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ADAM17-Deficient Mice Model the Transcriptional Signature of Human Atopic Dermatitis

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TO THE EDITOR

Atopic dermatitis (AD) is an inflammatory skin disease characterized by barrier defect, immune dysregulation, and microbial dysbiosis. AD frequently precedes atopic disease at other barrier tissues, such as food allergies and asthma (Dharmage et al., 2014). Despite the prevalence and impact of the disease, there are still few steroid-sparing treatment options and only one targeted therapy approved by the US Food and Drug Administration (Simpson et al., 2016), reflecting the limits of current knowledge and strategies for intervention.

To advance understanding of pathogenesis and treatment, a robust preclinical animal model is needed. Mice lacking *Adam17* in *Sox9*-expressing tissue including epidermis (*Adam17^{fl/fl} Sox9-Cre*, henceforth A17) have been previously identified as a model for severe human AD based on the eczematous phenotype and spontaneous

staphylococcal dysbiosis of A17 skin (Kobayashi et al., 2015). Although useful, these endpoints capture only the most grossly aberrant features of AD and cannot validate the model for as-yet-unknown mechanisms of pathogenesis. To address these limitations, we analyzed unbiased changes across the entire skin transcriptome in A17 mouse skin. All experiments were performed under an animal study proposal approved by the National Cancer Institute Animal Care and Use Committee.

We compared whole skin and epidermis RNA sequencing of three A17 mice with those of three age-matched wild-type controls. Back skin was enzymatically dissociated to obtain cell suspensions as described previously (Kobayashi et al., 2015). Total mRNA was used to generate cDNA libraries that were sequenced, filtered for low-quality reads, aligned with STAR (2.4.1d), and quantified to mm10 (Ensembl Transcripts, release 82) in

PartekFlow (Partek Inc., Chesterfield, MO). Read counts were normalized to total count and analyzed for differential expression using gene-specific analysis (GSA) per PartekFlow recommended settings. Sequencing data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus and are accessible through Gene Expression Omnibus Series accession number GSE107687.

Of 13,573 genes analyzed by gene-specific analysis, 2,514 differentially expressed genes (DEGs) were detected in A17 skin using a standard definition including both statistically significant change (P -value < 0.01, false discovery rate < 0.05) and effect size (fold change > 2 or < –2) (Figure 1a). These DEGs included genes important in lipid metabolism and keratinization, consistent with the understanding of barrier dysfunction in atopic inflammation, as well as significant immunologic pathways in adaptive and innate immunity previously identified in the human AD transcriptome (Figure 1b) (Ewald et al., 2015). Multiple up-regulated pathways implied dermal effector cell involvement, including those for T-cell activation, dendritic cell maturation, and cytokine signaling. Of particular



Abbreviations: A17, *Adam17^{fl/fl} Sox9-Cre* mouse; AD, atopic dermatitis; DEG, differentially expressed gene; hDEG, human ortholog of differentially expressed gene; MADAD, meta-analysis derived in atopic dermatitis (differentially expressed genes)

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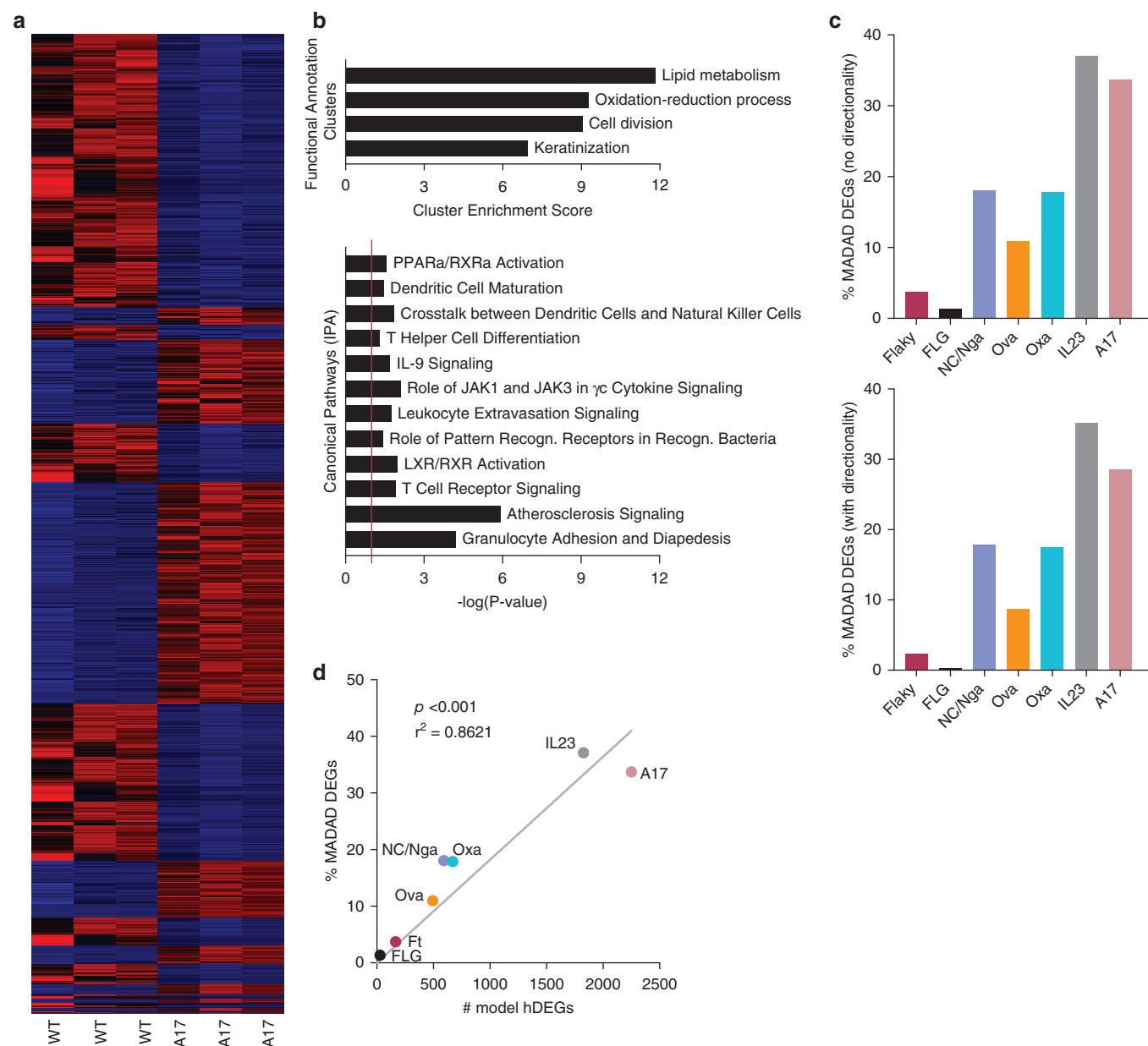


Figure 1. Differentially expressed genes (DEGs) in ADAM17 mice model human AD. (a) Hierarchical clustering of 2514 DEGs in 3 A17 and 3 wild type mice; (b) top scoring functional annotation clusters (DavidGO) and notable, statistically significant ($P < 0.05$) up- and downregulated Canonical Pathways (Ingenuity Pathway Analysis) from the 2514 ADAM17 DEGs, red line denotes significance cutoff; (c) percentage of MADAD DEGs found in the human orthologues of DEGs (hDEGs) for 7 mouse models of AD, without (upper) or with (lower) directionality of gene expressions; (d) Regression analysis showing variation in percent of MADAD genes in human orthologues of mouse DEG lists (hDEGs) by total number of hDEGs in mouse models. IL-23 injected mice (IL23); NC/Nga mice (NC/Nga); Oxazolone-challenged mice (Oxa); ovalbumin-challenged mice (Ova); Ft: flaky tail mice (*Tmem79^{ma/ma} Flg^{fl/fl}*); filaggrin-mutated (*Flg^{iv/m}*) mice (FLG).

interest was the up-regulation of JAK signaling pathways, which are targetable via inhibitors in humans. Nearly identical lists of DEGs were obtained from whole skin and epidermis samples (see [Supplementary Table S1](#) online). Transcriptomic data obtained from whole skin samples were further analyzed to reflect skin biopsies performed in humans.

To assess the similarity of A17 mouse skin and human AD skin more directly,

we queried human gene symbols corresponding to mouse gene symbols using the bioinformatics package bioMART and investigated the overlap between these human orthologs of mouse DEGs (hDEGs) in A17 whole skin ($n = 2,250$) and a previously published, meta-analysis-derived DEG list ("MADAD") of 595 genes from human AD skin ([Ewald et al., 2015](#)). We found that the A17 hDEGs contained 34% of human DEGs in MADAD ([Figure 1c](#)),

on the upper end of overlap in other murine AD models for which published DEGs were available ([Ewald et al., 2017](#)). The overall trend was maintained even when directionality was taken into account ([Figure 1c](#)). Among overlapping genes were those important for mitosis, disulfide bond formation, chemotaxis, and innate immunity ([Table 1](#), and see [Supplementary Table S2](#) online). Greatest enrichment was seen in genes

Table 1. Functional annotation clusters for MADAD and human orthologs of A17 DEGs

Annotations	Cluster Enrichment Score
DEGs in both MADAD and human ortholog of A17 (n = 200)	
Mitosis, cell cycle, cell division	7.84
Signal peptide, disulfide bond, glycoprotein	3.12
Inflammatory response, chemotaxis, cytokine	2.93
Innate immunity	2.67
DEGs in A17 only (n = 2,050)	
Chemical carcinogenesis, metabolism of xenobiotics by cytochrome P450	5.03
NAD (nicotinamide adenine dinucleotide), nucleotide phosphate-binding region: NAD	4.38
Cell cycle, mitosis	4.00
Retinol metabolism, retinol dehydrogenase activity	3.51
DEGs in MADAD only (N = 383)	
Signal peptide, disulfide bond, glycoprotein	9.64
Innate immunity, antiviral defense	7.57
Adaptive immune response	7.16
Chemotaxis, chemokine signaling pathway	3.47
DEGs in MADAD, A17, and IL-23 (n = 96)	
Signal peptide, disulfide bond, glycoprotein	4.88
Innate immune response	4.35
Leukocyte migration, neutrophil chemotaxis	4.10
DEGs in A17 and IL-23 (n = 596)	
Signal peptide, disulfide bond, glycoprotein	7.91
Oxidoreductase activity	4.52
Cytokine activity, IL-1	4.34

Abbreviations: A17, *Adam17^{fl/fl}Sox9-Cre* mice; DEG, differentially expressed gene; MADAD, meta-analysis derived in atopic dermatitis.

related to cell division, suggesting that A17 uniquely captures a proliferative phenotype within MADAD not reported for other models (Ewald et al., 2017). Both A17 and IL-23, the model with the highest MADAD coverage, captured innate immune activation seen in MADAD, with more direct comparison of the two models showing enriched IL-1 activity (Table 1, and see Supplementary Table S2). Most human DEGs captured in MADAD remain unrepresented in A17 or any other mouse model, speaking to the challenges of recapitulating the full range of change seen in polygenic diseases like AD with models that manipulate single variables in a controlled fashion.

For all mouse models, the extent of differential expression, or number of hDEGs in each model, predicted the extent of overlap with MADAD ($r^2 = 0.821$, $P < 0.001$) (Figure 1d). This raised the question of whether an increasingly extreme but nonspecific model of skin disease might, by sheer number of DEGs alone, capture more human AD hDEGs. To address this concern, we analyzed the A17 hDEGs

that did not overlap with MADAD. We found that these genes played a role in cytochrome P450 metabolism, nicotinamide adenine dinucleotide, cell division, and retinol metabolism (Table 1), all of which have been associated with AD previously (Drucker et al., 2017; Ohno et al., 2015; Rollman and Vahlquist, 1985; Ungar et al., 2017). Although these associations cannot prove the absence of off-target, non-AD-like inflammation, they also do not provide evidence of an additional, wholly unrelated skin pathology driving differential gene expression in A17 skin.

Critically, the absence of any A17 hDEG in MADAD may also reflect low sensitivity of MADAD itself to detect subtle transcriptional change in human AD, because failure to detect low-expression genes on whole-tissue microarrays would carry through the meta-analysis approach. Indeed, the human orthologs of several nonoverlapping A17 hDEGs, including filaggrin (*FLG*), inflammasome regulator NLRP10 (*NLRP10*), putative anti-oxidant *CCDC80* (*CCDC80*), and IL-2

receptor alpha (*IL2RA*), were missing from MADAD but have been implicated in human AD genome-wide association studies (Paternoster et al., 2015) and other work (Elias et al., 2017; Furue et al., 1994; Miyai et al., 2016).

Our results suggest that A17 shows among the greatest degree of transcriptional similarity with human AD and are consistent with eczematous phenotypes seen previously (Kobayashi et al., 2015). Similarities to other inflammatory processes as seen in psoriasis remain to be explored and may elucidate common features of these diseases.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Therese Woodring¹,
Tetsuro Kobayashi^{1,2}, Doyoung Kim²
and Keisuke Nagao^{1,2,*}

¹Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; and ²Current affiliation: Dermatology Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland, USA

*Corresponding author e-mail: keisuke.nagao@nih.gov

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2018.04.021>.

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Serum Soluble CD48 Levels as a Prognostic Marker in Mycosis Fungoides and Sézary Syndrome

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TO THE EDITOR

CD48 is a member of the signaling lymphocyte activation molecule family and is expressed on most hematopoietic cells (Latchman et al., 1998). CD48 has two different ligands, CD2 and CD244, and the latter binds to CD48 with a much higher affinity (Brown et al., 1998). Through interactions with these ligands, CD48 plays important roles in immune cell function and activation, such as enhancing T-cell activation, facilitating adhesion of antigen-presenting cells, and modulating cytotoxic T-cell and natural killer-cell immunity, which are associated with a variety of diseases including autoimmunity, allergy, infection, and cancer (McArdel et al., 2016). In addition to the membrane-bound form, CD48 has also existed in a soluble form in human serum and plasma (Smith et al., 1997), although it is still unknown how soluble CD48 (sCD48) is generated. Plasma sCD48 levels were first reported to be increased in patients with advanced lymphoid malignancies and arthritis (Smith et al., 1997). A recent study showed that serum sCD48 levels were increased in mild atopic asthma (Gangwar et al., 2017). On the other hand, there have been no studies

investigating serum sCD48 levels in skin disorders. Here, we measured serum sCD48 levels in patients with cutaneous T-cell lymphoma and atopic dermatitis (AD) to investigate whether sCD48 levels can be a disease marker.

Serum samples were obtained from 34 patients with mycosis fungoides (MF) (stage IA 9, stage IB 7, stage IIA 1, stage IIB 9, stage IIIA 3, stage IVA1 3, and stage IVA2 2; mean age $\pm$ standard deviation [SD] = 58.4 $\pm$ 16.5 years; 22 males and 12 females), 12 patients with Sézary syndrome (SS) (stage IVA1 9 and stage IVA2 3; mean age $\pm$ SD = 64.1 $\pm$ 14.8 years; 10 males and 2 females), 33 patients with AD (mild, n = 11; moderate, n = 11, severe, n = 11; mean age $\pm$ SD = 36.8 $\pm$ 13.1 years; 21 males and 12 females), and 14 healthy control individuals (mean age $\pm$ SD = 43.9 $\pm$ 11.4 years; 8 males and 6 females). All patients with MF/SS were given diagnoses according to International Society for Cutaneous Lymphomas/European Organisation for Research and Treatment of Cancer criteria (Olsen et al., 2007; Willemze et al., 2005). As for patients with MF, clinical stage IIB or greater was defined as advanced stage, and clinical stage I and IIA were defined as early stage. All patients with

AD were enrolled according to the criteria of Hanifin and Rajka (1980). The disease severity of AD was determined by Physician Global Assessment. The healthy control individuals had no history of allergy, AD, psoriasis, or malignancies. The medical ethics committee of the University of Tokyo approved all described studies. Written informed consent was obtained to use blood samples. Immunoreactive sCD48 was quantified by human ELISA kits (Sino Biological Inc., Beijing, China). Significance of difference was analyzed with Kruskal-Wallis test. Correlation coefficients were determined by using the Spearman rank correlation test. P -values of less than 0.05 were considered statistically significant.

Serum sCD48 levels in patients with advanced stage of MF were significantly elevated compared with those of normal controls (Figure 1a). Protein levels of sCD48 in sera of patients with SS were also significantly increased relative to those of normal control individuals (Figure 1a). On the other hand, serum sCD48 levels in patients with any severity of AD were comparable with those of healthy control individuals (Figure 1b). In patients with stage IIB of MF, sCD48 levels of patients with multiple tumors were significantly higher than those with single tumor (Figure 1c). In sera of patients with MF/SS, serum sCD48 levels positively correlated with serum soluble IL-2 receptor and lactate dehydrogenase

Abbreviations: AD, atopic dermatitis; MF, mycosis fungoides; sCD48, soluble CD48; SS, Sézary syndrome

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