Enhanced Expression of Genes Related to Xenobiotic Metabolism in the Skin of Patients with Atopic Dermatitis but Not with Ichthyosis Vulgaris

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Previous transcriptome analyses underscored the importance of immunological and skin barrier abnormalities in atopic dermatitis (AD). We sought to identify pathogenic pathways involved in AD by comparing the transcriptomes of AD patients stratified for filaggrin (*FLG*)-null mutations to those of both healthy donors and patients with ichthyosis vulgaris. We applied RNA sequencing to analyze the whole transcriptome of nonlesional skin. We found that 607 genes (476 up-regulated and 131 down-regulated by >2-fold) and 193 genes (172 up-regulated and 21 down-regulated by >2-fold) were differentially expressed when all AD or ichthyosis vulgaris patients were compared with healthy donors, respectively. Expression of genes involved in RNA/protein turnover and adenosine triphosphate synthesis, as well as genes involved in cell death, response to oxidative stress, DNA damage/repair, and autophagy, were significantly enriched in AD skin and, to a lesser extent, in ichthyosis vulgaris skin. *FLG*-null mutations appear to hardly interfere with current observations. Genes related to xenobiotic metabolism were up-regulated in AD skin only, as were genes related to arachidonic, linoleic, and α-linolenic acid metabolism. Thus, this work newly links AD pathogenesis to aberrant expression of genes related to xenobiotic metabolism.

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INTRODUCTION

Atopic dermatitis (AD) is one of the most common dermatoses, affecting 2–5% of adults and 10–20% of children worldwide (International Study of Asthma and Allergies in Childhood Steering Committee, 1998). AD is characterized by pruritus, eczematous lesions, and skin dryness and is commonly associated with allergic conditions such as allergic rhinitis and asthma (Leung and Guttman-Yassky, 2014). It was long assumed that most childhood cases of AD undergo full remission before adolescence (Bieber, 2008). However, data published in 2011 show that, in the United States, 10.7% of children and 10.2% of adults are affected by AD, suggesting that most children with AD continue to be affected into adulthood (Shaw et al., 2011). Similarly in Europe, 5–20% of all school children and 2–18% of all adults have AD, thus confirming the same clinical pattern (Asher et al., 2006; Flohr and Mann, 2014; International Study of Asthma and Allergies in Childhood Steering Committee, 1998; Harrop et al., 2007; Odhiambo et al., 2009).

The pathogenesis of AD is not yet fully understood, but there is consensus that immune hyper-responsiveness and epidermal barrier impairment are predominant etiologic factors (Leung and Guttman-Yassky, 2014). Several studies have linked impaired epidermal barrier function resulting from null mutations in the filaggrin (*FLG*) gene, which give rise to ichthyosis vulgaris (IV) as a major driver of the development of AD (Palmer et al., 2006; Weidinger et al., 2006). *FLG* is located on chromosome 1 in the epidermal differentiation complex, a region carrying various genes encoding epidermal structural proteins and repeatedly found to be linked to AD (Hoffjan and Stemmler, 2007). However, in various AD patient cohorts, IgE-mediated allergic reactions, irritants, or psychosomatic disorders appear to be of major importance (Leung and Guttman-Yassky, 2014). Moreover, air pollutants, endocrine disruptors, or components of cigarette smoke are positively associated with AD (Ahn, 2014). Hence, the deleterious impact of the environment on the course of AD may have recently increased (Hidaka et al., 2017; Kabashima et al., 2016).

Previous transcriptome analyses have been carried out in lesional versus nonlesional skin of adults with AD not stratified for *FLG*-null mutations and in nonlesional skin of infants stratified for *FLG* both refining and extending previous data

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Abbreviations: AD, atopic dermatitis; ATP, adenosine triphosphate; ctrl, control; FA, fatty acid; FLG, filaggrin; GO, gene ontology; IV, ichthyosis vulgaris; MUT, mutated; UGT, uridine 5′-diphospho-glucuronosyltransferase; WT, wild type

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obtained by microarray analyses (Ewald et al., 2015). Paller et al. (2016) have recently shown that patients with ichthyosis, including congenital ichthyosiform erythroderma, lamellar ichthyosis, epidermolytic ichthyosis, and Netherton syndrome exhibit a T helper type 17 skin immune profile, similar to that observed in patients with psoriasis. Data on IV, which is the most frequent form of ichthyosis, are still missing; however, inclusion of IV skin is clearly of importance to better understand the pathogenesis of AD, because patients with IV, despite having *FLG* mutations, do not exhibit overt skin inflammation. Thus, comparing results obtained from skin of AD patients with those from skin of IV patients might identify previously unrecognized pathways involved in the transition from noninflammatory dry and scaly skin to nonlesional AD skin, including the proinflammatory mechanisms involved in this sequence. And although emollients, immunosuppressants, and anxiolytic drugs do ameliorate AD, they are not curative (Hashizume and Takigawa, 2006). Therefore, elucidation of as-yet unexplored mechanisms involved in the pathogenesis and maintenance of AD could lead to new treatments and potential prophylactic strategies. With this goal, we aimed to identify potential pathways involved in the development of AD by comparing nonlesional AD skin from patients with or without *FLG*-null mutations with skin from patients with IV.

RESULTS

Gene expression signature in AD and IV patient skin is different, regardless of *FLG* mutations

We performed global transcriptional profiling to identify gene expression patterns that could distinguish AD patients with or without *FLG*-null mutations from control subjects and IV patients. By this approach, we also expected to uncover new pathways involved in AD pathogenesis. We used skin biopsy samples from four patient groups to carry out the analyses: controls (Ctrl wild type [WT]/WT), AD patients with or without *FLG* mutations (AD *FLG* MUT and AD WT/WT, respectively), and patients with IV presenting a dry and scaly skin without signs of overt inflammation. Furthermore, to avoid variations related to age or disease background, we recruited only adult patients with a history of AD since childhood. Moreover, to rule out differences due to tissue heterogeneity, the biopsy samples of nonlesional skin were taken from the same body region. We performed RNA sequencing in whole skin from a total of 20 patients and 9 healthy adult control subjects (cohort I) (see Supplementary Table S1 online). To validate our in silico findings, we performed quantitative real-time reverse transcriptase–PCR (see Supplementary Figure S1 online). The variability in gene expression within groups is supplied in Supplementary Figure S2 online. Heatmap and clustering analyses based on gene expression levels show clear variations between AD patients and control subjects, regardless of *FLG* status (see Supplementary Figures S3 and S4 online). In contrast, there were no significant differences between AD *FLG* MUT patients and AD WT/WT patients according to statistical analysis using *DESeq2* and as illustrated in Supplementary Figures S3 and S4. One patient with AD *FLG* MUT displayed an atypical gene expression profile when compared with other AD patients that could not be explained by *FLG* genotype or by other parameters including sex, age, serum IgE level, Eczema Area and Severity Index (EASI) score, and differential blood counts (see Supplementary Figures S3 and S4, and data not shown). The gene expression profile of the skin of two IV patients was similar to that of AD patients whereas others (n = 5) were similar to the control profile (see Supplementary Figures S3 and S4). This was not dependent on the type of *FLG* mutations. Therefore, *FLG* mutations do not determine the gene expression signature in the skin of AD or IV patients.

The machinery for RNA and protein synthesis is enhanced in AD and IV skin

To identify specific genes that are differentially modulated in various disease groups, we compared all groups by using an adjusted *P* (false discovery rate) of less than 0.1 and a change in gene expression greater than 2-fold. We found 607 differentially expressed genes (476 up-regulated and 131 down-regulated) when all AD patients (n = 13) were compared with control subjects (n = 9) (see Supplementary Tables S3 and S4 online), 618 differentially expressed genes (489 up-regulated and 129 down-regulated) when AD WT/WT patients (n = 7) were compared with control volunteers (n = 9) (see Supplementary Tables S3–S5 online), and 193 differentially expressed genes (172 up-regulated and 21 down-regulated) when IV patients (n = 7) were compared with control volunteers (n = 9) (see Supplementary Tables S3–S6 online). No significant differences were found when comparing AD WT/WT with AD *FLG* MUT patients (see Supplementary Table S3). Overall gene ontology (GO) and pathway analyses are summarized in Table S8 online. They showed that the cellular machineries involved in RNA transcription, splicing, and processing as well as in protein translation and elongation were significantly up-regulated in all AD skin classes compared with control samples (Figure 1a–c, and see Supplementary Table S4). Consistent with this, the expression of genes involved in metabolism of proteins was enhanced as well (Figure 1d and e, and see Supplementary Table S4). Results of in silico protein network analysis of the up-regulated genes related to RNA and protein metabolism are shown in Figure 1b and 1d, respectively. Increased protein metabolism and turnover were further shown by an increase in ubiquitination processes (see Supplementary Figure S5 online). Moreover, GO and network analyses showed increased RNA and protein metabolism also in the skin of IV patients when compared with control subjects, although the extent of up-regulation was not as high as that observed in the skin of AD patients (Figure 1c and e, and see Supplementary Tables S6 and S7 and Supplementary Figure S6 online). Accordingly, ubiquitination was not induced in IV skin (see Supplementary Tables S6–S8 online). Thus, there seems to be a progression of up-regulation of cellular metabolism in the skin, from IV to AD patients, that is associated with impaired epidermal barrier function and is further triggered by inflammation.

ATP synthesis-related genes are enhanced in AD and IV skin

GO analyses comparing all AD patients with healthy control subjects showed up-regulated expression of key genes involved in adenosine (ATP) synthesis (Figure 2a and b), consistent with the high energy demand of RNA and protein synthesis. Genes of ATP synthesis were up-regulated in AD patients (Figure 2b, and see Supplementary Tables S4, S5,

a

Gene	CTRL WT/WT N=9	all AD N=10	P value
FUS	6.89± 0.15	7.94± 0.10	P<0.0001
POLR2H	4.91± 0.13	5.97± 0.12	P<0.0001
DAZAP1	4.40± 0.10	5.05± 0.08	P<0.0001
RNASET2	3.86± 0.10	4.35± 0.09	P=0.0013
U2AF1	4.19± 0.05	5.02± 0.07	P<0.0001
FAU	4.95± 0.22	6.02± 0.12	P=0.0001
SNRNP70	5.82± 0.08	6.64± 0.09	P<0.0001
NOL3	4.25± 0.08	4.98± 0.08	P<0.0001
SF3B14	4.55± 0.15	5.48± 0.08	P<0.0001
EXOSC7	5.21± 0.13	6.12± 0.09	P<0.0001
POLR2G	3.76± 0.11	4.53± 0.08	P<0.0001
HNRNPH3	7.33± 0.08	8.07± 0.13	P=0.0002
CIRBP	6.69± 0.13	7.73± 0.09	P<0.0001
SNRPC	3.26± 0.13	4.04± 0.08	P<0.0001
NHP2	3.50± 0.12	4.18± 0.06	P<0.0001
PUF60	4.58± 0.10	5.36± 0.08	P<0.0001
SNRPD2	4.23± 0.13	5.02± 011	P=0.0002
SF3B5	3.97± 0.12	4.90± 0.09	P<0.0001
DDX39	3.71± 0.12	4.51± 0.06	P<0.0001
FRG1	4.16± 0.15	4.86± 0.07	P=0.0001
DUS1L	3.99± 0.09	5.29± 0.11	P<0.0001
LENG9	2.37± 0.15	3.48± 0.13	P<0.0001
NOP10	3.72± 0.12	4.45± 0.07	P<0.0001
ADRM1	3.95± 0.09	4.64± 0.09	P<0.0001
C1ORF66	3.60± 0.10	4.31± 0.06	P<0.0001
IRF3	4.09± 0.07	4.84± 0.08	P<0.0001
POP5	3.59± 0.07	4.53± 0.06	P<0.0001
SNRPF	2.81± 0.09	3.54± 0.07	P<0.0001
SSU72	4.56± 0.10	5.28± 0.11	P=0.0002
ELL	4.35± 0.17	5.31± 0.06	P<0.0001
SNRPB	3.92± 0.12	4.73± 0.07	P<0.0001
UBA52	6.62± 0.16	7.80± 0.10	P<0.0001
TAF1C	4.29± 0.08	5.16± 0.11	P<0.0001
CBX3	4.85± 0.20	5.70± 0.08	P=0.0002
TCEAL4	6.40± 0.07	7.38± 0.12	P<0.0001
MCM7	5.02± 0.10	5.83± 0.08	P<0.0001
ACTR5	3.45± 0.11	4.07± 0.09	P=0.0003
PQBP1	4.15± 0.14	4.97± 0.09	P<0.0001
NME2	3.38± 0.14	4.26± 0.09	P<0.0001
ZNF692	4.17± 0.13	4.69± 0.09	P=0.0024
RUVBL2	3.70± 0.06	4.33± 0.09	P<0.0001
DHPS	4.04± 0.11	4.77± 0.08	P<0.0001
UBB	5.52± 0.13	6.37± 0.13	P=0.0002

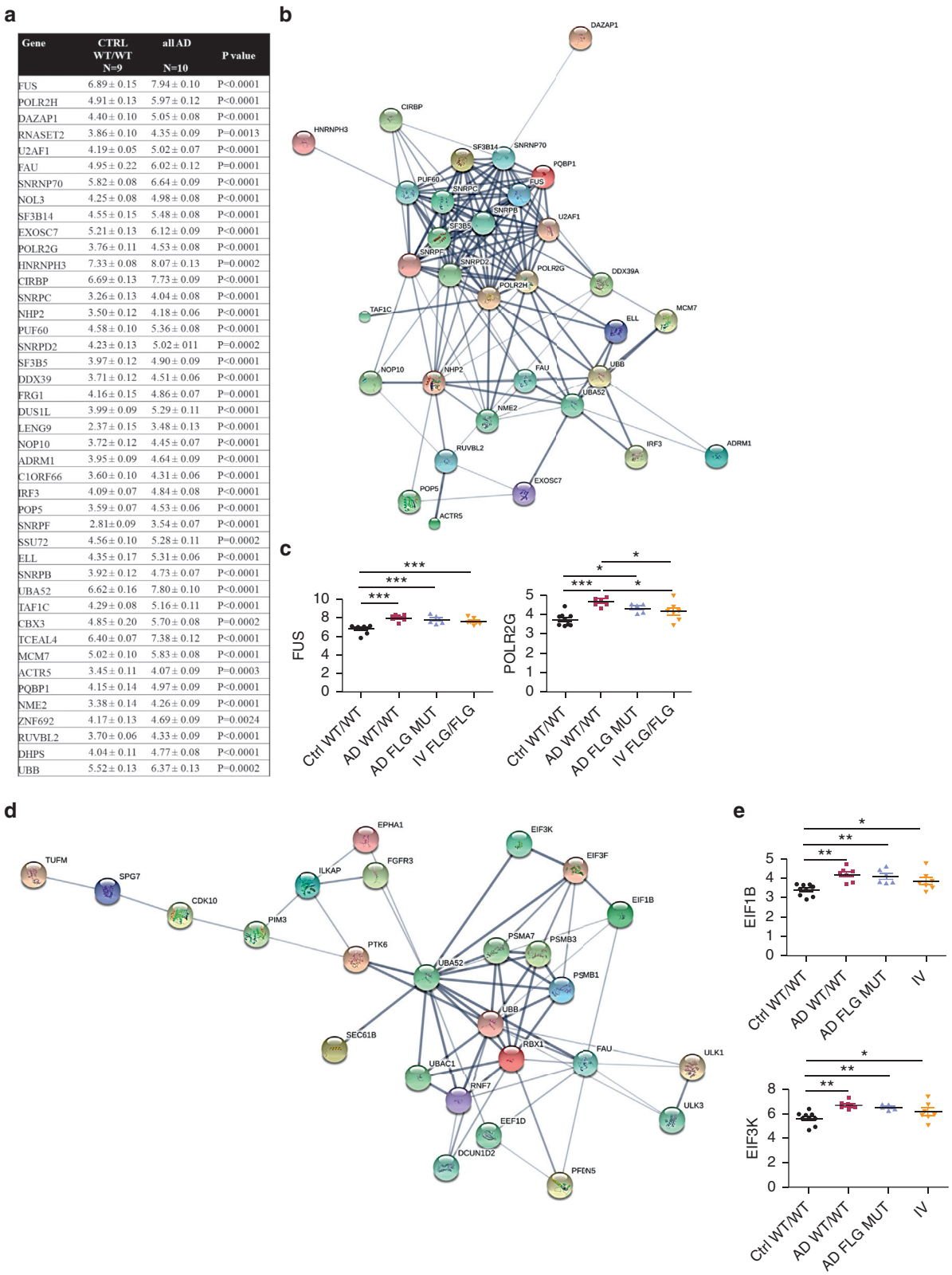

Figure 1. Expression of genes encoding machinery for RNA and protein synthesis is enhanced in AD and IV skin. Network analysis of genes differentially expressed in all AD patients (including those with and without *FLG* loss-of-function mutations) compared with healthy control subjects. Genes were included in analyses as defined by FC > 2 and adjusted $P < 0.1$. (**a**) Expression of genes involved in RNA machinery in the skin of all AD patients versus healthy donors. (**b**) Thirty-two selected genes were classified into the RNA machinery category with the GO terms *Gene expression, RNA binding, RNA splicing, RNA processing, mRNA processing, RNA metabolic process, mRNA metabolic process, Spliceosome, Processing of Capped Intron-Containing Pre-mRNA,* and *ncRNA processing.* (**c**) Histograms show changes in expression of *FUS* and *POLR2G.* (**d**) Twenty-six selected genes were classified into the protein machinery

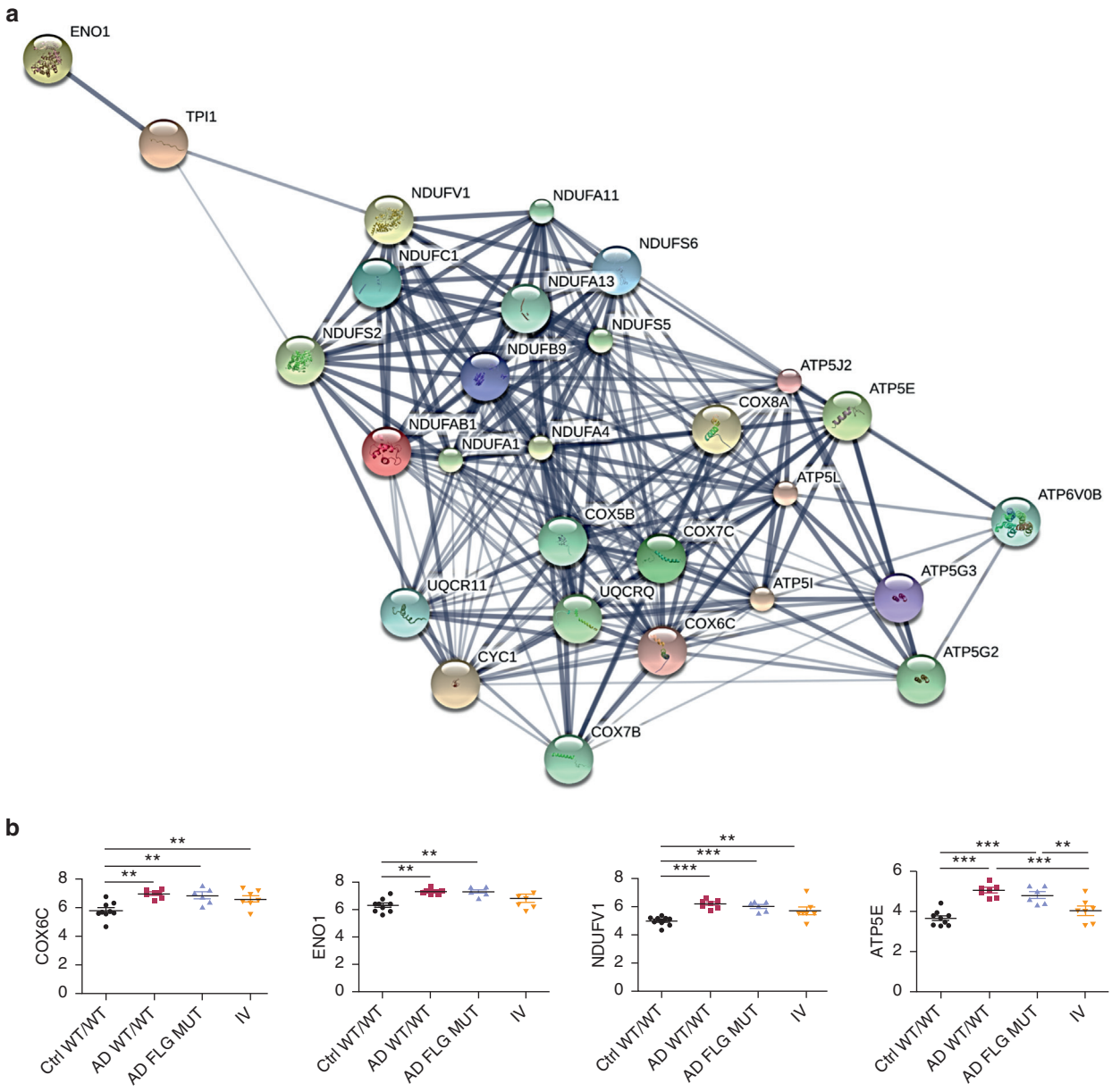

Figure 2. Expression of ATP synthesis-related genes is enhanced in AD and IV skin. Network analysis of genes differentially expressed in all AD patients (including those with and without *FLG* loss-of-function mutations) compared with healthy control subjects. Genes were included in analyses as defined by FC > 2 and adjusted $P < 0.1$. (**a**) Twenty-nine genes were classified into the ATP synthesis category with the GO terms *ATP synthesis coupled electron transport, Mitochondrial proton-transporting ATP synthase complex, ATP biosynthetic process,* and *Generation of precursor metabolites and energy*. (**d**) Histograms show changes in expression of *COX6C*, *ENO1*, *NDUFV1*, and *ATP5E*. Data were analyzed using a one-way analysis of variance test followed by a Newman and Keuls post hoc test. $^{**}P < 0.01$, $^{***}P < 0.0001$. Healthy control subjects are designated as Ctrl WT/WT, patients with AD without *FLG*-null mutations as AD WT/WT, AD patients with *FLG*-null mutations as AD *FLG* MUT, and IV patients as IV. AD, atopic dermatitis; ATP, adenosine triphosphate; Ctrl, control; FC, fold changes; FLG, filaggrin; GO, gene ontology; IV, ichthyosis vulgaris; MUT, mutated; WT, wild type.

and S8) and in IV patients when compared with control subjects, yet to a lesser extent than in AD (Figure 2b, and see Supplementary Tables S6–S8). Genes involved in fatty acid (FA) metabolism were up-regulated, including *ACAA1*, *ECHDC2*, *FABP4*, *SLC27A3,* and *ECHS1*, as were genes related to cholesterol biosynthesis such as *TM7SF2* and *CYP5R1* in AD patients when compared with control subjects (Figure 3a and b, and see Supplementary Tables S4 and S5). Moreover, genes of arachidonic, linoleic, and α-linolenic acid metabolism were triggered in AD but not in IV patients

category with the GO terms *Translational elongation, Translation, Metabolism of proteins,* and *Protein metabolic processes.* (**e**) Histograms show changes in expression of *EIF1B* and 3K *EIF3K*. Data were analyzed using a one-way analysis of variance test followed by a Newman and Keuls post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$. Healthy control subjects are designated as Ctrl WT/WT, patients with AD without *FLG*-null mutations as AD WT/WT, AD patients with *FLG*-null mutations as AD *FLG* MUT, and IV patients as IV. AD, atopic dermatitis; Ctrl, control; FC, fold changes; FLG, filaggrin; GO, gene ontology; IV, ichthyosis vulgaris; MUT, mutated; WT, wild type.

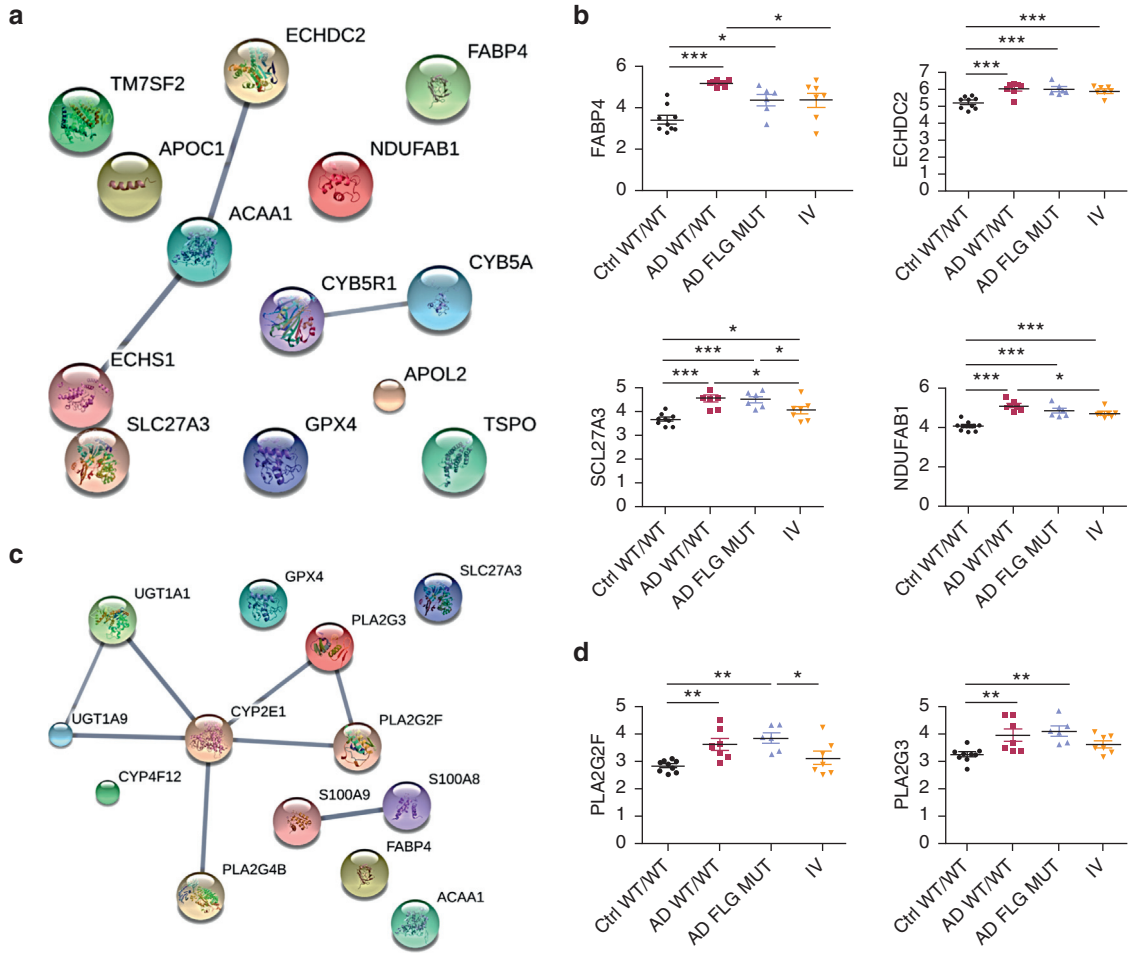

Figure 3. Expression of genes related to lipid metabolism is enhanced in AD and IV skin. Network analysis of genes differentially expressed in all AD patients (including patients with and without *FLG* loss-of-function mutations) compared with healthy control subjects. Genes were included in analyses as defined by FC > 2 and adjusted $P < 0.1$. (**a**) Thirteen genes were classified into the lipid metabolism category with the GO terms *Lipid metabolic process, Fatty acid metabolic process, Cellular lipid catabolic process, Small molecule metabolic process,* and *Lipid biosynthetic pathways*. (**b**) Histograms show changes in expression of *FABP4, ECHDC2, SLC27A3,* and *NDUFAB1*. (**c**) Thirteen genes were classified into the lipid catabolism category with the GO terms *Arachidonic metabolism, Linoleic acid metabolism, Alpha-linolenic acid metabolism* and *Catabolic process*. (**d**) Histograms show changes in expression of *PLA2G2F* and *PLA2G3*. Data were analyzed using a one-way analysis of variance test followed by a Newman Keuls post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$. Healthy control subjects are designated as Ctrl WT/WT, patients with AD without *FLG*-null mutations as AD WT/WT, AD patients with *FLG*-null mutations as AD *FLG* MUT, and IV patients as IV. AD, atopic dermatitis; Ctrl, control; FC, fold changes; FLG, filaggrin; GO, gene ontology; IV, ichthyosis vulgaris; MUT, mutated; WT, wild type.

(Figure 3c and d, and see Supplementary Tables S7 and S8), as suggested earlier in epidermal equivalents generated with patient keratinocytes (Blunder et al., 2017). Moreover, the expression of genes related to FA metabolism (*FABP4, TM7SF2, ACAA1, NDUFAB1*) was less induced in IV skin than in AD skin (Figure 3, and see Supplementary Tables S4–S7). In summary, RNA and protein synthesis that both require enhanced ATP production likely via FA catabolism is enhanced in AD skin at a higher rate than in IV skin.

Cell death is promoted in nonlesional AD and IV skin

Comparison of AD patients with control subjects shows enhanced expression of key genes involved in apoptosis and cell death (Figure 4a, and see Supplementary Tables S4 and S5). In IV, expression of cell death genes was moderately altered (*BLCAP, PDCD6, TNFRSF25, NGFRAP1, TRADD*) when compared to control subjects and to AD patients (Figure 4, and see Supplementary Tables S6–S8). Cell death can result from various cellular abnormalities including DNA damage, oxidative stress, or elevated levels of extracellular ATP. Increased oxidative stress has been shown in AD (Nakai et al., 2009; Tsukahara et al., 2003), thereby triggering anti-oxidative cellular responses via multiple pathways (Rinnerthaler et al., 2015). Expression of genes conferring resistance to oxidative stress (*ATOX1, GSTP1, GPX4, PRDX5, RNF7*) was increased in the skin of AD patients (Figure 4b, and see Supplementary Tables S4 and S5), and to a lesser extent in the skin of IV patients compared with control subjects or with AD patients (Figure 4b, and see Supplementary Tables S6 and S7). Thus, we here show that the transcriptional response to oxidative stress is not only significantly triggered in the skin of AD patients but also of IV patients. Expression of DNA damage/repair genes (*RAD9A, CIRBP, RBX1, UBA52, UBB, UBE2B, POLD4, DDIT4, MUM1)* was up-regulated in AD patients and, to a lesser degree, in IV patients (*RAD9A, UBA52, DDIT4, MUM1*) when compared with control subjects or with

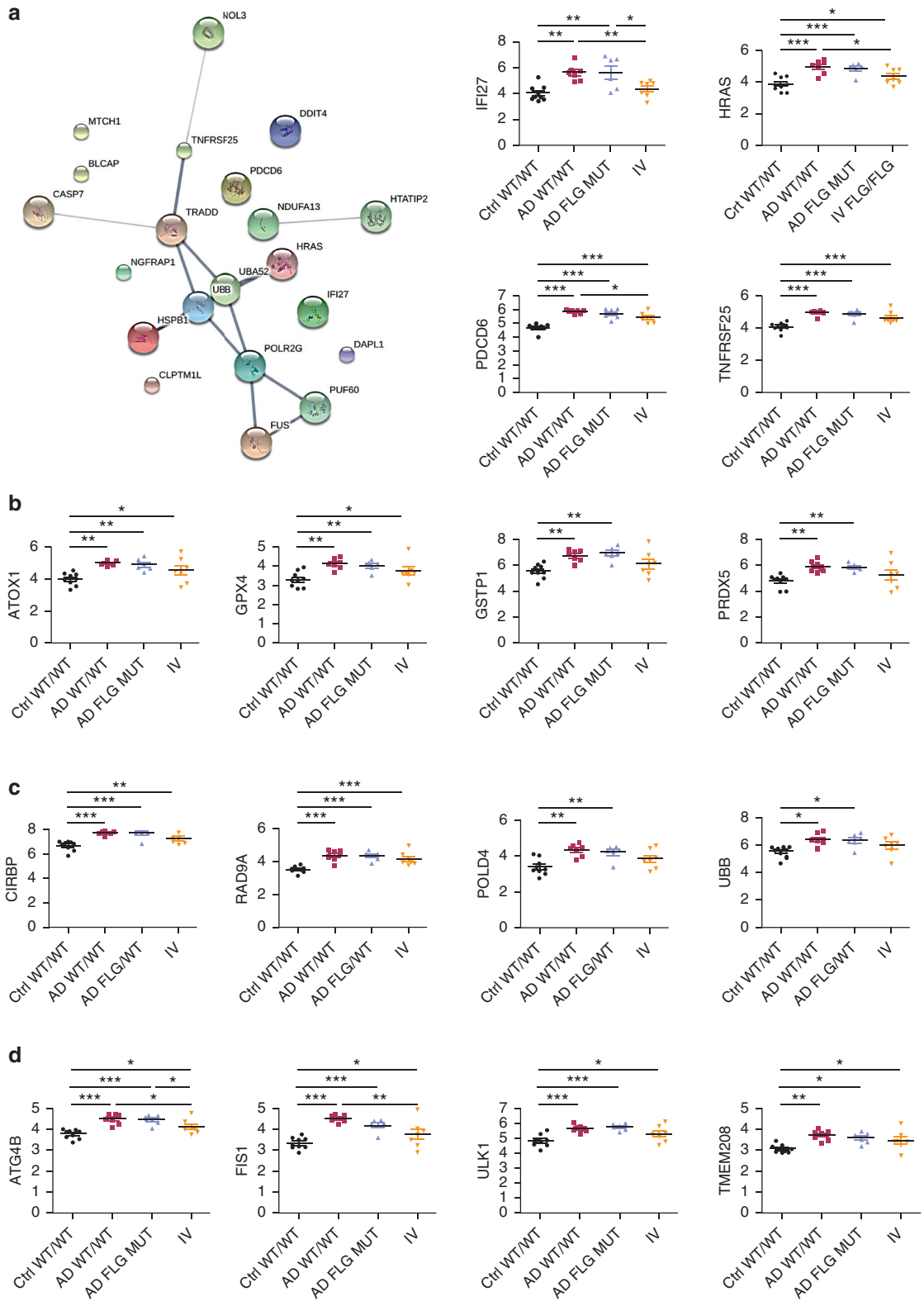

Figure 4. Expression of genes related to cell death, oxidative stress, DNA damage/repair, and autophagy is enhanced in AD and IV skin. Network analysis of genes differentially expressed in all AD patients (including patients with and without *FLG* loss-of-function mutations) compared with healthy control subjects. Genes were included in analyses as defined by FC > 2 and adjusted $P < 0.1$. (**a**) Twenty-one selected genes were classified into the apoptosis category with the GO terms *Apoptotic process, Cellular response to stimulus,* and *Apoptosic signaling pathway*. Histograms show changes in expression of *IFI27, HRAS, PDCD6,* and *TNFRSF25*. Histograms show changes in expression of *ATOX1, GPX4, GSTP1,* and *PRDX5*. (**b**) *CIRBP, RAD9A, POLD4,* and *UBB*. (**c**) *ATG4D, FIS1, ULK1,* and *TMEM208*. (**d**) Data were analyzed using a one-way analysis of variance test followed by a Newman Keuls post hoc test $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$. Healthy controls are designated as Ctrl WT/WT, patients with AD without *FLG* null mutations as AD WT/WT, AD patients with *FLG* null mutations as AD *FLG* MUT, and IV patients as IV. AD, atopic dermatitis; Ctrl, control; FLG, filaggrin; GO, gene ontology; IV, ichthyosis vulgaris; MUT, mutated; WT, wild type.

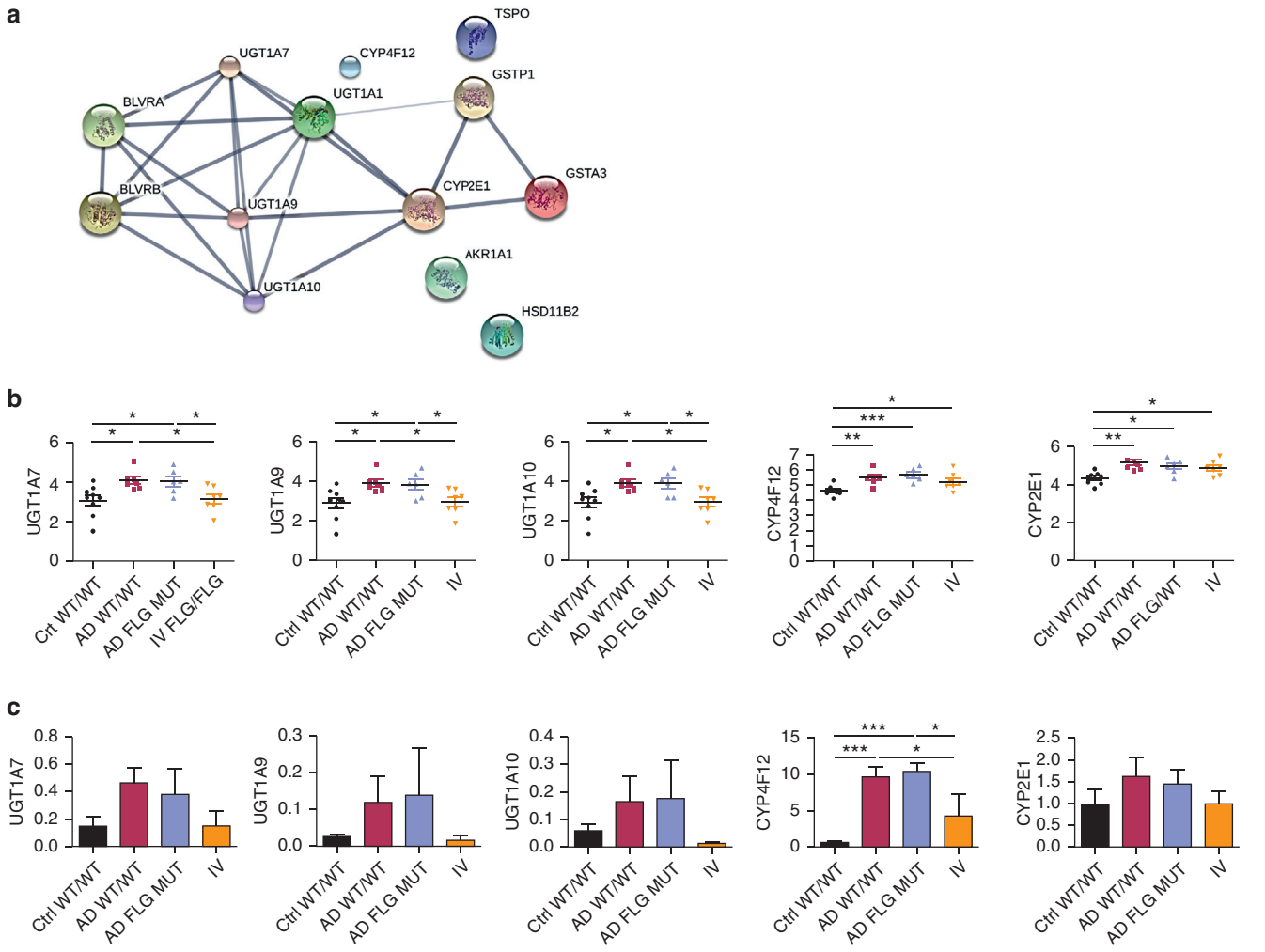

Figure 5. Xenobiotic metabolism is triggered in AD but not in IV skin. Network analysis of genes differentially expressed in all AD patients (including patients with and without *FLG* loss-of-function mutations) compared with healthy control subjects. Genes were included in analyses as defined by FC > 2 and adjusted $P < 0.1$. (**a**) Thirteen selected genes were classified into the xenobiotic metabolism category with the GO terms *Xenobiotic metabolic process, Xenobiotic glucuronidation, Cellular response to chemical stimulus, Pigment metabolic process, Small molecule metabolic process,* and *Drug metabolic process.* (**b**) Histograms show changes in expression of *UGT1A7, UGT1A9, UGT1A10, CYP4F12,* and *CYP2E1.* (**c**) Histograms show changes in expression of *UGT1A7, UGT1A9, UGT1A10, CXP4F12,* and *CYP2E1* in the skin of patients from cohort II. Data were analyzed using a one-way analysis of variance test followed by a Newman and Keuls post hoc test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$. Healthy control subjects are designated as Ctrl WT/WT, patients with AD without *FLG*-null mutations as AD WT/WT, AD patients with *FLG*-null mutations as AD *FLG* MUT, and IV patients as IV. AD, atopic dermatitis; Ctrl, control; FC, fold changes; FLG, filaggrin; GO, gene ontology; IV, ichthyosis vulgaris; MUT, mutated; WT, wild type.

AD patients (Figure 4c, and see Supplementary Tables S4–S8). Programmed cell death, oxidative stress, and repair mechanism induce autophagy. The up-regulation of autophagy-related genes such as *ATG16L2*, *FIS1*, *ATG4s*, *ULK1*, *TMEM208*, *S1008,* and *S100A9* was only observed in AD skin (Figure 4d, and see Supplementary Tables S4–S7). These results indicate that cell death, oxidative stress, DNA damage, and autophagy are enhanced in nonlesional AD, regardless of *FLG*-null mutation status and only marginally in IV.

Xenobiotic metabolism is triggered in AD but not in IV skin

Further analyses of the acquired results showed unexpected data on xenobiotic metabolism. Genes involved in the processing of xenobiotics, pollutants, and endocrine disruptors were up-regulated in the skin of AD patients (Figure 5 a and b, and see Supplementary Tables S4 and S5). Specifically, genes implicated in xenobiotic glucuronidation were induced (Figure 5a and b, and see Supplementary Tables S4 and S5). Hence, the expression of key genes involved in skin detoxification is enhanced in AD patients, independent of *FLG* status. In contrast, the expression of xenobiotic metabolism genes remained unaltered in the skin of IV patients compared with control subjects (Figure 5b, and see Supplementary Table S6). Thus, up-regulation of xenobiotic metabolism key genes seems to be specific for AD skin (Figure 5b, and see Supplementary Tables S7 and S8). To further validate these data, we performed additional quantitative real-time reverse transcriptase–PCR on skin biopsy samples from a distinct cohort of patients (cohort II). Similar to RNA sequencing analysis, in this second cohort of patients we observed a global increase in the expression of *UGT1A7, UGT1A9, UGT1A10, CYP2E1,* and *CYP4F12* in the skin of patients with AD, in contrast to healthy control subjects and IV patients (Figure 5c). Thus, these data suggest that inflammation, but not defective epidermal barrier

function, is associated with increased elimination of xenobiotics, pollutants, and endocrine disruptors in the skin of AD patients. Hence, one can hypothesize that the transition from subinflammatory, dry and scaly skin to overt inflamed skin might be related to triggered local metabolism of noxious molecules, as suggested in other work (Acciani et al., 2013; Hidaka et al., 2017; Kabashima et al., 2016; Kim 2015; Miller and Peden, 2014).

Further insights from gene profiling comparison of AD and IV skin

Genes involved in keratinocyte differentiation, including the *S100A*, *KRT,* and *SPPR* families, were induced in AD but not in IV, similarly to the expression of genes involved in immunity (*CCL13*, *CCL18*, *CCL27, B2M, S100A8, S100A9, IFI27*, *IFR3*, *LCN2*) (see Supplementary Table S7). Several genes that were down-regulated specifically in AD are related to inflammation (*IL1F8, ADIPOQ, KDM6B*) and to apoptosis (*CSRNP3*, *G2E3*, *MTRNR2L4*, *PEG3*) (see Supplementary Table S7). Thus, epidermal homeostasis is more altered in AD than in IV, suggesting that counteracting responses are not sufficient to dampen ongoing inflammation and to normalize the keratinization process.

DISCUSSION

The skin is in daily contact with foreign compounds that are potentially harmful. Endocrine disruptors, pollutants, pesticides, and other chemicals are found in water but also in skin care products such as shampoos and cosmetics. The skin of patients with AD is more reactive to these compounds and more prone to develop irritation after exposure to chemicals (Al-Jaberi and Marks, 1984). Moreover, increased concentrations of air pollutants are positively associated with AD, and the levels of endocrine disruptors such as phthalates are elevated in the dust collected from the bedrooms of children with AD (Ahn, 2014; Hidaka et al., 2017; Kabashima et al., 2016). Furthermore, recent work has shown the presence of metabolites generated by phase II enzymes in the skin, including glucuronides of drugs and pollutants (Manevski et al., 2015). These molecules are capable of inducing epigenetic modifications that promote deregulated immunity associated with a T helper type 2/T helper 17 immune response, higher serum IgE levels, and increased numbers of activated dendritic cells in the lungs (Acciani et al., 2013; Kim, 2015; Miller and Peden, 2014). Previous studies using photoacoustic spectrometry have shown that the skin of AD patients is more permeable to both hydrophilic and hydrophobic chemicals compared with the skin of healthy donors (Hata et al., 2002). The penetration of hydrophilic compounds into the skin correlates with both the severity of AD symptoms and serum IgE levels (Hata et al., 2002). Thus, several lines of evidence strongly suggest a link between AD and xenobiotics. Our results support this hypothesis. We found increased expression of several key genes involved in xenobiotic metabolism, including uridine-5′-diphospho (UDP)-glucuronosyltransferases (UGTs), in the skin of AD patients from two distinct cohorts, regardless of their *FLG* mutation status (Figure 5, and see Supplementary Tables S4 and S5). UGTs are a family of membrane-bound enzymes expressed in various tissues, including human skin, that catalyze the transfer of the glucuronic acid moiety of UDP-glucuronic acid to a large number of endogenous and exogenous compounds (Sumida et al., 2013). We show here that AD is associated with increased expression of glucuronidation-related genes in the skin. Thus, these results support previous work suggesting a link between AD and exposure to pollutants/xenobiotics (Ahn, 2014; Hidaka et al., 2017; Jedrychowski et al., 2011; Kabashima et al., 2016; Kim et al., 2013; Kim, 2015; Miller and Peden, 2014). In contrast to AD, expression of UGT1As and other genes involved in pollutant metabolism remained unchanged in the skin of IV patients (Figure 5, and see Supplementary Tables S4–S8). Whereas epidermal barrier function is compromised in both IV and AD patients, only AD skin exhibits activated pathways involved in pollutant detoxification. Thus, it can be assumed that impaired barrier function because of *FLG* null mutations does not, per se, render the skin more permeable to environmental pollutants. In line with our RNA sequencing data, increased levels of xenobiotics were detected in the serum of AD patients (Kim, 2015; Miller and Peden, 2014). Because enhanced xenobiotic metabolism is only observed in AD and not in IV, one can hypothesize that this might be a consequence of the inflammation and not its cause. However, up-regulation of key genes involved in drug, pollutants, or chemical metabolism has never been reported in psoriasis, another common inflammatory skin disease, thus likely ruling out this hypothesis (Coda et al., 2012; Keermann et al., 2015; Kõks et al., 2016; Li et al., 2014; Szabó et al., 2014). Therefore, we suggest that enhanced xenobiotic metabolism in the skin of individuals predisposed to AD triggers skin inflammation and is thus critically involved in the development of AD (Acciani et al., 2013; Hidaka et al., 2017; Kabashima et al., 2016; Kim, 2015; Miller and Peden, 2014).

Excess water loss through the skin in AD and IV (Gruber et al., 2011) induces compensatory mechanisms such as epidermal hyperplasia to restore the stratum corneum (SC) to limit excess transepidermal water loss and heat loss (Proksch et al., 1993). Keratinocyte hyperproliferation requires increased synthesis of DNA, RNA, and proteins, and thus energy provided by ATP. Accordingly, we observed in both AD and IV patient skin increased expression of several key genes involved in RNA and protein synthesis, modification and turnover, and ATP synthesis and transport (Figures 1 and 2, and see Supplementary Tables S4–S6). This profile of enhanced metabolism is not directly linked to *FLG*-null mutations, because AD WT/WT patients exhibit similar gene expression patterns (Figures 1 and 2, and see Supplemental Tables S4 and S5). Oxidation of FAs that originate from various pools is a major generator of cellular ATP. Because *FABP4* and *SCL27A3* were up-regulated in the skin of AD patients (Figure 3a, and see Supplementary Tables S4 and S5), we postulate that the high demand for ATP in AD skin, regardless of *FLG* mutation status, requires the mobilization of FAs from both adipose tissue and intracellular pools. In line with this, we found increased expression of genes involved in FA synthesis (*ACCA1*, *ECHDC2*) in the skin of AD patients (Figure 3a, and see Supplementary Tables S4 and S5). Thus, in AD, we speculate that FAs, including structural FAs, are used as substrates to produce ATP to provide energy for

keratinocyte renewal. These findings support prior work showing reduced long-chain ceramides in the stratum corneum of AD patients, suggesting that they may serve as substrates for ATP production (Cole et al., 2014; Janssens et al., 2012). In the skin of IV patients, genes of the FA metabolism were up-regulated, yet to a lesser extent than in AD (see Supplementary Tables S6 and S7). Thus, impaired epidermal barrier rather than inflammation induces increased cellular metabolism in the epidermis and subsequent deregulated FA metabolism, potentially sustaining abnormal stratum corneum FA composition. Moreover, the expression of genes involved in the metabolism of arachidonic, linoleic, and α-linolenic acid is only triggered in AD skin (Figure 3c and d, and see Supplementary Tables S4–S7), which may promote de-regulated keratinocyte differentiation and local inflammation (Blunder et al., 2017). Receptors controlling xenobiotic metabolism such as aryl hydrocarbon receptor, pregnane x receptor, and peroxisome proliferator-activated receptor also control the expression of genes involved in FA metabolism including *CYP2E1*, *CYP4F12*, *UGT1As*, *S100A9,* and *PLA2s* (Barbier et al., 2003; Beck et al., 2003; Buckley and Klaassen, 2009). Thus, xenobiotic metabolism may interfere with arachidonic, linoleic, and α-linolenic acid metabolism, hereby sustaining abnormal lipid metabolism, altered keratinization, and inflammation as observed in AD.

Previous studies reported increased expression of pro-apoptotic genes in the skin of AD patients associated with deregulated protein metabolism, and it is widely accepted that keratinocyte apoptosis is a major cause for spongiosis in lesional AD (Rebane et al., 2012). Here, we found enhanced expression of genes involved in cell death not only in the nonlesional skin of AD patients, regardless of *FLG* status, but also in the skin of IV patients, although more moderately (Figure 4a, and see Supplementary Tables S4–S8). Increased cell death in IV and in AD is mediated via both the intrinsic and extrinsic pathways (Figure 4a, and see Supplementary Tables S4–S7). Increased apoptosis observed in AD seems to originate from multiple cell death pathways, including oxidative stress and the IFN-γ and TNF-α signaling pathways (Rebane et al., 2012). Expression of *IFI27*, which encodes a protein that mediates IFN-induced apoptosis via the release of cytochrome C from the mitochondria and the activation of BAX and caspases 2, 3, 6, 8, and 9, is enhanced in AD skin, as reported previously (Cole et al., 2014), but not in IV skin (Figure 4a, and see Supplementary Tables S4–S7). The response to oxidative stress is markedly up-regulated in AD and moderately in IV (Figure 4b, and see Supplementary Tables S4–S7). Increased oxidative stress has already been observed in AD patients, including young children (Peroni et al., 2012; Tsuboi et al., 1998; Tsukahara et al., 2003). DNA damage can be induced by oxidative stress and leads to the up-regulation of genes involved in DNA repair as observed in AD skin and, to a lesser degree, in IV skin (Figure 4c, and see Supplementary Tables S4–S8). Thus, cell death, oxidative stress, DNA damage, and resulting autophagy are more pronounced in AD skin than in IV skin (Figure 4, and see Supplementary Tables S4–S8), similar to cellular metabolism and ATP production, suggesting a causal relationship. Moreover, the metabolism of xenobiotics not only generates deleterious metabolites but also by-products such as reactive oxygen species in the skin (Slominski et al., 2012). Thus, high cellular metabolism coupled with the metabolism of pollutants, endocrine disruptors, or other noxious chemicals in the skin may favor AD via reactive oxygen species production and deregulated lipid metabolism.

In conclusion, this work comparing the transcriptome profiles of nonlesional skin from AD patients to the skin of IV patients and of healthy control subjects uncovers a new potential pathogenic pathway involved in AD. Increased drug, pesticide, chemical, and endocrine disruptor metabolism in the skin, resulting from systemic or topical exposure, might trigger oxidative stress and abnormal lipid metabolism, especially, arachidonic, linoleic, and α-linolenic acid metabolism, hereby reaching a threshold favoring the transition from dry and scaly to inflamed AD skin. However, the complete mechanism remains to be elucidated.

METHODS

RNA sequencing

RNA extraction and quality control is described in the Supplementary Methods online. 50 ng of total RNA were amplified by applying the Ovation RNA-Seq System V2 (NuGen, Emeryville, CA). The resulting cDNAs were pooled in equal amounts and used to prepare the DNA fragment library with SOLiD System chemistry (Life Technologies, Carlsbad, CA). Sequencing was performed using the SOLiD 5500W platform and DNA sequencing chemistry (Life Technologies). Raw reads (75 base pairs) were color-space mapped to the human genome hg19 reference using the Maxmapper algorithm implemented in the Lifescope software (Life Technologies). Mapping to multiple locations was permitted. Reads with a score less than 10 were excluded. Average mapping quality was 30. Analysis of the RNA content and gene-based annotation was done with the whole-transcriptome workflow of Lifescope.

Data analysis

To identify significantly differentially expressed genes between patient groups, non-normalized mapped raw counts were used and analyzed using the R package DESeq2, which is based on a negative-binomial distribution. Two different group comparisons were performed: (i) comparison of AD, IV, and control samples and (ii) comparison of patient groups with *FLG* mutation status included: these are AD patients without *FLG* mutation (AD WT/WT), AD patients with *FLG* mutation (AD *FLG* MUT), IV patients, and control samples (Ctrl WT/WT). Different sequencing runs were considered by including a batch variable in the respective design. Genes with a mean of mapped reads greater than 10 were considered for further analyses. For comparison of more than two groups, likelihood ratio tests were performed; for pairwise group comparison, negative binomial Wald tests were performed. *P*-values were adjusted for multiple testing based on the false discovery rate according to the Benjamini-Hochberg method. Genes with at least a 2-fold change and an adjusted *P*-value less than 0.1 were considered as significantly differentially expressed. GO and pathways analyses were performed using the tool DAVID (Huang da et al., 2009) and ConsensusPathDB (Kamburov et al., 2011). Functional protein association networks were constructed using STRING based on the lists of differentially expressed genes (Szklarczyk et al., 2015). For further analysis (i.e., the principal component analysis) the remove Batch Effect of the R package limma on the regularized log-transformed normalized data were used. Heatmaps were visualized and hierarchical clustering analysis with average linkage were performed using

Genesis (Sturn et al., 2002) on gene-wise *z*-transformed data across all patient samples. Only variable genes with interquartile range greater than 0.8 were considered.

Statistical analysis

Statistical analyses for gene expression validation were performed using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA). Data are presented as mean ± standard error of the mean. Statistical significance was determined between groups using an one-way analysis of variance test followed by a Newman and Keuls post hoc test with $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$.

The study was approved by the Ethics Committee of the Medical University of Innsbruck and conducted in accordance with the Declaration of Helsinki principles. All study subjects gave written informed consent and participated voluntarily.

Data have been preceded and are now deposited in a public repository with an accession number (GSE102628). The entries are scheduled to be released on Nov 03, 2017.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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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.2017.08.036.

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