therapies. In addition, the slight photoprotective effect seen with commercial creams containing idebenone may be due to the sunscreen ingredients that they contain. Idebenone specifically does not increase the photoprotective value of an established antioxidant combination of C + E + ferulic acid. The results of this study also validate an earlier study showing that C + E + ferulic acid offers eight-fold UV photoprotection to skin (Lin *et al.*, 2005).

CONFLICT OF INTEREST

Joshua Tournas is on the speaker's board for SkinCeuticals Inc., Garland, TX, USA.

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DISCLOSURES

Joshua Tournas, MD, is on the speaker's board for SkinCeuticals, Inc., Garland, TX, USA.
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Identification of the Target Genes of Atopic Dermatitis by Real-Time PCR

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TO THE EDITOR

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease by typically distributed eczematous skin lesions with lichenification, pruritic excoriation, and severe dry skin. During the past year, there have been some significant advances concerning our understanding of the cellular and immunologic mechanisms that underlie AD as well as the immunologic triggers involved in its pathogenesis. Complex interactions among a multitude of genetic, environmental, skin barrier, pharmacologic, and immunologic factors contribute to the pathogenesis of AD (Kay, 2001; Cookson, 2004; Leung *et al.*, 2004). In addition to the epidermal differentiation complex on chromosome 1q21, several candidate genes located on different chromosomes have been introduced via linkage analyses (MacLean and Eidelman, 2001; Bowcock and Cookson, 2004).

The recent development of gene knockout and transgenic mouse models may contribute to developing effective drugs and to elucidating AD's associated factors (Heinzmann and Daser, 2002). The techniques used in proteomics and genomics have allowed researchers to understand many diseases and disease-associated factors regardless of their drawbacks (Banks *et al.*, 2000; de Hoog and Mann, 2004; Kramer and Cohen, 2004). Several techniques to identify the differentially expressed genes have been established, such as differential display PCR, representational difference analysis, cDNA microarray, serial analysis gene expression and suppression subtractive hybridization. To gain understanding on the biological implication of AD, we first explored specific genes by performing SSH and then chose several genes from these data to performe real-time RT-PCR.

Abbreviations: AD, atopic dermatitis; SSH, suppression subtractive hybridization

The AD samples were collected from nonasthmatic atopic patients according to our previous reports (Jeong *et al.*, 2003; Park *et al.*, 2004). The nonatopic control samples were selected and taken from the foreskins and skin from plastic surgery of adults who did not have a personal/family history or any sign of AD. None of the subjects had received systemic or topical corticosteroids, or immunomodulators for at least 2 weeks prior to the study. As a criterion for AD patients, we checked the serum IgE levels. The serum IgE levels of all 22 AD patients were from 250 to over 20,000 IU/ml by the Cap system (Pharmacia Biotech, Buckingham, UK, Average 3,700 IU). All the patients showed a positive prick test to dust and mites. Prior to performing the subtraction library construction, we checked the IL-4 expression levels in the AD skin tissues. The IL-4 mRNA levels were expressed in all AD samples, but this was not detected in the normal samples (data not shown). The mean SCORAD (SCORing Atopic Dermatitis) indexes of AD for SSH (8 patients) and real-time PCR (14 patients) were 45.7±13.4 and 53.6±17.6. Punch biopsies 5 mm in diameter were individually obtained from the lesional skin in the posterior thigh or lower back of AD and psoriasis patients. The medical ethical committee of the Samsung Biomedical Research Institute approved all described studies. This study was conducted according to the Declaration of Helsinki Principles and written informed consent obtained from all participants.

Table 1. List of genes subjected into real-time RT-PCR

Gene name	Function	Accession no.
Sphingosine-1-phosphate lyase 1 (SPL)	Lipid metabolism	BC052991
Proline endopeptidase (PREP)	Proteolysis	NM_002726
Cathepsin B (CTSB)	Immunoregulatory and inflammatory process	NM_004887
Coxsackie virus and adenovirus receptor (CXADR)	Virus receptor	AF242865
Delta sleep-inducing peptide, immunoreactor (DSIP)	Immunoregulatory and inflammatory process	BC018148

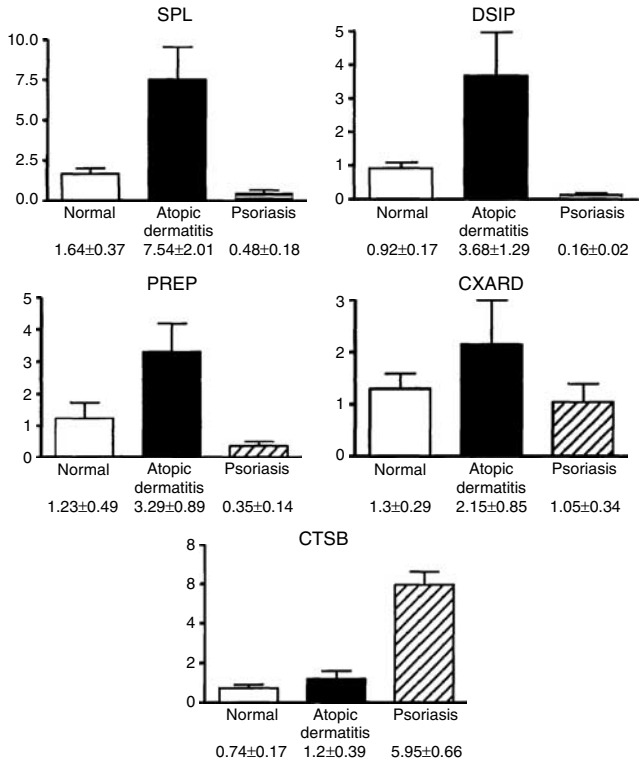

Figure 1. Real time RT-PCR reveals the selected clones from the SSH library. The total RNA was isolated from 14 different skin biopsy samples from AD patients, 10 samples from psoriasis patients, and 10 samples from normal controls. Each bar shows the relative gene expressions of the target genes compared to the expression of the β-actin gene. The results are means±SEM.

For the SSH, we used one normal control and eight AD samples. The cDNAs were synthesized from 2 μg of total RNA by using a Super SMART PCR cDNA kit and the SSH was performed with a CLONTEC PCR-Select cDNA Subtraction kit (BD Bioscience, Palo Alto, CA) according to the manufacturer's protocol. Approximately 1,000 clones from the SSH library were arrayed in duplicate by spotting them onto nylon membranes, and they were screened by reverse Northern blotting using the subtracted cDNA probes. After sequencing and the database searches, the genes could be divided into two groups: those with known functions and those with unknown functions. We found 450 different genes, and then excluded the unidentified and ribosomal genes and, finally, we selected 134 genes (http://www.sdgrc.re.kr/). Most of the genes with known functions could be classified into 12 groups based on their functions. To support and complement the result of SSH screening, five genes were selected to confirm their expression levels. We used real-time RT-PCR to check their expression levels among AD patients, psoriasis patients, and normal control subjects. We recruited 10 psoriasis patients with typical skin lesions who had not received any treatment for at least 2 weeks and also used 10 normal control samples. Five genes of special interest, such as sphingosine-1-phosphate lyase, cathepsin B, coxsackie virus and adenovirus receptor, prolyl endopeptidase and delta sleep inducing peptide were selected for their potential functionality with AD as based on the references search (Table 1).

As the AD patients have a reduced skin barrier function and dry skin, and sphingosine-1-phosphate lyase, which are related to lipid metabolism, they

were chosen for validation. Intense pruritus is another major feature of AD. The pathogenesis of cutaneous pruritus is not well understood, but it is thought to be induced by the various products from the inflammatory effector cells, including neuropeptides, histamine, leukotrienes, and proteolytic enzymes. The prolyl endopeptidase and cathepsin B might be genes responsible for producing the inflammatory agents. Finally, coxsackie virus and adenovirus receptor and delta sleep inducing peptide are genes that were found repeatedly in the SSH library and their expression levels were confirmed. Five genes showed increased expression levels in the AD samples compared to normal control samples (Figure 1). The coxsackie virus and adenovirus receptor, prolyl endopeptidase, delta sleep inducing peptide, and sphingosine-1-phosphate lyase genes showed markedly increased expression levels in the AD samples compared to the normal and psoriasis samples, but their expression levels in the psoriasis samples were lower than in the normal control samples. CTBS was the only gene that had a greater expression in the psoriasis samples than in the AD and normal samples (Figure 1).

In conclusion, this study was the first attempt to identify differentially expressed genes in lesional AD skin to help increase our understanding of AD with its complicated disease mechanism. Although the known and unknown genes found by using the SSH method may be helpful in understanding the complicated atopic pathophysiology, there is a drawback that we used only one normal sample. However, among the 5 genes that were examined at the transcription level, sphingosine-1-phosphate lyase, coxsackie virus and adenovirus receptor, prolyl endopeptidase, and delta sleep inducing peptide are genes that have not yet been studied for the other skin diseases as well as AD. Therefore, besides the known factors, these genes are thought to provide an important clue in analyzing the complex mechanism of dry skin and itching. In recent years, single-nucleotide polymorphism studies have made active progress to grasp the individual genetic causes of AD. However, most of the studies focused on the known genes. Thus, we adopted several tools to cover as many genes as possible and complement the significance of the selected genes with the SSH method. We are currently in progress on an SNP study centering on the genes included in the SSH library. We hope that the selected genes can be used for the treatment and diagnosis of this common allergic skin disease.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Elevated Serum CTACK/CCL27 Levels in CTCL

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TO THE EDITOR

Cutaneous T-cell-attracting chemokine (CTACK), also called CCL27, belongs to the CC chemokine family and is a ligand for CC chemokine receptor (CCR) 10. It is selectively and constitutively produced in skin by epidermal keratinocytes (Morales *et al.*, 1999) and displayed on the surface of dermal endothelial cells (Homey *et al.*, 2002). It selectively attracts cutaneous lymphocyte antigen positive, CCR10-positive memory T cells into inflammatory sites (Morales *et al.*, 1999). We and other researchers previously reported that serum levels of this chemokine reflect disease activity of atopic dermatitis (Kakinuma *et al.*, 2003a; Hijnen *et al.*, 2004; Hon *et al.*, 2004).

Abbreviations: CCR, CC chemokine receptor; CTACK, cutaneous T-cell-attracting chemokine; CTCL, cutaneous T-cell lymphoma; MF, mycosis fungoides; TBI, tumor burden index