

Large-scale DNA microarray analysis of atopic skin lesions shows overexpression of an epidermal differentiation gene cluster in the alternative pathway and lack of protective gene expression in the cornified envelope

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Summary

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None declared.

Background Atopic dermatitis (AD)-specific genes have not yet been clarified.

Objectives To identify gene expression specific to active atopic skin lesions.

Methods We analysed 23 000 genes in skin biopsy samples from 17 patients with AD and four normal controls using Affymetrix oligonucleotide arrays.

Results Four of the 10 genes with the greatest differences in expression between patients and controls, S100A8 and S100A7 (upregulated), and loricrin and filaggrin (downregulated), were epidermal differentiation genes located on 1q21, a locus previously reported to have a genetic linkage with AD.

Conclusions Our results, showing downregulation of the cornified envelope genes and upregulation of the alternative keratinization pathway, are the first to suggest abnormal epidermal differentiation and defective defences as key abnormalities in AD.

Atopic dermatitis (AD) is a very common disease that is prevalent in up to 20% of the general population during childhood. Until now, the pathogenesis of AD has been attributed primarily to IgE elevation, and the disease has been classified as an atopic disease similar to allergic rhinitis or bronchial asthma. However, there have been few reports about skin-specific abnormalities in AD. Our large-scale microarray analysis of atopic skin lesions is the first to show overexpression of the epidermal keratin genes in the alternative pathway and lack of protective gene expression in the cornified envelope (CE). Four of the genes with the largest changes in expression, S100 calcium-binding protein (S100) A8, loricrin, S100A7 and filaggrin, are epidermal differentiation genes located on chromosome 1q21. Chromosome 1q21 is a common locus with genetic linkages to both AD and psoriasis,¹ and upregulation of the epidermal differentiation complex genes in psoriatic skin has been demonstrated in large-scale gene expression studies.² We discuss the differences in keratinocyte terminal differentiation mechanisms between AD and psoriasis. An understanding of the upregulation of the epidermal differentiation complex genes in active skin lesions of AD may provide new insight into the use of these genes as novel targets for the treatment of AD.

Patients and methods

Seventeen patients with AD (13 men and four women; mean age 27 years), who had not been treated with topical or systemic corticosteroids for at least 1 month prior to the examination, and four healthy controls (three men and one woman; mean age 34 years) were enrolled in this study after informed consent was obtained. All patients fulfilled the diagnostic criteria of Williams *et al.*,³ and the study protocol was approved by the Ethics Committee of Shiga University of Medical Science. Biopsy specimens were taken from involved skin of the 17 patients with AD and from normal skin of the four controls. Biopsies of normal-appearing skin were also taken from five of the 17 patients with AD. The specimens were sectioned in a cryostat for RNA extraction.

RNA preparation, DNA microarray experiment and statistical analysis

Total RNA was prepared from each biopsy specimen section using TRIzol (Invitrogen, Carlsbad, CA, U.S.A.) and an RNeasy Kit (Qiagen, Valencia, CA, U.S.A.), according to the manufacturers' protocols. The derived RNA was amplified as described

by Luo *et al.*⁴ and was used as a template for cRNA labelling. cRNA labelling and hybridization for the Gene Chip HG-U133A arrays (Affymetrix, Santa Clara, CA, U.S.A.) were carried out as described in the *Gene Chip Expression Analysis Technical Manual* (Affymetrix). We analysed 23 000 genes in skin biopsy samples from 17 patients with AD and four normal controls using Affymetrix oligonucleotide arrays.

Array images were quantified using Micro Array Suite 5.0 software (Affymetrix), and the cell files were used with the Affy 1.1 package (Bioconductor, <http://www.bioconductor.org>) to deduce expression values. In order to perform background correction, normalization, probe-specific correction and summarization, we used the algorithms *mas*, *invariantset*, *pmonly* and *liwong*, respectively. A Mann–Whitney U-test was performed to compare each gene between normal and AD samples, and those that showed a significant difference ($P < 0.05$) were chosen.

Results

We compared expression profiles between skin of healthy controls and lesional skin of patients with AD. Only 10 genes showed at least a fivefold difference in expression, with statistical significance ($P < 0.05$) as shown in Table 1. Among the 10 genes, four were epidermal differentiation genes located on 1q21. Of these four genes, S100A8 and S100A7 were

upregulated and loricrin and filaggrin were downregulated (Table 1). Among the 10 genes showing greatest differences in expression, three keratins, keratin 6A (12q13), keratin 6B (12q13) and keratin 16 (17q21), were upregulated (Table 1). In addition to these 10 genes, small proline-rich protein (SPRR) 1A, S100A2 and S100A9 were further 1q21 genes that were significantly upregulated (Table 1). Table 2 shows 20 representative CE-related genes, including epithelial host defence proteins and epithelial differentiation-associated keratins. Among the CE-related genes, the elafin (20q13), human β -defensin 2 (8p23) and SPRR2C (1q21) genes, which are epithelial host defence proteins, were not differentially expressed in atopic skin lesions. We also performed comparison between normal-appearing skin and lesional skin in the patients with AD. Interestingly, most of the significant changes mentioned above were observed in both comparisons (Tables 1 and 2), and seven of the 10 most changed genes were common to the two comparisons.

Discussion

The present study has clarified that four of the 10 genes with the greatest differences in expression, S100A8 and S100A7 (upregulated), and loricrin and filaggrin (downregulated), are epidermal differentiation genes located on 1q21. The 1q21 locus was previously reported as a locus with genetic linkage

Table 1 Ten genes that showed the greatest degrees of change and other significantly upregulated 1q21 genes in skin lesions of atopic dermatitis. Of the 10 most highly changed genes in comparison between normal controls and involved skin, S100A8 (upregulated 32-fold), loricrin (downregulated 6.5-fold), S100A7 (upregulated 6.0-fold) and filaggrin (downregulated 5.1-fold) were epidermal differentiation genes located on 1q21. Other significantly upregulated (at least 2.0-fold) genes in the 1q21 region included SPRR1A (3.7-fold), S100A2 (3.1-fold) and S100A9 (2.7-fold). Results of comparison between normal-appearing skin of patients with atopic dermatitis and involved skin are also shown in accordance with the order of the results of the normal controls vs. involved skin comparison

Gene name	Probe set	Accession	Location	Involved/normal		Involved/ normal-appearing	
				Fold change, direction	P-value	Fold change, direction	P-value
Ten most greatly changed genes							
S100 calcium-binding protein A8	202917_s_at	NM_002964	1q21.3	32, up	0.003	33. up	0.001
Keratin 6A	209125_at	J00269	12q13.13	7.5, up	0.003	4.5, up	0.001
	214580_x_at	AL569511	12q13.13	6.3, up	0.003	6.1, up	0.002
Serine proteinase inhibitor, clade B, member 13	211361_s_at	AJ001696	18q21.33	6.6, up	0.014	6.4, up	0.010
Loricrin	207720_at	NM_000427	1q21.3	6.5, down	0.006	3.5, down	0.008
S100 calcium-binding protein A7	205916_at	NM_002963	1q21.3	6.0, up	0.003	5.7, up	0.001
Chondroitin sulphate proteoglycan 2	221731_x_at	J02814	5q14.3	5.6, down	0.035	1.8, down	0.389
Keratin 6B	213680_at	AI831452	12q13.13	5.4, up	0.003	4.4, up	0.005
Fibronectin 1	211719_x_at	BC005858	2q35	5.1, down	0.028	1.7, down	0.638
Filaggrin	215704_at	AL356504	1q21.3	5.1, down	0.035	2.3, down	0.072
Keratin 16	209800_at	AF061812	17q21.2	5.0, up	0.003	4.1, up	0.001
Other significantly changed 1q21 genes							
Small proline-rich protein 1A	213796_at	AI923984	1q21.3	3.7, up	0.003	2.8, up	0.008
S100 calcium-binding protein A2	204268_at	NM_005978	1q21.3	3.1, up	0.006	2.7, up	0.008
S100 calcium-binding protein A9	203535_at	NM_002965	1q21.3	2.7, up	0.003	2.3, up	0.002

Table 2 Expression of cornified envelope-related genes in skin lesions of atopic dermatitis. In active skin lesions of atopic dermatitis, SPRR1A was upregulated (3.7-fold), other small proline-rich proteins were not changed, and loricrin (6.5-fold) and filaggrin (5.1-fold) were downregulated. In non-1q21 regions, keratins 6A (7.5-fold), 6B (5.4-fold) and 16 (5.0-fold) were upregulated. Overexpression of keratins 5, 14, 1 and 10, elafin (20q13) and human β -defensin 2 (8p23) was not detected. Results of comparison between normal-appearing skin and involved skin are also shown

Gene name	Probe set	Accession	Location	Involved/normal		Involved/normal-appearing	
				Fold change, direction	P-value	Fold change, direction	P-value
Cornified envelope components							
Filaggrin	215704_at	AL356504	1q21.3	5.1, down	0.035	2.3, down	0.072
Involucrin	214599_at	NM_005547	1q21.3	1.2, up	0.054	1.2, up	0.273
Loricrin	207720_at	NM_000427	1q21.3	6.5, down	0.006	3.5, down	0.008
Small proline-rich protein 1A	214549_x_at	NM_005987	1q21.3	1.2, up	0.098	1.2, up	0.072
	213796_at	AI923984	1q21.3	3.7, up	0.003	2.8, up	0.008
Small proline-rich protein 1B	205064_at	NM_003125	1q21.3	1.6, up	0.003	1.6, up	0.001
Small proline-rich protein 2B	208539_x_at	NM_006945	1q21.3	1.4, up	0.004	1.1, up	0.085
Small proline-rich protein 2C	220664_at	NM_006518	1q21.3	1.3, up	0.054	1.2, up	0.028
Small proline-rich protein 3	218990_s_at	NM_005416	1q21.3	1.2, down	0.098	1.1, down	0.531
Trichohyalin	213780_at	N30878	1q21.3	1.1, up	0.502	1.0, up	0.875
Epithelial host defence proteins							
β-defensin 2	207356_at	NM_004942	8p23.1	1.2, up	0.018	1.4, up	0.003
Elafin	203691_at	NM_002638	20q13.12	1.2, up	0.035	1.0, down	0.531
	41469_at	L10343	20q13.12	1.1, up	0.395	1.1, up	0.875
Epidermal differentiation-associated keratins							
Keratin 1	205900_at	NM_006121	12q13.13	2.3, down	0.502	1.1, up	0.875
Keratin 2A	207908_at	NM_000423	12q13.13	1.5, down	0.303	1.1, down	0.695
Keratin 5	201820_at	NM_000424	12q13.13	1.1, down	0.964	1.4, up	0.210
Keratin 6A	209125_at	J00269	12q13.13	7.5, up	0.003	4.5, up	0.001
	214580_x_at	AL569511	12q13.13	6.3, up	0.003	6.1, up	0.002
Keratin 6B	213680_at	AI831452	12q13.13	5.4, up	0.003	4.4, up	0.005
	209126_x_at	L42612	12q13.13	1.3, up	0.022	1.3, up	0.010
Keratin 10	207023_x_at	NM_000421	17q21.2	2.4, down	0.395	1.0, down	0.938
	210633_x_at	M19156	17q21.2	1.9, down	0.502	1.1, down	0.938
	213287_s_at	X14487	17q21.2	1.9, down	0.502	1.1, down	1.000
Keratin 14	209351_at	BC002690	17q21.2	1.1, down	0.823	2.5, up	0.100
Keratin 16	209800_at	AF061812	17q21.2	5.0, up	0.003	4.1, up	0.001
Keratin 17	205157_s_at	NM_000422	17q21.2	1.5, up	0.008	1.2, up	0.015
	212236_x_at	Z19574	17q21.2	1.4, up	0.011	1.4, up	0.019

to AD.¹ The normal CE consists of loricrin, SPRRs and filaggrin, along with keratins.⁵ We found that the downregulation of loricrin and filaggrin was accompanied by upregulation of some keratins in active skin lesions of AD. Our results suggest that deterioration of epidermal differentiation associated with altered expression of genes located on 1q21 might be a key abnormality in AD.

We also found that keratin 6 (locus 12q13) and keratin 16 (locus 17q21) were upregulated, whereas keratins 5, 14, 1 and 10 were not. Keratins 6 and 16 are markers of an alternative pathway of keratinocyte differentiation,⁶ and keratins 5, 14, 1 and 10 are expressed during the normal process of keratinization in normal skin.⁷ The alternative pathway, which is characterized by hyperproliferation and distinct differentiation from the normal pathway, is a functional physiological response to conditions that require rapid epidermal

regeneration and protective gene expression. Therefore, the present findings suggest that this alternative pathway may be inappropriately regulated in AD and may cause this pathological skin condition. On the other hand, the elafin (20q13) and human β -defensin 2 (8p23) genes, both epithelial host defence proteins,^{8,9} were not overexpressed in atopic skin lesions (Table 2). Consistent with our results, the expression of human β -defensin 2 mRNA in atopic skin lesions was reported to be significantly lower than in psoriatic lesions.^{10,11} Elafin is highly expressed in psoriatic epidermal keratinocytes compared with AD,¹¹ but is scarcely expressed in normal skin.¹² Nomura *et al.* recently reported that SPRR2C, which is a component of the CE and has a protective skin barrier function, showed the largest (11-fold) increase in psoriatic skin lesions as compared with AD.¹¹ SPRR2C showed only modest changes, if any, in involved skin of AD (Table 2). Taken

together, these results suggest that abnormal epidermal differentiation and defective defences are key abnormalities in lesions of AD. Similar changes in expression were also detected when normal-appearing skin and involved skin were compared in patients with AD (Tables 1 and 2). This further supports our hypothesis that abnormal epidermal differentiation and defective defences are key abnormalities in lesions of AD.

A similar upregulation of S100 genes located at 1q21 regions in both AD and psoriasis² might explain the remarkable findings of Cookson *et al.*, which showed a chromosome 1q21 linkage to both AD and psoriasis.¹ Compared with psoriasis,² in the present study the expression of S100A8 (32-fold increase) and S100A7 (sixfold increase) were prominent; however, S100A9 (31-fold increase) and S100A7 (12-fold increase) showed the greatest increases in psoriasis.² Besides the S100 genes, two CE-related genes, loricrin and filaggrin, were substantially downregulated in AD, which was not observed in psoriasis.² Therefore, these differences in expression of 1q21 genes might play a role in the development, at least in part, of the distinctly different appearance of skin lesions in AD and psoriasis.

It is well known that several chromosomal abnormalities occur on human chromosome 1q21.¹³ Genomic sequencing of epidermal differentiation complex genes in the 1q21 region is needed to identify mutations involved in AD. Furthermore, clear identification of the locus of abnormal differentiation genes specific to the disease could ultimately lead to the identification of novel targets for developing treatments.

References

- 1 Cookson WO, Ubhi B, Lawrence R *et al.* Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. *Nature Genet* 2001; **27**:372–3.
- 2 Bowcock AM, Shannon W, Du F *et al.* Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. *Hum Mol Genet* 2001; **10**:1783–805.
- 3 Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's diagnostic criteria for atopic dermatitis III. Independent hospital validation. *Br J Dermatol* 1994; **131**:406–16.
- 4 Luo L, Salunga RC, Guo H *et al.* Gene expression profiles of laser-captured adjacent neuronal subtypes. *Nature Med* 1999; **1**:117–22.
- 5 Steinert PM, Marekov LN. The proteins elafin, filaggrin, keratin intermediate filaments, loricrin, and small proline-rich proteins 1 and 2 are isopeptide cross-linked components of human epidermal cornified cell envelope. *J Biol Chem* 1995; **270**:17702–11.
- 6 Olsen E, Rasmussen HH, Celis JE. Identification of proteins that are abnormally regulated in differentiated cultured human keratinocytes. *Electrophoresis* 1995; **16**:2241–8.
- 7 Fuchs E. Epidermal differentiation: the bare essentials. *J Cell Biol* 1990; **111**:2807–12.
- 8 Pol A, Pfundt R, Zeeuwen P *et al.* Transcriptional regulation of the elafin gene in human keratinocytes. *J Invest Dermatol* 2003; **120**:301–7.
- 9 Dinulos JGH, Mentele L, Fredericks LP *et al.* Keratinocyte expression of human β -defensin 2 following bacterial infection: role in cutaneous host defense. *Clin Diagn Lab Immunol* 2003; **10**:161–6.
- 10 Ong PY, Ohtake T, Brandt C *et al.* Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 2002; **347**:1151–60.
- 11 Nomura I, Gao B, Boguniewicz M *et al.* Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol* 2003; **112**:1195–202.
- 12 Nakane H, Ishida-Yamamoto A, Takahashi H, Iizuka H. Elafin, a secretory protein, is cross-linked into the cornified cell envelopes from the inside of psoriatic keratinocytes. *J Invest Dermatol* 2002; **119**:50–5.
- 13 Heizmann CW, Fritz G, Schaefer BW. S100 proteins: structure, functions and pathology. *Front Biosci* 2002; **7**:1356–68.