metrics, such as FastQC and MultiQC, sequence alignment based on STAR RNA-sequencing aligner and sequencing reads assignment to genomic features by featureCounts and *voom*-transformed. Mixed effect model on R's limma framework was used and p-values were adjusted by Benjamini-Hochberg procedure. Differentially expressed genes were defined by fold-change (FCH)>2.0 and false discovery rate

238 (FDR)< 0.05. Gene-set over-expression analysis was performed with XGR software
239 using canonical/KEGG/REACTOME/BioCarta pathways.[21]
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Results

Normal skin changes from infancy through adulthood

To better understand AD-related changes in different age-groups, we evaluated changes in healthy skin throughout maturation, using RNA-seq data presented as heatmaps of previously reported immune and barrier gene-set (Fig E1-E2 and Table E4). Comparisons between age-groups were considered differentially expressed genes (DEGs) using a threshold of fold-change (FCH)>2 and false discovery rate (FDR)<0.05. Overall, while most immune- and barrier-related markers were comparable between age-groups, a mixed expression pattern was detected, with the largest number of DEGs found in the adult versus infant comparison. A *green* box highlights genes that show a trend of increase with age, as visualized by a *blue* to *red* transition from infancy to adulthood (Fig E1). Among these, the negative regulatory marker IL-37 showed significant down-regulation in infants compared to adults (FDR<0.05). *Pink* boxes indicate clusters of genes that are most up-regulated in infants. These include regulatory/T-regulatory/Treg markers such as CTLA4, IL-10 and TGFβ3, as well as Th17/Th22-related genes, such as CXCL2 and S100P/A7/A9 (FDR<0.05). Using a Gene Set Variation Analysis (GSVA) curated from previously published datasets,[22-24] we found that while most immune axes were comparable between age-groups (Th1/Th2/Th22, p>0.05), Th17 pathway was significantly up-regulated in infants (p<0.05) (Fig E3A).

Barrier-related genes were slightly intensified in the older age-groups, including terminal differentiation-related (late cornified envelope/LCE1F/2B/2C/2D, FLG, SCEL), tight junction-related (CLDN8/11/23) and lipid-related genes (ELOVL5, FADS1/2,

264 FABP7, SGPP1). Only few lipid-related markers were significantly increased in infants
265 versus adults (FA2H, LPL, CIDEC, and PPARG) (FDR<0.05) (Fig E2).

266 Spearman correlation between mRNA expressions (by RT-PCR) with age
267 similarly showed that multiple Th17/Th22-related markers were inversely correlated with
268 age (IL-20, IL-22, S100A7/9/12/8, CCL20, PI3) ($r \leq -0.4$, $p<0.05$), while positive
269 correlation with age were found for barrier-related markers (loricrin/LOR,
270 CLDN1/CLDN23) and negative regulatory markers (IL-37, IL-34) ($r>0.4$, $p<0.05$) (Table
271 E5).

272 IHC evaluating for epidermal hyperplasia and immune cell infiltrates revealed that
273 healthy infants had significantly thinner epidermis, with greater counts of $TRAIL^+$
274 dendritic cells/DCs, eosinophils (major basic protein/MBP^+ cells), and neutrophils
275 (neutrophil elastase/NE^+ cells). On the other hand, older age-groups showed greater
276 counts of $Fc\varepsilon R1^+$ DCs (Fig E3B-F).

277

278 <u>Shared and uniquely differentially expressed genes unfold the common versus age-</u>
279 <u>specific features</u>

280 Global molecular profiling of the age-specific AD transcriptomes by RNA-seq
281 identified 9,829 DEGs (3,859 up-regulated, 5,970 down-regulated) in lesional AD skin
282 versus skin of similar-age healthy controls across all age-groups (using the same
283 threshold of FCH>2 and FDR<0.05) (Fig ~~1~~<u>4</u>A). Of these, 362 DEGs (3.7%) were shared
284 among lesional AD skin in all age-groups (154 up-regulated, 208 down-regulated).
285 Lesional infant and adult AD had a larger number of uniquely expressed genes as

compared to children and adolescents: 3,060 uniquely expressed DEGs were detected in infants, 236 in children, 643 in adolescents, and 2,766 in adults.

A principal component analysis (PCA) of all lesional and normal samples across ages using RNA-seq data shows that while the normal skin samples overlap, lesional AD samples present wider variations with separation seen between adults and pediatric age-groups (Fig ~~1~~4B). No clear trends were observed in non-lesional PCA (Fig E4C-D).

The global immune phenotype of AD across age-groups in lesional skin presented key Th2- (IL-13, CCL17/TARC, CCL18/PARC, IL-4R) and Th22- (IL-22, S100A7/8/9/12) related markers (Fig 1~~2~~) (FDR<0.05).[25,26] Additional Th2-related products including TSLPR/CRLF2, CCL26, CCL7, and CCL27/CTACK were shared considering FDR<0.1 and FCH>2 criteria (Table E4). Other shared up-regulated DEGs across ages in lesional AD included general inflammation (MMP12/25)[27] and T-cell activation/migration/NK-cell (TNFRSF9, CCR7, GZMB, CSF2) markers, while commonly down-regulated DEGs included the negative regulators IL-34 and IL-37 (Fig 1 and Table E4) (FDR<0.05).

Nevertheless, specific age-groups also showed distinct phenotypes. In infant AD uniquely/higher up-regulated genes included those related to innate immunity (IL-1B, IL-8), and aryl hydrocarbon receptor/AHR (FDR<0.05).[28] Infant-specific DEGs also included Th2-related cytokines, including the itch cytokine IL-31,[29] cytokines implicated in atopy initiation (IL-5 and IL-33),[30,31] and multiple Th17-related markers, including the key Th17 cytokines IL-17A and IL-17F (Fig 1) (FDR<0.05). These are in line with the dominant Th17-polarization characterizing AD in infants,[4] and further define robust Th17-related up-regulations as particularly characteristic of infant AD. Of note, many of

these are significantly higher in infants versus children (e.g, IL-17A, CXCL1, CCL20, IL-19) (FDR<0.05).

Except for IL-16 and CXCL12, both related with T-cell activation and recruitment (FDR<0.05),[32,33] significant up-regulations of key immune DEGs were not found uniquely in child or adolescent AD. These age-groups mostly exhibited comparable expression of many Th2 and Th22-related DEGs (e.g, IL-13, CCL17/TARC and IL-22), lower levels of Th17-related markers, as detailed above, or slight higher levels of T-cell migration (CCL19, CCR7), DCs (ITGAX/CD11c), and Th1-related markers (CCL3, CCL4, CXCL16) compared to infants, but not to the levels found in adults.

Conversely, lesional adult AD presented numerous unique DEGs with intensification of the inflammatory tone across various immune axes. Specifically, multiple Th1-related markers were uniquely expressed or showed significantly higher expression levels in adults, including CXCL9/10, MX1, interferon regulatory factor-1 (IRF1), IL-12B/p40, and CCR1 (FDR<0.05; Fig 1).

Heatmaps with clustering analysis of non-normalized gene-expression results of lesional and non-lesional AD skin across age-groups are presented in Fig E5-E8 and detailed in the supplementary materials.

Using a GSVA on previously published AD-related pathways, including the Meta-Analysis Derived AD transcriptome/MADAD,[24] and key immune pathways,[22,23] we observed significant enrichment across AD age-groups in the MADAD and Th2 and Th22 pathways compared to controls (Fig 2A-D). However, Th17 axis showed highest expression in infants, while Th1 pathway significantly up-regulated only in adults ($p<0.05$; Fig 2).

For a broader perspective on age-specific AD-related changes of gene expression, all DEGs in lesional AD versus controls by age-groups were also analyzed using function-based pathway databases (canonical/KEGG/REACTOME/BioCarta pathways), which revealed significantly enriched pathways in all age-groups (FDR<0.05). Of these, some of the pathways were common to two and more age-groups, and some unique for a specific age (Table E6). Pathways that were significantly enriched across all ages including those related with cytokine and chemokine signaling, the JAK-STAT pathway, and adaptive immune system, while pathways only enriched in children, adolescents and adults included those related with T-cytotoxic/Tc-cell, Th-cell, and other T-cell receptor/TCR-signaling (FDR<0.05) (Fig E9). IFNγ signaling showed gradual intensification with age, starting in children. The top up-regulated pathways detected in the infant AD signature included various aspects of cell cycle and replication and were substantially enriched in that age-group versus other age-groups.

Barrier characteristics differ across atopic dermatitis age-groups

As disrupted epidermal barrier is an important characteristic of AD, genes of epidermal barrier components, including epidermal differentiation, lipid biosynthesis/metabolism, tight and gap junctions, and keratins were assessed using a previously reported epidermal barrier gene-set (Fig 3 and Table E4).[20,34-36]

The common signature of barrier impairment included key AD-associated lipid biosynthesis/metabolism-related genes, significantly down-regulated across all age-groups, including elongation of very long chain fatty acids protein 3 (ELOVL3),[24] reported to improve with dupilumab treatment,[12] acyl-CoA wax alcohol acyl transferase

1 (AWAT1),[37] and fatty acid 2-hydroxylate (FA2H) (FDR<0.05). Additionally, significant attenuation of tight junction-related genes in lesional skin, including cadherin (CDH20, CDH12),[38] claudin (CLDN1),[39] and paracingulin (CGNL1),[40] was shared across age-groups (FDR<0.05). Epidermal differentiation genes that are considered a hallmark of the barrier dysregulation in AD were not commonly down-regulated across age-groups. While in adults, multiple terminal differentiation genes showed significant down-regulations versus controls (LCEs [LCE1B/1C/1E/1F/2C/2D], filaggrin/FLG, PSORC1C2[41]) (FDR<0.05), less significant or similar but non-significant trends were largely observed for other age-groups.

Many markers showed exclusive down-regulation in infants with AD, but not in other AD age-groups. These included lipid biosynthesis/metabolism-related genes, such as 7-dehydrocholesterol reductase (DHCR7) and peroxisome proliferator activated receptor gamma (PPARG), both are also negatively associated with mast cell activation and hyper-degranulation,[42,43] perpilin 1 (PLIN1), suppressed by the topical drug adapelene that causes sebum inhibition,[44] CIDEC, associated with reduced skin surface lipids and dry skin,[45] and leptin (LEP), reported to be lower in serum of AD versus non-AD children (FDR<0.05).[46] Moreover, most of these genes were also significantly down-regulated in non-lesional infant AD.

<u>RT-PCR studies further define atopic dermatitis age-groups and developmental trajectories</u>

To validate RNA-seq data and evaluate mRNA expressions of key inflammatory and epidermal differentiation markers in AD, some of which may be poorly detected by

RNA-seq due to relatively low levels, we performed RT-PCR on a large panel of 71 genes (Fig E10).

PCR was generally in line with the RNA-seq data, and further defined the common Th2/Th22-skewing across age-groups as well as the unique inflammatory milieu of each one. Markers of general inflammation (i.e, MMP12) were commonly up-regulated across ages, with adults displaying the greatest up-regulations (Fig 4). Some markers exhibited distinct expression patterns. For example IL-17C, an epithelial marker and a potential therapeutic-target for inflammatory skin disorders,[47,48] was gradually increased starting in adolescence, while IL-8/CXCL8, associated with childhood asthma,[49] was significantly highest in infants compared with other age-groups ($p<0.05$) (Fig E10).

Overall, Th2/Th22-related markers including IL-13, IL-31, CCL17/TARC, CCL18/PARC, CCL7, IL-26 and IL-22 showed highly significant up-regulations in lesional AD across all age-groups versus controls ($p<0.05$) (Fig 4 and E10). Other Th2-related chemokines, including CCL13, CCL22, and CCL26, were up-regulated in most AD age-groups. IL-4R (targeted by dupilumab),[50] showed significant up-regulations in lesional AD skin across all age-groups (for infants, significance was attained only for the non-lesional comparison, with increasing significance with age ($p<0.05$; Fig 4 and E10). In contrast, robust up-regulations were found in infants compared to children in IL-9, IL-33 and its receptor IL-1RL1/IL-33R (in non-lesional skin), associated with early mite sensitization, food allergies and asthma,[51-53] and in IL-5, associated with induction of eosinophilic inflammation[30] ($p<0.05$) (Fig 4 and E10). Infants also showed the highest

400 expression levels of multiple Th17-related products, including IL-17A, LL37/CAMP,
401 CCL20, PI3, DEFB4B, KYNU, and the Th17/Th22-regulated S100As (Fig 4 and E10).

402 As over-expression of Th1-related markers is associated with chronicity in long-
403 standing AD,[26] multiple key Th1-related immune mediators were significantly up-
404 regulated only in adults, including IFNγ, CXCL9/CXCL10/CXCL11, STAT1, and MX1
405 ($p<0.001$) (Fig 4 and E10). Moreover, IFNγ and CXCL9/CXCL10/CXCL11 were
406 significantly up-regulated in adult lesional skin, even as compared with adolescents,
407 suggesting that the adult AD phenotype is only achieved in adulthood.

408 We then analyzed key immune and barrier markers in lesional and non-lesional
409 AD in parallel with normal skin (Fig 5) to reflect developmental trajectories. The T-cell
410 migration marker CCL19 presented up-regulation with age only in lesional AD, while in
411 non-lesional and normal skin expression levels remained similar across ages. The Th2
412 markers, IL-31 and CCL17, did not show a consistent trend in normal skin, but
413 presented greater up-regulation in specific ages (infants and adults, respectively), with
414 up-regulation versus normal skin detected across all ages. While Th1-related markers
415 were intensified in adults only in AD skin (IFNγ, CXCL10), Th17-related markers were
416 most up-regulated in infants in both normal skin and AD (DEFB4B, PI3, S100A12), with
417 an increase towards adulthood in AD, but not in normal skin. Terminal differentiation
418 (FLG, LOR) markers were most down-regulated in adult AD versus controls, where
419 normal skin presented greater expression level with age, while AD skin presented only
420 modest increases in expression. The tight junction-related marker CLDN23 showed a
421 comparable expression in AD versus normal skin across ages, with minor gradual up-
422 regulation with age in all tissues.

Epidermal hyperplasia and compromised skin barrier characterize atopic dermatitis across ages

IHC was also performed to evaluate for protein expression of epidermal differentiation/proliferation markers, as well as immune cell infiltrates. All AD age-groups showed significant increases in epidermal hyperplasia in lesional skin, assessed by thickness, $Ki67^+$ cell counts, and K16 staining and mRNA expression ($p<0.01$; Fig 6A,C,E, and Fig E11-E12). Overall, the greatest abnormalities in non-lesional AD skin in terms of the proliferation marker K16 were seen in infants, with positive staining decreasing with age (14/19 in infants [74%], 5/10 in children [50%], 6/13 in adolescents [46%], and 4/13 in adults [31%]). Healthy controls were negative for K16 staining across all ages (Fig 6A,C). Conversely, impairment of the key epidermal differentiation markers, FLG and LOR, was most significant in adult AD ($p<0.05$; Fig 6B,D and Fig E11B,D).

We next assessed for inflammatory infiltrates including $CD3^+$ T-cells, $CD11c^+$ myeloid DCs, $CD206^+$, $TRAIL^+$ and $Fc\varepsilon R1^+$ Inflammatory DCs, DC-$LAMP^+$ mature DCs, $CD1c^+$ resting DCs, as well as for polymorphonuclear infiltrates including eosinophils (major basic protein/MBP^+ cells), neutrophils (neutrophil elastase/NE^+ cells), and mast cells (tryptase$^+$ cells) (Fig 6, E11-E14). Overall, $CD3^+$ T-cells and all DC subsets were significantly increased in lesional AD across all age-groups starting at disease initiation in infants. The highest counts of MBP^+/eosinophils and NE^+/neutrophils were found in infants, with MBP^+ cell counts being higher in infant non-lesional AD than in lesional skin of other age-groups ($p<0.01$; Fig 6K-L, Fig E14).

446

447 Th2/Th22-related markers correlated with SCORAD across atopic dermatitis age-
448 groups

449 To define common AD as well as unique biomarkers in various age-groups, we
450 correlated mRNA expressions by RT-PCR with clinical variables (age, SCORAD, EASI,
451 pruritus AD) and TEWL, the functional barrier measure[54] using Spearman correlations
452 (Table 2 and Table E5).

453 In all pediatric age-groups SCORAD correlated robustly ($r>0.4$, $p<0.05$) with key
454 Th2 and Th17/Th22-related markers, but these markers differed between lesional and
455 non-lesional samples as well as in different age-groups, as presented in Table 2. For
456 example, IL-22 significantly correlated with SCORAD in non-lesional adolescent AD and
457 in lesional child AD, and the Th17/Th22-related S100As correlated with severity in
458 lesional and non-lesional adolescent AD. Th2-related products positively correlated with
459 SCORAD included cytokines (IL-31, IL-13), chemokines (CCL18, CCL13, CCL17,
460 CCL22), and cytokine receptors (IL-4R, IL-1RL1/IL-33R/ST2), all implicated in AD
461 pathogenesis ($r>0.4$, $p<0.05$) (Table 2).[55-58]

462 Robust positive correlations with age were found only in lesional skin. These
463 included IL-17RA, which maintains skin barrier and immune homeostasis,[59]
464 CCL22/MDC, which was previously correlated with AD severity in serum,[60] and the T-
465 regulatory marker FOXP3 ($r>0.4$, $p<0.05$).[61] Among markers negatively correlating with
466 age was the Th17/Th2-regulated cytokine, IL-19 (in both lesional and non-lesional skin),
467 previously shown to be highly and significantly increased in early AD in infants,[3,4] and

IL-33 (in lesional skin), associated with early allergy sensitization ($r<-0.4$, $p<0.05$) (Table E5).[62]

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Discussion

Despite the significant burden of AD in both pediatric and adult populations, the molecular signature of AD skin in different pediatric age-groups has not been fully characterized. We performed comprehensive profiling of AD across age-groups (infants/toddlers with disease duration of <6 months, 0-5y/o; children, 6-11y/o; adolescents, 12-17y/o; and adults ≥18y/o) to identify age-specific versus shared immune and barrier dysregulations, taking into account changes related to normal maturation in healthy individuals. Thus, our results portray the evolution of AD-related changes in the skin throughout life. Moreover, while reports on immune-aging and immune-senescence show up-regulation of proinflammatory markers with aging even in response to non-pathogenic skin challenge,[63-68] the molecular characteristics of normal skin throughout maturation is still elusive. Previous blood studies suggest that infants present over-expression of Tregs[69,70] and a greater Th17 lineage capacity versus adults,[71] and the latter present a greater Th1-development potential.[71] In lymphatic tissues a developmental Th switch was postulated to occur after three years of age.[72] Our healthy control data provide a unique glimpse into these processes in human skin. In our youngest age-groups we found the greatest decrease in the negative regulators (i.e, IL-37 and IL-34, both significantly correlated with age across all age-groups), with the largest increase in Treg-associated markers (IL-10, TGFβ3, CTLA4) displayed in infants, simulating physiologic or pathogenic states of impaired immunity and inflammation.[69,70,73-78] Infants also presented an enriched Th17 pathway compared to other age-groups. These findings may reflect the developing cutaneous immune system in infants, with age-dependent changes in regulatory and protective or defensive

mechanisms.[69-71,79] Analysis presenting key immune and barrier markers as developmental trajectories in both normal and AD skin further reveals how AD alters normal skin development. For example, the greatest down-regulation of terminal differentiation genes (FLG, LOR) in AD versus controls was detected in adults, a result of a gradual up-regulation of these markers in normal skin but not in AD skin, where expression levels increases were less prominent.

Generally, all AD age-groups express a common genomic fingerprint, featuring robust Th2 and Th22 skewing. Common Th2-related markers included IL-13, CCL17/TARC, CCL18/PARC, and IL-4R. Common Th22-related markers included IL-22 and the S100As. The shared immune signature also included genes of general inflammation (e.g, MMP12), T-cell activation/migration (e.g, TNFRSF9, CCR7), and negative regulators (IL-34 and IL-37). We also found positive and significant correlations of Th2/Th22-related markers with SCORAD in all pediatric age-groups, further highlighting their pathogenic significance in AD regardless of age.

Despite these shared molecular abnormalities, major variations between age-groups were detected. Overall, infant and adult AD skin displayed the most significant dysregulations when compared to other AD age-groups. While infants exhibit the greatest Th17-related enrichment (e.g, IL-17A, IL-17F), chronic, long-standing adult AD reveal unique Th1-skewing (e.g, IFNγ, CXCL9/CXCL10/CXCL11). Analyzing publicly available function-based pathway databases,[21] we found the greatest representation of genes related to cell growth and proliferation in infant AD versus controls, suggesting the rapid development of lesional AD in infancy. Thus, in agreement with findings

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517 recently published based on flow-cytometry in AD blood,[80] the adult skin phenotype of
518 AD is achieved only at adulthood.

519 Although the levels of Th17-related products in infant AD skin are comparable to
520 those found in psoriasis,[3,4] we found these expression levels are not persistent in older
521 pediatric age-groups. In fact, enrichment of the Th17 pathway was also detected in
522 normal infant skin, and even after normalization of AD skin by controls of the same age-
523 groups, these results were sustained. In addition, despite pathway-level up-regulation
524 versus controls, AD skin of children exhibits the lowest levels of Th17-related genes as
525 compared to other AD age-groups. These results in our youngest age-group could be
526 attributed to the role of Th17-related cytokines and chemokines in the developing
527 immune system, first encountering multiple extrinsic pathogens early in life. Th17 cells-
528 generated products such as IL-17A and IL-17F, which showed the greatest up-
529 regulations in infant AD, are of pivotal importance for anti-microbial defenses by
530 recruiting and expanding immune response, both cellular and humoral.[81]

531 During early life and soon after AD starts to manifest, the initiation of other atopic
532 diseases, including food-allergy, allergic rhinitis, and asthma, also takes place and has
533 been termed the atopic march.[82] Indeed, in the infant AD age-group, multiple markers
534 associated with these atopic comorbidities were up-regulated significantly versus
535 children and older age-groups. For example, IL-33 and IL-9 are associated with early
536 mite and peanut allergic sensitization,[51,52] IL-33 and its receptor IL-1RL1/IL-33R alter
537 mast cell and basophil functions and induce asthma and allergic rhinitis,[83] IL-5 plays a
538 key role in asthma induction and eosinophilic inflammation,[30,84] and IL-8 was suggested
539 as an important mediator in childhood asthma.[85]

AD skin is also characterized by a disrupted epidermal barrier, with reported abnormalities of epidermal differentiation, keratinocyte junctions, and lipid composition.[25,86] These components were down-regulated in all AD age-groups, most prominently in lesional skin. Although epidermal differentiation markers such as FLG and LCEs were most down-regulated in adults, multiple barrier-related genes were most down-regulated in lesional and non-lesional infant AD, despite the short duration of the disease in this age-group. Positive staining of the entire epidermis by the epidermal hyperplasia marker K16 was most prevalent in non-lesional infant skin versus other age-groups, supporting our gene expression findings. These findings argue that barrier impairment plays an important role at disease onset. Although our results cannot resolve the dispute of whether AD originates as immune vs. barrier dysregulation (i.e, inside-out versus the outside-in hypotheses),[49] these findings suggest both are particularly robust very early in AD development.

Differences in expression levels of various therapeutic targets across ages, most of which are Th2-related markers, may potentially reflect differing responses to therapeutics based on age. For example, the itch cytokine IL-31, known to perpetuate the itch-scratch cycle of AD and other AD-related features, such as epidermal terminal differentiation disruption,[87-91] is a target for inhibition by AD therapeutics. IL-31 antagonists have shown greater improvement of pruritus-related assessments than clinical scores in adult AD and are currently being tested in adolescents.[92,93] The even greater increases that were found in infants and children vs. adolescents and adults suggests additional benefit from IL-31 targeting in younger pediatric patients. On the other hand, IL-13 cytokine is consistently and significantly up-regulated versus controls

563 in all AD age-groups. As such, IL-13 antagonists, which are in clinical trials,[94,95] may
564 benefit AD patients across all ages. Nevertheless, these speculations need to be
565 carefully investigated in age-specific clinical trials to establish whether age-related
566 differences in efficacy exist and determine underlying mechanisms.

567 This study has a few limitations. Despite inclusion of different age-groups, this is
568 not a longitudinal, prospective study and reflects a single time-point measurement of
569 different patients in different ages. *FLG* mutations were not assessed in our cohort, and
570 different races were included. Additionally, the pathogenicity of immune axes and
571 specific cytokines and chemokines presented in different age-groups cannot be further
572 validated without future targeted clinical trials.

573 In conclusion, our findings shed light on the development of AD skin throughout
574 life and offer, for the first time, a broader view of the molecular dysregulations resulting
575 in the AD phenotype during maturation. Moreover, our results provide a glimpse into the
576 initiation of other atopic comorbidities as part of the atopic march. The molecular
577 characterization of adult AD facilitated the development of AD-targeted treatments by an
578 applicable bench-to-bedside approach. Our skin profiling portrays the immune and
579 barrier abnormalities of different pediatric AD age-groups and suggests age-related
580 differences that may guide the development of targeted, age-specific therapies. Specific
581 cytokine and barrier abnormalities characterize different AD age-groups and should be
582 carefully considered when future therapeutic agents are being developed or tested for
583 younger AD patients.

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References

1. Silverberg JI. Public Health Burden and Epidemiology of Atopic Dermatitis. Dermatol Clin 2017; 35:283-9.
2. Tsai TF, Rajagopalan M, Chu CY, Encarnacion L, Gerber RA, Santos-Estrella P, et al. Burden of atopic dermatitis in Asia. J Dermatol 2019; 46:825-34.
3. Esaki H, Brunner PM, Renert-Yuval Y, Czarnowicki T, Huynh T, Tran G, et al. Early-onset pediatric atopic dermatitis is TH2 but also TH17 polarized in skin. J Allergy Clin Immunol 2016.
4. Brunner PM, Israel A, Zhang N, Leonard A, Wen HC, Huynh T, et al. Early-onset pediatric atopic dermatitis is characterized by TH2/TH17/TH22-centered inflammation and lipid alterations. J Allergy Clin Immunol 2018; 141:2094-106.
5. Czarnowicki T, He H, Canter T, Han J, Lefferdink R, Erickson T, et al. Evolution of pathologic T-cell subsets in patients with atopic dermatitis from infancy to adulthood. J Allergy Clin Immunol 2020; 145:215-28.
6. Saeki H, Nakahara T, Tanaka A, Kabashima K, Sugaya M, Murota H, et al. Clinical Practice Guidelines for the Management of Atopic Dermatitis 2016. J Dermatol 2016.
7. Brunner PM, Leung DYM, Guttman-Yassky E. Immunologic, microbial, and epithelial interactions in atopic dermatitis. Ann Allergy Asthma Immunol 2018; 120:34-41.
8. Renert-Yuval Y, Guttman-Yassky E. Monoclonal antibodies for the treatment of atopic dermatitis. Curr Opin Allergy Clin Immunol 2018; 18:356-64.

Journal Pre-proof

9. Davidson WF, Leung DYM, Beck LA, Berin CM, Boguniewicz M, Busse WW, et al. Report from the National Institute of Allergy and Infectious Diseases workshop on "Atopic dermatitis and the atopic march: Mechanisms and interventions". J Allergy Clin Immunol 2019; 143:894-913.

10. Glines KR, Stiff KM, Freeze M, Cline A, Strowd LC, Feldman SR. An update on the topical and oral therapy options for treating pediatric atopic dermatitis. Expert Opin Pharmacother 2019; 20:621-9.

11. de Bruin-Weller M, Thaci D, Smith CH, Reich K, Cork MJ, Radin A, et al. Dupilumab with concomitant topical corticosteroid treatment in adults with atopic dermatitis with an inadequate response or intolerance to ciclosporin A or when this treatment is medically inadvisable: a placebo-controlled, randomized phase III clinical trial (LIBERTY AD CAFE). Br J Dermatol 2018; 178:1083-101.

12. Guttman-Yassky E, Bissonnette R, Ungar B, Suarez-Farinas M, Ardeleanu M, Esaki H, et al. Dupilumab progressively improves systemic and cutaneous abnormalities in atopic dermatitis patients. J Allergy Clin Immunol 2018.

13. Renert-Yuval Y, Guttman-Yassky E. What's New in Atopic Dermatitis. Dermatol Clin 2019; 37:205-13.

14. Cork MJ, Thaci D, Eichenfield LF, Arkwright PD, Hultsch T, Davis JD, et al. Dupilumab in adolescents with uncontrolled moderate-to-severe atopic dermatitis: results from a phase IIa open-label trial and subsequent phase III open-label extension. Br J Dermatol 2019.

15. Simpson EL, Paller AS, Siegfried EC, Boguniewicz M, Sher L, Gooderham MJ, et al. Efficacy and Safety of Dupilumab in Adolescents With Uncontrolled Moderate

631 to Severe Atopic Dermatitis: A Phase 3 Randomized Clinical Trial. JAMA
632 Dermatol 2019.
633 16. Al-Temimi MH, Griffee M, Enniss TM, Preston R, Vargo D, Overton S, et al.
634 When is death inevitable after emergency laparotomy? Analysis of the American
635 College of Surgeons National Surgical Quality Improvement Program database. J
636 Am Coll Surg 2012; 215:503-11.
637 17. Laudanska H, Reduta T, Szmitkowska D. Evaluation of skin barrier function in
638 allergic contact dermatitis and atopic dermatitis using method of the continuous
639 TEWL measurement. Rocz Akad Med Bialymst 2003; 48:123-7.
640 18. Suarez-Farinas M, Ungar B, Noda S, Shroff A, Mansouri Y, Fuentes-Duculan J,
641 et al. Alopecia areata profiling shows TH1, TH2, and IL-23 cytokine activation
642 without parallel TH17/TH22 skewing. J Allergy Clin Immunol 2015; 136:1277-87.
643 19. Tintle S, Shemer A, Suarez-Farinas M, Fujita H, Gilleaudeau P, Sullivan-Whalen
644 M, et al. Reversal of atopic dermatitis with narrow-band UVB phototherapy and
645 biomarkers for therapeutic response. J Allergy Clin Immunol 2011; 128:583-93
646 e1-4.
647 20. Suarez-Farinas M, Tintle SJ, Shemer A, Chiricozzi A, Nograles K, Cardinale I, et
648 al. Nonlesional atopic dermatitis skin is characterized by broad terminal
649 differentiation defects and variable immune abnormalities. J Allergy Clin Immunol
650 2011; 127:954-64 e1-4.
651 21. Fang H, Knezevic B, Burnham KL, Knight JC. XGR software for enhanced
652 interpretation of genomic summary data, illustrated by application to
653 immunological traits. Genome Med 2016; 8:129.

22. Dhingra N, Shemer A, Correa da Rosa J, Rozenblit M, Fuentes-Duculan J, Gittler JK, et al. Molecular profiling of contact dermatitis skin identifies allergen-dependent differences in immune response. J Allergy Clin Immunol 2014; 134:362-72.

23. Guttman-Yassky E, Ungar B, Noda S, Suprun M, Shroff A, Dutt R, et al. Extensive alopecia areata is reversed by IL-12/IL-23p40 cytokine antagonism. J Allergy Clin Immunol 2016; 137:301-4.

24. Ewald DA, Malajian D, Krueger JG, Workman CT, Wang T, Tian S, et al. Meta-analysis derived atopic dermatitis (MADAD) transcriptome defines a robust AD signature highlighting the involvement of atherosclerosis and lipid metabolism pathways. BMC Med Genomics 2015; 8:60.

25. Czarnowicki T, Krueger JG, Guttman-Yassky E. Skin barrier and immune dysregulation in atopic dermatitis: an evolving story with important clinical implications. J Allergy Clin Immunol Pract 2014; 2:371-9; quiz 80-1.

26. Gittler JK, Shemer A, Suarez-Farinas M, Fuentes-Duculan J, Gulewicz KJ, Wang CQ, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. J Allergy Clin Immunol 2012; 130:1344-54.

27. Sun Y, Sheshadri N, Zong WX. SERPINB3 and B4: From biochemistry to biology. Semin Cell Dev Biol 2017; 62:170-7.

28. Smith SH, Jayawickreme C, Rickard DJ, Nicodeme E, Bui T, Simmons C, et al. Tapinarof Is a Natural AhR Agonist that Resolves Skin Inflammation in Mice and Humans. J Invest Dermatol 2017; 137:2110-9.

29. Kato A, Fujii E, Watanabe T, Takashima Y, Matsushita H, Furuhashi T, et al. Distribution of IL-31 and its receptor expressing cells in skin of atopic dermatitis. J Dermatol Sci 2014; 74:229-35.

30. Pelaia C, Paoletti G, Puggioni F, Racca F, Pelaia G, Canonica GW, et al. Interleukin-5 in the Pathophysiology of Severe Asthma. Front Physiol 2019; 10:1514.

31. Toki S, Goleniewska K, Zhang J, Zhou W, Newcomb DC, Zhou B, et al. TSLP and IL-33 reciprocally promote each other's lung protein expression and ILC2 receptor expression to enhance innate type-2 airway inflammation. Allergy 2020.

32. Kumar A, Humphreys TD, Kremer KN, Bramati PS, Bradfield L, Edgar CE, et al. CXCR4 physically associates with the T cell receptor to signal in T cells. Immunity 2006; 25:213-24.

33. Laberge S, Ghaffar O, Boguniewicz M, Center DM, Leung DY, Hamid Q. Association of increased CD4+ T-cell infiltration with increased IL-16 gene expression in atopic dermatitis. J Allergy Clin Immunol 1998; 102:645-50.

34. Guttman-Yassky E, Diaz A, Pavel AB, Fernandes M, Lefferdink R, Erickson T, et al. Use of Tape Strips to Detect Immune and Barrier Abnormalities in the Skin of Children With Early-Onset Atopic Dermatitis. JAMA Dermatol 2019.

35. Guttman-Yassky E, Bissonnette R, Ungar B, Suarez-Farinas M, Ardeleanu M, Esaki H, et al. Dupilumab progressively improves systemic and cutaneous abnormalities in patients with atopic dermatitis. J Allergy Clin Immunol 2019; 143:155-72.

36. Sanyal RD, Pavel AB, Glickman J, Chan TC, Zheng X, Zhang N, et al. Atopic dermatitis in African American patients is TH2/TH22-skewed with TH1/TH17 attenuation. Ann Allergy Asthma Immunol 2019; 122:99-110.e6.

37. Turkish AR, Henneberry AL, Cromley D, Padamsee M, Oelkers P, Bazzi H, et al. Identification of two novel human acyl-CoA wax alcohol acyltransferases: members of the diacylglycerol acyltransferase 2 (DGAT2) gene superfamily. J Biol Chem 2005; 280:14755-64.

38. Charfeddine C, Dallali H, Abdessalem G, Ghedira K, Hamdi Y, Elouej S, et al. Identification of a CDH12 potential candidate genetic variant for an autosomal dominant form of transgrediens and progrediens palmoplantar keratoderma in a Tunisian family. J Hum Genet 2020; 65:397-410.

39. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol 2011; 127:773-86 e1-7.

40. Vasileva E, Citi S. The role of microtubules in the regulation of epithelial junctions. Tissue Barriers 2018; 6:1539596.

41. Abbas Zadeh S, Mlitz V, Lachner J, Golabi B, Mildner M, Pammer J, et al. Phylogenetic profiling and gene expression studies implicate a primary role of PSORS1C2 in terminal differentiation of keratinocytes. Exp Dermatol 2017; 26:352-8.

42. Zhang G, Yang J, Li P, Cao J, Nie H. Rosiglitazone inhibits HMC-1 cell migration and adhesion through a peroxisome proliferator-activated receptor gamma-dependent mechanism. Iran J Allergy Asthma Immunol 2014; 13:11-8.

722 43. Kovarova M, Wassif CA, Odom S, Liao K, Porter FD, Rivera J. Cholesterol
723 deficiency in a mouse model of Smith-Lemli-Opitz syndrome reveals increased
724 mast cell responsiveness. J Exp Med 2006; 203:1161-71.
725 44. Sato T, Akimoto N, Kitamura K, Kurihara H, Hayashi N, Ito A. Adapalene
726 suppresses sebum accumulation via the inhibition of triacylglycerol biosynthesis
727 and perilipin expression in differentiated hamster sebocytes in vitro. J Dermatol
728 Sci 2013; 70:204-10.
729 45. Gao G, Chen FJ, Zhou L, Su L, Xu D, Xu L, et al. Control of lipid droplet fusion
730 and growth by CIDE family proteins. Biochim Biophys Acta Mol Cell Biol Lipids
731 2017; 1862:1197-204.
732 46. Seo S, Yoon WS, Cho Y, Park SH, Choung JT, Yoo Y. Leptin and Atopic
733 Dermatitis in Korean Elementary School Children. Iran J Allergy Asthma Immunol
734 2016; 15:138-44.
735 47. Vandeghinste N, Klattig J, Jagerschmidt C, Lavazais S, Marsais F, Haas JD, et
736 al. Neutralization of IL-17C Reduces Skin Inflammation in Mouse Models of
737 Psoriasis and Atopic Dermatitis. J Invest Dermatol 2018.
738 48. Guttman-Yassky E, Krueger JG. IL-17C: A Unique Epithelial Cytokine with
739 Potential for Targeting across the Spectrum of Atopic Dermatitis and Psoriasis. J
740 Invest Dermatol 2018; 138:1467-9.
741 49. Silverberg NB, Silverberg JI. Inside out or outside in: does atopic dermatitis
742 disrupt barrier function or does disruption of barrier function trigger atopic
743 dermatitis? Cutis 2015; 96:359-61.

Journal Pre-proof

744 50. Beck LA, Thaci D, Hamilton JD, Graham NM, Bieber T, Rocklin R, et al.
745 Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. N Engl
746 J Med 2014; 371:130-9.
747 51. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, et
748 al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and
749 peanut allergic sensitization. J Allergy Clin Immunol 2013; 131:187-200 e1-8.
750 52. Brough HA, Cousins DJ, Munteanu A, Wong YF, Sudra A, Makinson K, et al. IL-9
751 is a key component of memory TH cell peanut-specific responses from children
752 with peanut allergy. J Allergy Clin Immunol 2014; 134:1329-38 e10.
753 53. Cayrol C, Girard JP. Interleukin-33 (IL-33): A nuclear cytokine from the IL-1
754 family. Immunol Rev 2018; 281:154-68.
755 54. Alexander H, Brown S, Danby S, Flohr C. Research Techniques Made Simple:
756 Transepidermal Water Loss Measurement as a Research Tool. J Invest Dermatol
757 2018; 138:2295-300 e1.
758 55. Nograles KE, Zaba LC, Shemer A, Fuentes-Duculan J, Cardinale I, Kikuchi T, et
759 al. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic
760 dermatitis despite reduced IL-17-producing TH17 T cells. J Allergy Clin Immunol
761 2009; 123:1244-52 e2.
762 56. Morita E, Takahashi H, Niihara H, Dekio I, Sumikawa Y, Murakami Y, et al.
763 Stratum corneum TARC level is a new indicator of lesional skin inflammation in
764 atopic dermatitis. Allergy 2010; 65:1166-72.

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765 57. Kagami S, Saeki H, Komine M, Kakinuma T, Tsunemi Y, Nakamura K, et al.
766 Interleukin-4 and interleukin-13 enhance CCL26 production in a human
767 keratinocyte cell line, HaCaT cells. Clin Exp Immunol 2005; 141:459-66.
768 58. Ito T, Wang YH, Duramad O, Hori T, Delespesse GJ, Watanabe N, et al. TSLP-
769 activated dendritic cells induce an inflammatory T helper type 2 cell response
770 through OX40 ligand. J Exp Med 2005; 202:1213-23.
771 59. Floudas A, Saunders SP, Moran T, Schwartz C, Hams E, Fitzgerald DC, et al. IL-
772 17 Receptor A Maintains and Protects the Skin Barrier To Prevent Allergic Skin
773 Inflammation. J Immunol 2017; 199:707-17.
774 60. Nakazato J, Kishida M, Kuroiwa R, Fujiwara J, Shimoda M, Shinomiya N. Serum
775 levels of Th2 chemokines, CCL17, CCL22, and CCL27, were the important
776 markers of severity in infantile atopic dermatitis. Pediatr Allergy Immunol 2008;
777 19:605-13.
778 61. Ito Y, Adachi Y, Makino T, Higashiyama H, Fuchizawa T, Shimizu T, et al.
779 Expansion of FOXP3-positive CD4+CD25+ T cells associated with disease
780 activity in atopic dermatitis. Ann Allergy Asthma Immunol 2009; 103:160-5.
781 62. Yi L, Cheng D, Zhang K, Huo X, Mo Y, Shi H, et al. Intelectin contributes to
782 allergen-induced IL-25, IL-33, and TSLP expression and type 2 response in
783 asthma and atopic dermatitis. Mucosal Immunol 2017; 10:1491-503.
784 63. Zhou L, Leonard A, Pavel AB, Malik K, Raja A, Glickman J, et al. Age-specific
785 changes in the molecular phenotype of patients with moderate-to-severe atopic
786 dermatitis. J Allergy Clin Immunol 2019; 144:144-56.

Journal Pre-proof

64. Ventura MT, Casciaro M, Gangemi S, Buquicchio R. Immunosenescence in aging: between immune cells depletion and cytokines up-regulation. Clin Mol Allergy 2017; 15:21.

65. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 2000; 51:245-70.

66. Giuliani N, Sansoni P, Girasole G, Vescovini R, Passeri G, Passeri M, et al. Serum interleukin-6, soluble interleukin-6 receptor and soluble gp130 exhibit different patterns of age- and menopause-related changes. Exp Gerontol 2001; 36:547-57.

67. Agrawal A, Agrawal S, Cao JN, Su H, Osann K, Gupta S. Altered innate immune functioning of dendritic cells in elderly humans: a role of phosphoinositide 3-kinase-signaling pathway. J Immunol 2007; 178:6912-22.

68. Vukmanovic-Stejic M, Chambers ES, Suarez-Farinas M, Sandhu D, Fuentes-Duculan J, Patel N, et al. Enhancement of cutaneous immunity during aging by blocking p38 mitogen-activated protein (MAP) kinase-induced inflammation. J Allergy Clin Immunol 2018; 142:844-56.

69. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. Proc Biol Sci 2015; 282:20143085.

70. Mold JE, Venkatasubrahmanyam S, Burt TD, Michaelsson J, Rivera JM, Galkina SA, et al. Fetal and adult hematopoietic stem cells give rise to distinct T cell lineages in humans. Science 2010; 330:1695-9.

71. Black A, Bhaumik S, Kirkman RL, Weaver CT, Randolph DA. Developmental regulation of Th17-cell capacity in human neonates. Eur J Immunol 2012; 42:311-9.

72. Knolle J, Pierau M, Hebel K, Lampe K, Jorch G, Kropf S, et al. Children From the Age of Three Show a Developmental Switch in T-Cell Differentiation. Front Immunol 2020; 11:1640.

73. Pan Y, Wen X, Hao D, Wang Y, Wang L, He G, et al. The role of IL-37 in skin and connective tissue diseases. Biomed Pharmacother 2020; 122:109705.

74. Esaki H, Ewald DA, Ungar B, Rozenblit M, Zheng X, Xu H, et al. Identification of novel immune and barrier genes in atopic dermatitis by means of laser capture microdissection. J Allergy Clin Immunol 2015; 135:153-63.

75. Okamura T, Sumitomo S, Morita K, Iwasaki Y, Inoue M, Nakachi S, et al. TGF-beta3-expressing CD4+CD25(-)LAG3+ regulatory T cells control humoral immune responses. Nat Commun 2015; 6:6329.

76. Fujio K, Yamamoto K, Okamura T. Overview of LAG-3-Expressing, IL-10-Producing Regulatory T Cells. Curr Top Microbiol Immunol 2017; 410:29-45.

77. Lai C, August S, Behar R, Polak M, Ardern-Jones M, Theaker J, et al. Characteristics of immunosuppressive regulatory T cells in cutaneous squamous cell carcinomas and role in metastasis. Lancet 2015; 385 Suppl 1:S59.

78. Pitt JM, Stavropoulos E, Redford PS, Beebe AM, Bancroft GJ, Young DB, et al. Blockade of IL-10 signaling during bacillus Calmette-Guerin vaccination enhances and sustains Th1, Th17, and innate lymphoid IFN-gamma and IL-17

responses and increases protection to Mycobacterium tuberculosis infection. J Immunol 2012; 189:4079-87.

79. Xu R, Sun HF, Williams DW, Jones AV, Al-Hussaini A, Song B, et al. IL-34 Suppresses Candida albicans Induced TNFalpha Production in M1 Macrophages by Downregulating Expression of Dectin-1 and TLR2. J Immunol Res 2015; 2015:328146.

80. Czarnowicki T, He H, Canter T, Han J, Lefferdink R, Erickson T, et al. Evolution of pathologic T-cell subsets in atopic dermatitis from infancy to adulthood. J Allergy Clin Immunol 2019.

81. Ochs HD, Oukka M, Torgerson TR. TH17 cells and regulatory T cells in primary immunodeficiency diseases. J Allergy Clin Immunol 2009; 123:977-83; quiz 84-5.

82. Simpson EL, Eichenfield LF, Ellis CN, Mancini AJ, Paller AS. Current issues in atopic comorbidities and preventing the atopic march. Semin Cutan Med Surg 2012; 31:S6-9.

83. Saluja R, Ketelaar ME, Hawro T, Church MK, Maurer M, Nawijn MC. The role of the IL-33/IL-1RL1 axis in mast cell and basophil activation in allergic disorders. Mol Immunol 2015; 63:80-5.

84. Xu X, Xu JF, Zheng G, Lu HW, Duan JL, Rui W, et al. CARD9(S12N) facilitates the production of IL-5 by alveolar macrophages for the induction of type 2 immune responses. Nat Immunol 2018; 19:547-60.

85. Charrad R, Kaabachi W, Rafrafi A, Berraies A, Hamzaoui K, Hamzaoui A. IL-8 Gene Variants and Expression in Childhood Asthma. Lung 2017; 195:749-57.

852 86. Leung DY. New insights into atopic dermatitis: role of skin barrier and immune
853 dysregulation. Allergol Int 2013; 62:151-61.
854 87. Sonkoly E, Muller A, Lauerma AI, Pivarcsi A, Soto H, Kemeny L, et al. IL-31: a
855 new link between T cells and pruritus in atopic skin inflammation. J Allergy Clin
856 Immunol 2006; 117:411-7.
857 88. Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M,
858 Presnell SR, et al. Interleukin 31, a cytokine produced by activated T cells,
859 induces dermatitis in mice. Nat Immunol 2004; 5:752-60.
860 89. Kim BE, Leung DY, Boguniewicz M, Howell MD. Loricrin and involucrin
861 expression is down-regulated by Th2 cytokines through STAT-6. Clin Immunol
862 2008; 126:332-7.
863 90. Leung DY, Guttman-Yassky E. Deciphering the complexities of atopic dermatitis:
864 shifting paradigms in treatment approaches. J Allergy Clin Immunol 2014;
865 134:769-79.
866 91. Cornelissen C, Marquardt Y, Czaja K, Wenzel J, Frank J, Luscher-Firzlaff J, et al.
867 IL-31 regulates differentiation and filaggrin expression in human organotypic skin
868 models. J Allergy Clin Immunol 2012; 129:426-33, 33 e1-8.
869 92. Kabashima K, Furue M, Hanifin JM, Pulka G, Wollenberg A, Galus R, et al.
870 Nemolizumab in patients with moderate-to-severe atopic dermatitis:
871 Randomized, phase II, long-term extension study. J Allergy Clin Immunol 2018;
872 142:1121-30 e7.

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873 93. Mihara R, Kabashima K, Furue M, Nakano M, Ruzicka T. Nemolizumab in
874 moderate to severe atopic dermatitis: An exploratory analysis of work productivity
875 and activity impairment in a randomized phase II study. J Dermatol 2019.
876 94. Simpson EL, Flohr C, Eichenfield LF, Bieber T, Sofen H, Taieb A, et al. Efficacy
877 and safety of lebrikizumab (an anti-IL-13 monoclonal antibody) in adults with
878 moderate-to-severe atopic dermatitis inadequately controlled by topical
879 corticosteroids: A randomized, placebo-controlled phase II trial (TREBLE). J Am
880 Acad Dermatol 2018.
881 95. Wollenberg A, Howell MD, Guttman-Yassky E, Silverberg JI, Kell C, Ranade K,
882 et al. Treatment of atopic dermatitis with tralokinumab, an anti-IL-13 mAb. J
883 Allergy Clin Immunol 2018.

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Table 1. Patient characteristics

	Infant AD n=17	Infant controls n=13	P value	Child AD n=10	Child controls n=13	P value	Adolescent AD n=14	Adolescent controls n=5	P value	Adult AD n=13	Adult controls n=15	P value
Age (y), mean (SD)	1.2 (1.2)	1.2 (0.8)	0.9	7.8 (2.1)	7.9 (2.3)	0.94	14.8 (1.7)	14.2 (1.8)	0.5	45.2 (14.6)	35.6 (10.0)	0.06
Female sex, n (%)	8 (47.0)	7 (53.8)	1	6 (60.0)	7 (53.8)	1	6 (42.8)	3 (60.0)	0.6	7 (53.8)	8 (53.3)	1
Clinical severity measures												
SCORAD, mean (SD)	58.1 (12.4)	NA	NA	64.3 (15.7)	NA	NA	62.1 (20.0)	NA	NA	65.1 (13.3)	NA	NA
EASI, mean (SD)	15.3 (8.8)	NA	NA	19.8 (12.6)	NA	NA	27.4 (22.8)	NA	NA	NA	NA	NA
TEWL, lesional, g/h/m2 mean (SD)	32.9 (13.8)	NA	NA	36.8 (17.6)	NA	NA	51.2 (25.9)	NA	NA	NA	NA	NA
TEWL, non-lesional, g/h/m2 mean (SD)	16.2 (8.0)	NA	NA	25.6 (10.5)	NA	NA	25.6 (13.5)	NA	NA	NA	NA	NA
Pruritus ADQ (SD)	17.2 (9.3)	NA	NA	26.8 (5.5)	NA	NA	22.0 (9.4)	NA	NA	NA	NA	NA
IgE, IU/ml, mean (SD)	5830.6 (858.3)	NA	NA	1072 (915.8)	NA	NA	6136 (5943.6)	NA	NA	230 (291.0)	NA	NA

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Table 2. Spearman correlations of skin biomarkers with AD clinical severity based on SCORAD, r>0.4/<-0.4 and p<0.05

SCORAD															
LESIONAL SKIN								NON-LESIONAL SKIN							
INFANTS			CHILDREN			ADOLESCENTS			INFANTS			CHILDREN			ADOLESCENTS		
Marker	r	P value	Marker	r	P value	Marker	r	P value	Marker	r	P value	Marker	r	P value	Marker	r	P value
IL21	0.574	0.016	CCL21	0.842	0.004	CAMP	0.697	0.007	IL26	0.493	0.044	IL9	0.870	0.002	CXCL1	0.925	<0.001
IL34	-0.575	0.016	IL1B	0.830	0.006	K16	0.574	0.035	IL1RL1	0.485	0.049	IL19	0.850	0.006	S100A12	0.859	<0.001
PPL	-0.569	0.017	IL31	0.827	0.003	IL23A	0.560	0.040	IL37	-0.483	0.049	DEFB4B	0.800	0.014	DEFB4B	0.842	<0.001
LCN2	-0.510	0.036	IFNG	0.77	0.014	S100A8	0.552	0.044				IL17F	0.749	0.020	IL1B	0.789	0.001
CCL22	-0.499	0.041	MMP12	0.745	0.018	IL4R	0.538	0.050				PPL	0.700	0.043	S100A8	0.771	0.002
			IL22	0.733	0.021	FLG	-0.622	0.020				CLDN8	-0.883	0.003	CXCL11	0.758	0.003
			CXCL1	0.721	0.024							CLDN1	-0.833	0.008	S100A9	0.754	0.003
			IL4R	0.721	0.024							IL33	-0.783	0.017	CCL17	0.754	0.003
			IL15	0.709	0.028							IL34	-0.717	0.037	KRT16	0.749	0.003
			CXCL11	0.697	0.031										IL20	0.745	0.003
			OX40	0.697	0.031										CAMP	0.723	0.005
			IL10	0.697	0.031										CCL19	0.719	0.005
			IL17A	0.693	0.026										IL10	0.697	0.007
			CCL18	0.685	0.035										IL22	0.697	0.007
			CCL13	0.685	0.035										IL23A	0.692	0.008
			CCL7	0.673	0.039										CXCL9	0.675	0.010
			IL13	0.661	0.044										CCL7	0.666	0.011
			FOXP3	0.648	0.049										PI3	0.657	0.013
			CLDN1	-0.709	0.028										IL19	0.648	0.015
															CCL20	0.644	0.015
															LCN2	0.644	0.015
															CXCL8	0.640	0.016
															MMP12	0.635	0.017
															IFNG	0.631	0.018
															CCL13	0.626	0.019
															CXCL10	0.626	0.019
															KYNU	0.607	0.021
															IL31	0.603	0.022
															S100A7	0.596	0.028
															IL13	0.591	0.029
															CCL18	0.569	0.037
															CCL22	0.565	0.038

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887 **Figure Legends**

888 **Figure 1**

889 Immune genes. Summary heatmap of immune genes from multiple pathways in lesional
890 and non-lesional AD versus normal skin across age-groups using RNA-seq, by criteria
891 of fold change (FCH)>2 and false discovery rate (FDR)<0.05. Samples are sorted by
892 hierarchical clustering. Tables show DEGs presenting comparable (*top*) and differential
893 (*bottom*) expression across AD age-groups. N, normal; NL, non-lesional; LS, lesional.
894 ***FDR<0.001, **FDR<0.01, *FDR<0.05, $^{+}$FDR<0.1.

895

896 **Figure 2**

897 Gene-set variation analyses (GSVA) for major T-helper immune pathways (**A-E)**. Red
898 bars represent means. *Black symbols*: significance of comparison to normal*; red*
899 *symbols:* significance of comparison between lesional and non-lesional skin; *symbols in*
900 *other colors*: significance of comparison between respective groups. N, normal; NL,
901 non-lesional; LS, lesional. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1.

902

903 **Figure 3**

904 Epidermal barrier-related genes. Summary heatmap of barrier-associated genes in
905 lesional and non-lesional AD versus normal skin across age-groups using RNA-seq, by
906 criteria of fold change (|FCH|)>2 and false discovery rate (FDR)<0.05. Samples are
907 sorted by hierarchical clustering. N, normal; NL, non-lesional; LS, lesional.
908 ***FDR<0.001, **FDR<0.01, *FDR<0.05, $^{+}$FDR<0.1.

909

Figure 4

qRT-PCR analysis of selected inflammatory genes in skin of different AD age-groups and age-appropriate controls. Values show log2 expression and are presented as means ± SEMs. Red bars represent means. *Black symbols*: significance of comparison to normal*; red symbols:* significance of comparison between lesional and non-lesional skin; *symbols in other colors*: significance of comparison between respective groups. CON, controls; NL, non-lesional; LS, lesional. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1.

Figure 5

qRT-PCR analysis of selected inflammatory and barrier genes of different AD and normal skin age-groups presented as line charts, representing developmental trajectories. Values show log2 normalized expression and are presented as means ± SEMs. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1.

Figure 6

Immunohistochemistry studies assessing AD across pediatric and adult age-groups. **A-B,** Representative immunohistochemistry images of keratin 16 (K16) and filaggrin (FLG) staining at 10x magnification in AD age-groups and age-appropriate controls. **C-D,** Corresponding mRNA expression levels by qRT-PCR. Values show log2 expression presented as means ± SEMs. Red bars represent means. **E-L,** Boxplots depicting cell counts of skin infiltrates in non-lesional/lesional AD and healthy skin across age-groups. Values are presented as means ± SEMs. *Black symbols*: significance of comparison to normal*; red symbols:* significance of comparison between lesional and non-lesional

933 skin; *symbols in other colors*: significance of comparison between respective groups.

934 NL, non-lesional; LS, lesional. ***P<0.001, **P<0.01, *P<0.05, ^{+}P<0.1.

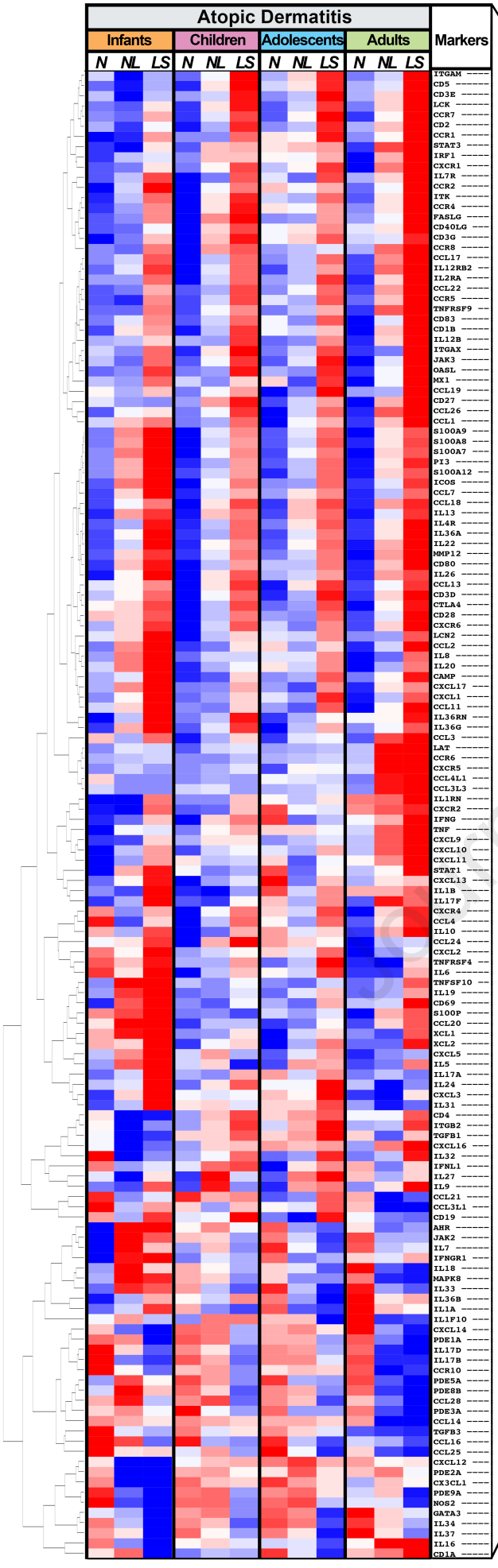
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FIG.1



SHARED DEGs

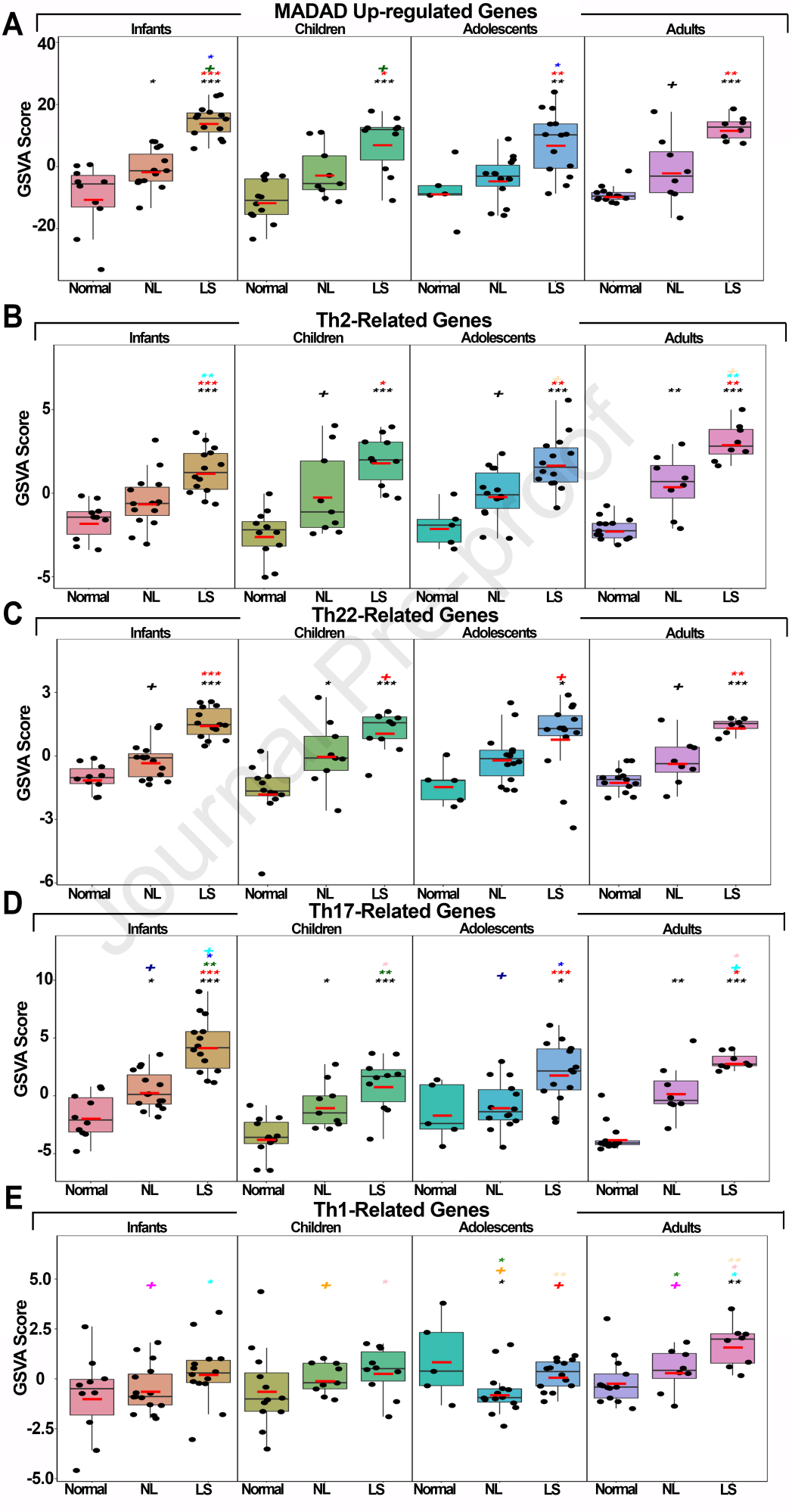
Markers	Infants		Children		Adolescents		Adults	
	NLvsN	LSvsN	NLvsN	LSvsN	NLvsN	LSvsN	NLvsN	LSvsN
CCR7	-1.14	2.10*	1.95	4.77***	1.97	5.27**	2.38*	7.94***
CXCR1	1.16	2.52*	1.98	4.68**	1.41	3.61*	9.44***	18.60***
CCL17	1.48	4.76**	3.83	12.60***	1.36	4.64*	5.58**	15.20***
IL12RB2	1.56	3.17***	2.11	3.87***	1.52	2.46*	2.88***	4.85***
IL2RA	-1.00	2.34*	2.14	4.21***	1.09	2.81*	2.51*	7.57***
CCL22	1.16	3.08**	1.45	4.51***	1.56	2.90*	3.22**	10.70***
CCR5	-1.17	2.09*	1.42	2.66**	-1.13	1.67	1.73	3.56***
TNFRSF9	1.00	5.34**	2.99	5.99**	1.52	3.58	9.80***	36.30***
ITGAX	-1.18	1.56+	1.75	3.01***	1.25	2.18*	1.19	3.22***
OASL	1.16	6.07***	1.20	6.50***	-1.15	4.67*	2.72+	12.50***
CCL26	1.89+	2.10*	1.66	3.71**	2.33	4.87**	4.30***	6.87***
S100A9	18.80***	171.00***	17.90**	109.00***	6.98	60.60***	33.60***	215.00***
S100A8	15.10***	144.00***	13.00*	69.60***	4.40	45.20***	19.60***	125.00***
S100A7	17.30***	86.90***	22.00***	90.60***	9.60+	43.80***	45.90***	173.00***
PI3	6.44**	47.80***	3.67	16.70***	2.38	17.10**	10.20**	62.10***
S100A12	7.44**	130.00***	5.23	23.30***	5.80	55.60***	18.40***	73.00***
ICOS	3.14**	5.55***	2.66	4.82***	1.02	2.85+	2.04	5.23***
CCL7	4.16**	33.40***	3.20	6.44**	1.98	3.47+	2.51	9.34***
CCL18	4.33**	16.40***	13.40**	52.30***	9.90+	29.50***	16.80***	55.50***
IL13	6.09***	11.00***	5.63*	7.99***	4.26	7.06**	3.38*	12.70***
IL4R	1.32	3.00***	1.54	2.82***	1.54	2.36**	1.82**	3.75***
IL36A	7.39**	136.00***	3.74	39.40***	5.36	26.80**	12.40**	136.00***
IL22	3.86*	54.80***	6.99+	20.10***	4.90	18.40***	16.10***	77.90***
MMP12	8.47**	36.60***	5.44	24.90***	2.54	12.00*	47.20***	251.00***
CD80	4.48***	13.10***	3.14	8.10***	-1.01	3.01+	5.29***	19.70***
IL26	4.70**	28.50***	6.99*	17.60***	1.27	2.94	7.95***	13.90***
CTLA4	1.15	2.03+	2.26	5.46***	1.26	3.54*	2.82*	6.75***
LCN2	1.37	5.40***	3.71	6.01**	-1.05	1.88	1.12	3.27*
IL20	3.32+	19.10***	2.96	4.92*	-1.66	1.76	5.21*	22.30***
IL36G	4.01***	13.70***	1.99	6.32***	2.96	5.92**	1.75	4.14**
IL19	50.30***	906.00***	1.36	4.69+	4.42	16.40**	2.32	22.00***
IL34	-2.15**	-7.06***	-1.19	-2.78***	-1.22	-4.18***	-1.79+	-2.92***
IL37	-1.42	-9.73***	-2.21	-3.81*	-1.87	-8.38**	-5.06**	-12.60***

DIVERSELY EXPRESSED DEGs

Markers	Infants		Children		Adolescents		Adults	
	NLvsN	LSvsN	NLvsN	LSvsN	NLvsN	LSvsN	NLvsN	LSvsN
STAT3	-1.13	1.50*	1.50	1.53*	1.07	1.15	1.92***	2.42***
IRF1	1.31	1.41+	1.36	1.38	-1.06	1.12	1.99***	3.65***
CCR4	1.59	3.85**	5.14*	12.00***	-1.19	1.34	1.80	4.74**
IL12B	1.06	1.56	-1.07	2.84	1.14	2.68	2.80	10.90***
JAK3	-1.23	1.75*	1.50	2.49***	1.43	2.67**	1.78*	3.63***
MX1	-1.09	1.52	-1.17	1.24	-1.64	1.40	1.88	3.96***
CCL19	-1.19	1.10	1.27	1.52	1.32	2.58+	1.02	2.38*
CCL1	1.98	2.86+	1.37	5.57*	7.10	13.40**	5.13*	25.50***
CCL13	1.14	1.67	3.97+	7.44***	2.62	5.10**	1.88	4.24**
IL8	5.43**	40.30***	2.13	2.26	1.12	3.04	2.23	18.70***
CAMP	1.42	11.60***	1.35	4.14*	1.32	5.03*	2.16	7.96***
CXCL1	2.24	22.00***	1.38	3.44+	-1.66	4.27+	3.19+	13.00***
CCR6	1.17	1.64	-1.10	1.68	1.10	1.37	75.20***	97.90***
CXCR5	1.31	1.05	1.15	1.43	2.18	1.99	4.72**	5.78***
CCL4L1	-2.06*	-1.98+	1.17	1.10	-1.10	-1.10	5.71***	15.50***
IL1RN	-1.08	1.87**	-1.00	1.30	-1.11	1.03	1.02	1.36
CXCL9	1.04	2.37	1.52	1.48	1.21	1.35	2.19	6.39**
STAT1	1.79*	2.27**	-1.18	-1.11	-1.60	-1.29	1.08	1.58
IL1B	-1.22	3.64*	-1.09	1.46	-1.97	-1.25	-1.03	1.20
CXCL10	2.94	6.03*	2.01	2.30	-1.38	1.64	2.94	10.20**
CXCL11	2.22	4.21*	-1.37	-1.29	-2.07	-1.26	1.47	4.13*
IL17F	1.37	3.44**	-1.08	1.60	-1.47	1.12	2.74+	2.23
TNFRSF4	-1.34	1.22	1.03	1.61	1.24	3.01*	-1.27	2.02+
CD69	2.16+	3.17**	-1.10	1.23	-1.16	1.37	1.03	1.89
CCL20	3.52*	4.64**	2.09	2.18	1.51	3.11	3.54+	6.60**
IL5	2.16	3.85**	1.34	-1.71	2.32	2.22	1.21	2.64+
IL17A	1.44	8.46***	-1.08	1.79	-1.63	1.04	1.25	-1.05
IL24	1.26	7.16***	2.33	4.81**	1.06	3.96*	-3.76*	-1.26
CXCL3	1.64	3.03*	-1.04	1.01	-1.08	1.48	-1.15	1.57
IL31	1.28	3.26**	1.01	-1.12	-1.02	1.31	-1.34	-1.10
AHR	2.41***	2.30***	1.18	-1.02	-1.45	-1.63	-1.28	-1.20
JAK2	3.83***	3.02***	-1.05	-1.42	-1.49	-2.07+	-1.79+	-1.84+
IL7	4.75***	2.55*	1.54	-1.02	-1.85	-2.85+	-1.74	-1.60
IL33	2.54**	2.17*	-1.46	-1.95+	-1.95	-3.05*	-2.30*	-2.70**
CCL14	-1.64+	-3.38***	-1.36	-2.10*	-1.04	-1.82	-337.00***	-364.00***
CXCL12	-2.39***	-2.29**	1.21	1.24	1.16	-1.11	-1.18	-1.14
IL16	-1.40*	-1.93***	1.05	-1.04	1.01	-1.21	1.40*	1.45*

FIG 2

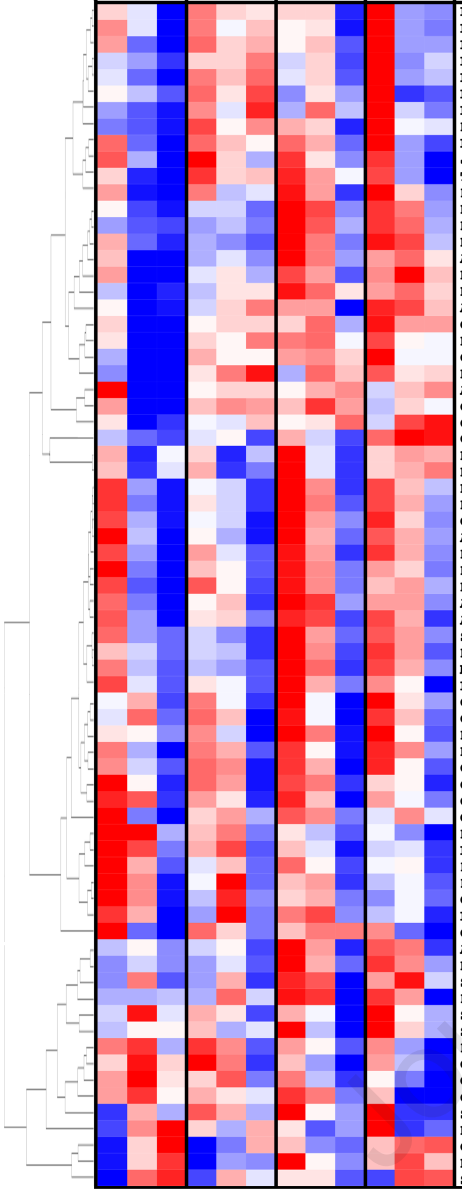
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FIG 3

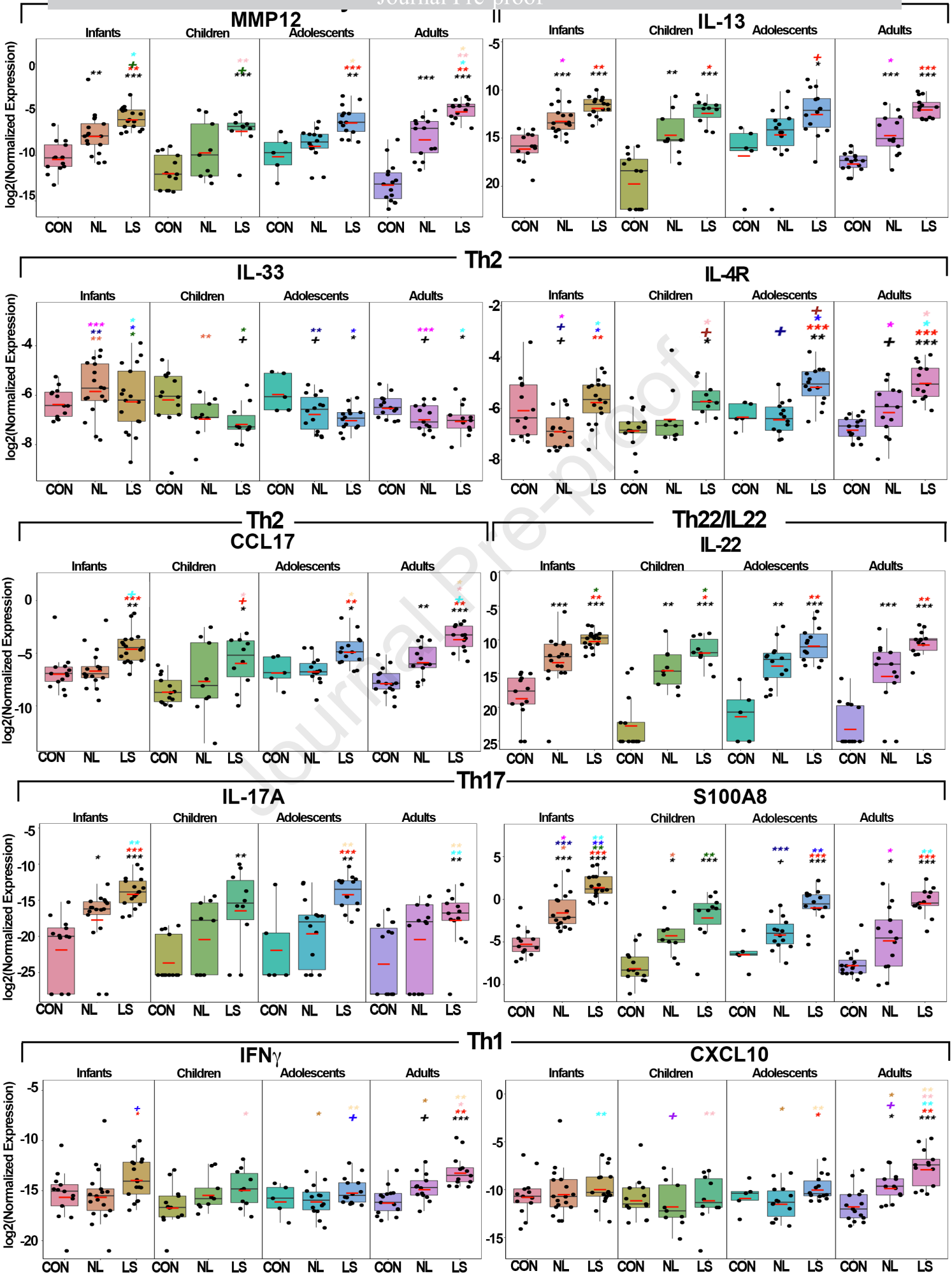
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Markers	Atopic Dermatitis							
	Infants		Children		Adolescents		Adults	
	NLvsN	LSvsN	NLvsN	LSvsN	NLvsN	LSvsN	NLvsN	LSvsN
LCE2B -----:	-1.25	-2.64*	-1.23	-1.31	1.02	-2.13	-4.38**	-4.66**
LCE1B -----:	-1.51	-3.02*	-1.50	-1.26	1.07	-2.04	-4.07**	-4.57**
LCE1C -----:	-2.05	-3.38**	-1.34	-1.22	1.17	-1.71	-4.09**	-4.12**
LCE2C -----:	-1.13	-1.46	-1.00	1.21	1.23	-1.36	-3.61*	-3.07*
LCE2D -----:	-1.33	-1.72	-1.16	1.04	1.21	-1.41	-3.55*	-3.22*
LCE1F -----:	-1.23	-1.60	-1.36	1.11	1.41	1.02	-4.66**	-4.25**
LCE1E -----:	-1.23	-1.57	-1.50	1.40	1.93	1.05	-3.36	-4.29*
FLG --------:	-1.13	-1.38	-1.73	-1.25	-1.14	-2.56	-3.06+	-3.39*
LOR --------:	-4.26**	-9.61***	-1.57	-2.09	-1.43	-4.43*	-5.77**	-8.83***
JAM2 -------:	-1.46*	-1.94***	-1.39	-1.64*	-1.32	-1.62+	-1.59*	-2.05***
TJP3 -------:	-1.71**	-1.82**	-1.40	-1.34	-1.29	-1.63+	-1.78**	-2.49***
PSORS1C2 --:	-3.23**	-3.45**	-1.95	-1.76	-1.63	-4.85*	-2.36+	-4.25**
FADS2 -----:	-4.81**	-6.97**	1.04	-2.19	-3.59	-35.90***	-1.66	-10.40***
FADS1 -----:	-1.54	-1.70	1.19	-1.22	-2.91	-11.40***	-1.29	-4.77**
HAO2 -------:	-6.29*	-10.40**	-1.37	-2.11	-4.20	-33.40***	-1.56	-10.30**
AGPAT3 ----:	-1.79**	-1.76**	1.11	-1.06	-1.53	-2.32**	1.11	-1.13
DGAT2 -----:	-2.74***	-3.30***	1.14	-1.15	-1.25	-2.58**	1.53	-1.19
DHCR7 -----:	-3.23***	-1.71*	1.36	1.36	-1.34	-2.03+	1.20	-1.24
ACER1 -----:	-1.75	-1.72	1.17	1.42	1.02	-2.35	-1.07	-1.40
GJB3 -------:	-1.83**	-2.19***	1.05	1.05	1.21	-1.27	-1.29	-1.31
DEGS2 -----:	-2.80***	-2.12*	-1.09	1.20	1.03	-1.40	-1.52	-1.60
CLDN11 ----:	-1.79*	-2.29**	-1.30	-1.29	1.04	-1.24	-3.21***	-3.26***
KRT2 -------:	-2.19	-2.36+	1.49	2.35	2.67	1.79	-1.82	-1.72
ANXA6 -----:	-2.62***	-2.68***	1.07	1.06	1.13	1.21	1.19	1.28
CDH5 -------:	-2.47***	-2.19***	1.11	1.05	1.33	1.07	1.22	1.09
CTSD -------:	-2.83***	-1.67*	-1.05	1.19	1.04	1.39	1.73*	1.95**
CLDN23 ----:	-1.81	-2.14	1.15	-2.54	-2.06	-4.69*	3.23*	1.72
KRT34 -----:	-5.31*	-1.61	-4.33	-1.83	-12.00	-31.10***	1.33	1.21
KRT33A ----:	-6.06*	-1.90	-7.55	-4.68	-20.00	-72.60**	1.32	2.04
KRT79 -----:	-9.36**	-28.60***	-1.18	-4.31+	-4.00	-43.00***	-2.60	-6.12*
ELOVL3 ----:	-22.60***	-62.60***	-2.00	-8.14*	-4.26	-60.50***	-2.58	-14.00**
GAL --------:	-19.70**	-101.00***	-1.59	-12.80*	-9.76	-95.50***	-5.93	-36.50***
AWAT1 -----:	-28.90***	-262.00***	-2.99	-14.70*	-9.73	-119.00**	-3.01	-18.00*
FAXDC2 ----:	-2.24***	-3.86***	-1.43	-2.18**	-1.54	-3.73***	-1.42	-2.59***
FA2H -------:	-9.11***	-20.90***	-1.30	-3.17*	-1.98	-6.72**	-1.36	-3.20*
HMGCS2 ----:	-4.99***	-9.44***	-2.01	-5.04**	-1.84	-5.50**	1.13	-1.90
ACOT2 -----:	-1.91**	-2.92***	1.10	-1.64	-1.14	-2.32*	-1.01	-1.61+
ACOX2 -----:	-2.08*	-3.54***	1.05	-1.56	-1.14	-3.09**	-1.29	-2.92***
SOAT1 -----:	-2.60*	-3.16**	-1.33	-1.89	-3.19	-8.19***	-1.33	-2.92*
FAR2 -------:	-1.66	-2.69*	-1.06	-1.96	-3.08	-11.50***	-1.64	-5.02***
MOGAT1 ----:	-3.79+	-8.62**	-1.26	-2.08	-4.38	-52.00***	-2.21	-8.44**
LPIN1 -----:	-1.48+	-1.87**	-1.05	-1.43	-1.68	-2.47**	-1.23	-2.03**
CLDN1 -----:	1.31	-1.71*	-1.52	-2.87***	-2.23	-5.24***	-2.33**	-3.46***
CLDN8 -----:	2.16*	-1.63	-1.38	-5.54***	-2.64	-7.80***	-1.87	-4.35***
FABP7 -----:	-1.12	-2.60+	-2.79	-13.00***	-2.94	-37.70***	-6.58**	-22.50***
KRT77 -----:	-2.45**	-7.04***	-1.32	-3.94***	-2.40	-7.56***	-1.72	-4.43***
CDH12 -----:	-2.04	-3.78**	-1.17	-7.08***	-1.99	-11.50***	-2.80+	-6.52***
CGNL1 -----:	-1.62*	-2.61***	-1.13	-2.22***	-1.11	-2.20**	-1.08	-1.78**
CDH20 -----:	-1.27	-5.71***	-1.36	-3.51**	-1.89	-8.43***	-1.56	-2.59*
ORMDL3 ----:	-1.63***	-2.32***	1.07	-1.18	-1.07	-1.45*	1.18	-1.00
FABP4 -----:	-1.53	-6.10**	1.29	-2.19	-1.42	-2.26	-1.56	-2.77
LPL --------:	-1.75	-5.55**	1.48	-2.54	-1.37	-3.09	1.06	-1.76
PPARG -----:	-2.28*	-4.07***	1.26	-1.32	-1.44	-2.31	1.05	-1.58
PLIN1 -----:	-2.13	-8.32**	3.04	-1.70	1.10	-2.93	1.26	-1.48
CIDEC -----:	-2.90	-12.50**	3.15	-1.24	1.21	-2.02	1.53	-1.13
LEP --------:	-1.68	-7.08*	5.51	-1.23	1.27	-2.86	1.29	-1.92
CLDN5 -----:	-3.44***	-4.71***	-1.36	-2.27+	1.22	1.24	-2.14+	-2.84**
ACSL1 -----:	1.14	-1.11	1.10	-1.32	-1.72	-3.11**	-1.07	-2.02**
ELOVL5 ----:	1.22	-1.01	1.23	-1.17	-2.66	-5.00**	-1.22	-2.18*
SEPP1 -----:	1.42	-1.08	1.27	-1.17	-1.08	-2.08*	1.23	-1.23
PNPLA3 ----:	-1.02	1.02	1.57	1.07	-1.09	-2.95*	-1.25	-2.55**
SCEL -------:	2.57*	1.06	-1.21	-2.41*	-1.35	-4.60**	-2.54*	-3.36**
SPTLC3 ----:	1.09	1.11	-1.22	-1.12	-1.55	-2.43*	-1.45	-1.72+
PLCB4 -----:	1.21	-1.65+	-1.22	-2.43**	-1.24	-1.91	-1.70	-3.09***
CDH19 -----:	1.68+	-1.01	-1.48	-3.63***	-1.52	-2.61*	-1.55	-2.57**
GPAM -------:	2.04	-1.21	1.40	-1.71	-1.78	-2.76	-1.48	-2.39+
CDH10 -----:	2.17	-1.72	-1.36	-2.10	-1.45	-9.92**	-8.52***	-20.10***
SGPP1 -----:	2.69**	1.60	-1.32	-2.42*	-2.30	-3.41*	-5.68***	-5.77***
EREG -------:	2.19	3.37*	-1.40	1.08	-1.63	-1.40	-3.79*	-3.28*
CERS3 -----:	1.60+	2.53***	1.21	1.73+	-1.33	-1.42	1.15	1.16
HMGCS1 ----:	2.21*	3.58***	1.75	1.60	-2.27	-3.32*	1.45	-1.03
SPTLC1 ----:	2.18***	2.43***	1.53	1.29	-1.01	-1.39	1.80*	1.78*

FIG.4

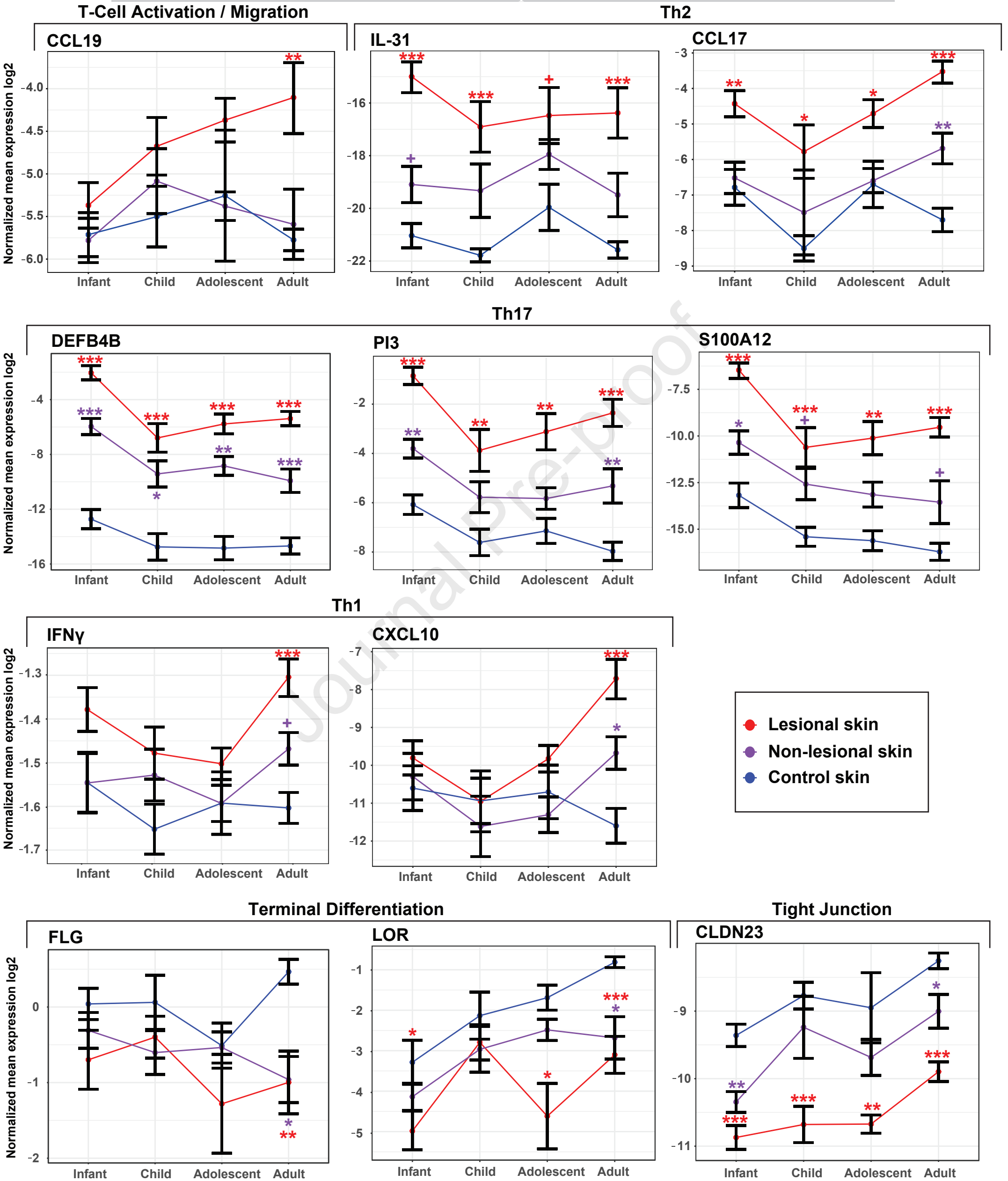
Journal Pre-proof



Journal Pre-proof

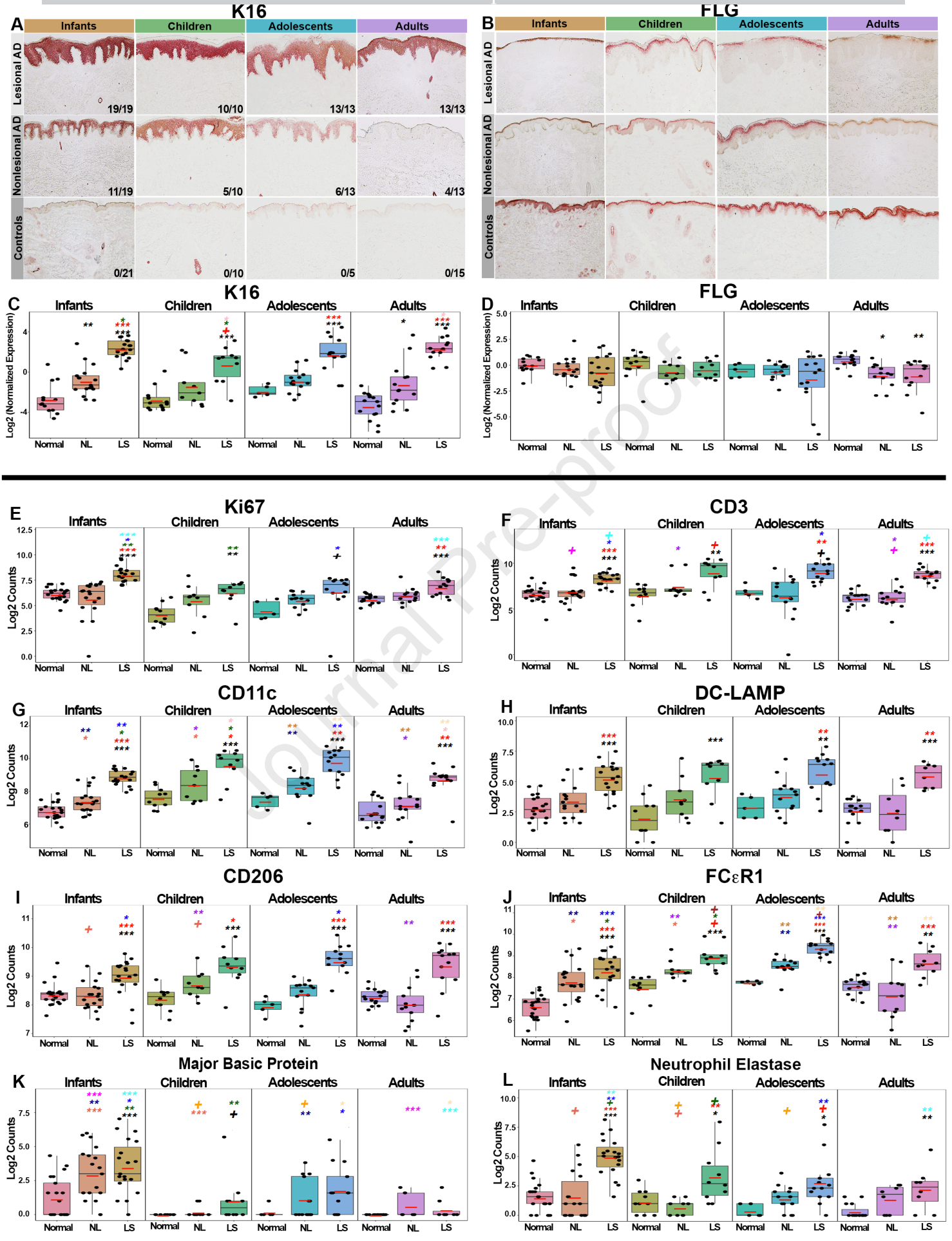
FIG. 5

Journal Pre-proof



Journal Pre-proof

FIG.



Journal Pre-proof