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Skin Tape Stripping Identifies Gene Transcript Signature Associated with Allergic Contact Dermatitis

Idy Tam^{1,2}, Kathryn R. Hill³, Jin Mo Park^{3,4}, JiaDe Yu^{2,4}

¹Tufts University School of Medicine, Boston, MA

²Department of Dermatology, Massachusetts General Hospital, Boston, MA

³Cutaneous Biology Research Center, Massachusetts General Hospital, Boston, MA

⁴Harvard Medical School, Boston, MA

Abstract

Background: Allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) are common skin conditions with overlapping clinical and histologic appearance but distinct underlying mechanisms. Patch testing is the gold standard for ACD diagnosis, yet the interpretation of its results may be confounded by weak and varying macroscopic reactions.

Objective: To examine whether gene transcript profiling of RNA sampled from patch-tested patient skin by tape stripping (TS) could differentiate ACD from ICD and the baseline skin state (control; CON).

Methods: Nine patients (7 females and 2 males; age 24–72 [mean 38.6]) with confirmed ACD through patch testing were recruited. Total RNA was isolated from TS samples and relative transcript abundance was determined by quantitative real-time PCR using 39 gene-specific primers.

Results: TS captured gene transcripts derived from diverse skin cell types including not only keratinocytes but also epidermal and dermal antigen-presenting cells. Among the genes analysed in transcript profiling, genes encoding epidermal barrier components and inflammatory mediators exhibited changes in transcript abundance in ACD skin compared to ICD or CON skin.

Conclusions: Our findings reveal the potential of skin TS for non-invasive biopsy during patch testing and molecular marker-based ACD diagnosis.

Keywords

Allergic contact dermatitis; irritant contact dermatitis; non-invasive method; tape strip skin biopsy; keratinocyte; RNA analysis

Corresponding Author: JiaDe Yu, MD, Department of Dermatology, Massachusetts General Hospital/ Harvard Medical School, Boston, MA 02114, Jiade.yu@mgh.harvard.edu.

Conflicts of interest:

J.Y. is on the board of the American Contact Dermatitis Society. J.M.P. is a consultant of Chong Kun Dang Pharmaceutical.

1. INTRODUCTION

Allergic and irritant contact dermatitis (ACD and ICD, respectively), together, is the 5th most common reason for dermatology consultation in the United States and the leading cause of occupational dermatoses.¹ While ICD is a nonspecific localized skin reaction caused by direct contact with irritants, ACD is a specific T cell-mediated hypersensitivity reaction that occurs with exposure to specific contact allergens.² The exact pathogenesis of ACD is not well understood but it is accepted that ACD occurs in two distinct phases: sensitization and elicitation. During the sensitization phase, contact allergens conjugate with proteins in the epidermis and are captured by skin-resident antigen-presenting cells (APC) leading to the priming of allergen-specific T cells and their differentiation into effector cells in the skin-draining lymph nodes.^{3,4} Upon re-exposure to the allergen, the primed effector T cells are recruited to the allergen-contacted skin and promote inflammation, leading to the clinical manifestation of ACD.^{3,4} In contrast to ACD, ICD does not entail adaptive immune components and is mainly driven by innate immune responses to irritant-induced skin tissue damage.² Although patch testing is the gold standard for diagnosis of ACD, it often fails to discriminate between ACD and ICD, as weak ACD reactions appear similar to ICD. In addition, the interpretation of patch test results is confounded by weak and at times, false-positive irritation, false-negative results, and patient factors such as immunosuppression that may affect final results. Crucial to addressing these limitations is the development of more sensitive and objective methods for assessing patch test reactions. Molecular marker-based diagnostic methods likely respond to this imperative, enabling more reliable ACD diagnosis and accurate identification of the contact allergen for each tested patient.

Tape stripping (TS) is a non-invasive method that has been used to study immune and barrier abnormalities in patients with atopic dermatitis.⁵⁻⁷ Compared to skin biopsies, TS is painless, does not lead to scarring, and can therefore garner increased patient acceptance. In this study, we sought to establish a protocol with which to isolate RNA from patch test sites by skin TS. Using tape strip-isolated RNA, we performed gene transcript profiling to identify molecular markers that allow us to differentiate ACD from ICD and the baseline skin state (control; CON).

2. METHODS

2.1 Patients and Patch Testing

All patients were patch tested to the North American Baseline-80 series (Dormer-Chemotechnique, Villenge, Sweden), an irritant of 2% and/or 4% sodium lauryl sulfate (SLS) (Chemical Connection) and negative vehicle control of 100% petroleum jelly. The patch tests were applied on patients' upper back, marked at the borders with skin markers to identify test sites, and affixed with Scanpor tape (Norgesplaster Alpha AS, Vennessla, Norway). Patches were removed on D2 after placement and final reading was performed on D4 after placement. The interpretation of patch testing results was performed in accordance with the International Contact Dermatitis Research Group recommendations.⁸ Patients with at least one positive ACD reaction on patch testing were enrolled. This study was approved by the Partners Healthcare Institutional Review Board.

2.2 Skin TS and primary human cells

Tape strips (D-Squame, 14.0 mm in diameter; CuDerm, Dallas, TX) were applied on the same site with gentle pressure for 5 to 10 seconds and removed with forceps. Twenty consecutive tape strips were collected from 13 ACD samples, 10 ICD samples, and 10 CON samples (vehicle [petrolatum]-applied samples from ACD patients) from 9 patients on D4. Each skin site displayed easily discernible positive allergic reactions as well as from the vehicle (petrolatum)-applied site. First 10 tape strips were discarded. Strips #11 through #20 were individually placed into tubes and immersed in 0.1 ml of Trizol reagent (Thermo Fisher Scientific) per strip. The tubes were vigorously agitated with a vortex mixer to maximize their exposure to the Trizol reagent. Primary keratinocytes (Gibco human epidermal keratinocytes from adult skin; Thermo Fisher Scientific) and peripheral blood mononuclear cells, purified by Ficoll gradient centrifugation from leukopaks (Massachusetts General Hospital Blood Donor Center), were used as cellular sources of RNA.

2.3 RNA analysis

Trizol extracts from the same skin site were combined and processed for RNA isolation according to the manufacturer's instruction (TRI Reagent Solution). Purified total RNA was subjected to automated TapeStation electrophoresis (Agilent) and converted into cDNA synthesis using the SuperScript IV VILO Master Mix (Thermo Fisher Scientific). Relative transcript abundance was determined by qPCR using the SYBR Green PCR Master Mix (Applied Biosystems) and gene-specific primers (Table 2).

2.4 Immunohistochemistry images

The immunohistochemistry images showing the distribution of specific proteins in human skin sections were obtained from the Human Protein Atlas and presented according to its guidelines.⁹

2.5 Statistical analysis

Data normality and log-normality were assessed using the Shapiro-Wilk test and confirmed in a QQ plot. *P* values were obtained by the paired *t*-test and the unpaired two-tailed Student's *t*-test with Welch's correction. The criterion of false discovery rate less than 0.1 was used to identify discoveries in multiple *t*-tests. Principal component analysis (PCA) and hierarchical clustering analysis were performed using the ClustVis web tool¹⁰ and the Morpheus software (Broad Institute), respectively.

3. RESULTS

3.1 Characterization of RNA obtained by skin TS

To assess the feasibility of using tape strip-isolated specimens for gene transcript profiling, we extracted RNA from tape strips collected from CON skin and examined their suitability to serve as a template for cDNA synthesis. To obtain information on the composition of RNA pools extracted from skin tape strips, we first determined their size ranges by automated TapeStation electrophoresis. Compared to total RNA isolated from primary keratinocytes and peripheral blood mononuclear cells, which contained transcripts with sizes

expected of intact 28S and 18S rRNA, tape strip-isolated RNA from unprovoked skin (CON samples) ranged in size from 20 to 200 nucleotides and mainly consisted of small fragments (Figure 1A). We next examined whether gene transcripts in skin-resident cells were sampled by TS and contributed to the repertoire of the RNA preparations. To this end, cDNA synthesized from tape strip-isolated RNA was analysed by quantitative real-time PCR (qPCR) using primers designed to generate 50–60 base-pair amplicons. This analysis revealed that skin TS sampled gene transcripts derived from diverse cell types (Figure 1B), including those associated with keratinocytes in both the suprabasal and the basal epidermal layers (*FLG*, *KRT10*, and *KRT14*) as well as with dermal hematopoietic-derived immune cell types (*ITGAM* and *FCGR3A*). We applied this tape strip-qPCR workflow to sampling skin sites in ACD patch tests and analysing their transcript profiles.

3.2 Analysis of gene transcript abundance in tape strip samples from CON, ICD and ACD skin

Patients recruited for this study underwent patch testing for evaluation of suspected ACD from June 2019 through January 2020. Tape strip samples were obtained from 9 adults (7 females and 2 males; age 24–72 [mean 38.6]) who had at least one positive reaction on patch testing (Table 1). Four out of nine patients (44.4%) reported a history of atopic dermatitis. All patients were treated with topical immunosuppressive agents (eg. corticosteroids and/or calcineurin inhibitors) only and none of the patients received any systemic immunosuppressive treatments prior to patch testing. Certain patients reacted to more than one allergen. Multiple positive patch test reactions in a patient were independently subjected to TS and analysed in parallel.

We sought to determine whether ACD skin sites showed a unique signature of gene transcript abundance that differentiated them from CON and ICD sites. For this investigation, we used a panel of qPCR primers for 39 genes that encoded markers of cell lineage and differentiation, immune effector function, and inflammation (Table 2). Transcript abundance for these genes relative to 18S rRNA abundance in tape strip-isolated RNA was calculated from qPCR amplification plots. We first compared the amounts of gene transcripts in the CON, ICD and ACD skin site in each patient. This within-patient paired analysis led us to identify four genes whose transcript abundance was significantly different ($P < 0.05$) in the three skin sites (Figure 2A). Of these genes, *LOR*, *KRT6A* and *MMP13* transcript abundance was lower in ACD compared to CON skin (Figure 2B). Genes showing a significant difference in ICD versus CON skin were also identified: the transcript abundance of *KRT17* was higher and that of *MMP13* lower in ICD compared to CON skin (Figure 2B). None of the genes examined showed a significant difference in ACD-ICD comparison. Of note, nine genes (*CD274*, *CXCL5*, *ITGAM*, *KRT14*, *LCE5A*, *LOR*, *RSAD2*, *S100A8*, and *S100A9*) displayed a trend of difference ($P < 0.1$) in the paired analysis, including in ACD-ICD comparison (*CD274*, *KRT14*, *LCE5A*, and *LOR*; Figure 2A).

In a second approach to identifying gene transcripts with differential abundance, we pooled the qPCR data obtained from the CON, ICD and ACD sample groups and compared their values without within-patient pairing. This between-group unpaired analysis revealed significant differences ($P < 0.05$) in *LOR* transcript abundance in ACD-CON and ACD-ICD

comparison (Figure 3A and 3B). In addition, three genes (*KRT14*, *KRT6A*, and *LCE5A*) exhibited a trend of difference ($P < 0.1$) in this unpaired analysis. Taken together, these results showed that TS could capture molecular signatures uniquely associated with ACD skin, and that specific gene transcripts in tape strip-isolated RNA could help identify ACD reactions in patch tests independently of or in conjunction with macroscopic clinical evaluation.

3.3 Assessment of heterogeneity in the pattern of gene transcript abundance in ACD skin

The ACD samples investigated in this study, 13 in total, were obtained from the back of nine patients that were reactive to 10 different allergens: nickel, potassium dichromate, fragrance mix I, cinnamal, hydroperoxides of linalool, methylisothiazolinone, quaternium-15, 4-tert-butylphenolformaldehyde resin, thiuram mix, and shellac. Although the ACD samples as a group displayed a shared pattern of gene transcript abundance that set them apart from the CON and ICD samples, individual ACD samples may be dissimilar in their transcript profiles for the entire set of genes examined. We performed hierarchical clustering analysis of the 12 ACD samples for which the full qPCR data set is available and found substantial heterogeneity in their transcript profiles (Figure 4A). In this analysis, the ACD samples showed a trend toward division into two clusters, each having similar transcript profiles among the cluster members. A PCA scatter plot constructed from the same data set also revealed segregation and clustering among the ACD samples (Figure 4B). An identical subset of ACD samples (7c, 7d, 8c, and 9c) grouped together in both hierarchical clustering and PCA. The clusters identified in either analysis, however, did not correlate with allergen type or other discernible clinical features associated with the ACD samples. Analysis on a larger scale involving greater patient and gene numbers would be essential to capture such correlations. In summary, our study revealed a correlative signature of gene transcript abundance specific to ACD skin sites as well as heterogeneity in their transcript profiles, both detected by non-invasive TS.

4. DISCUSSION

Our study highlights the ability of non-invasive TS to sample RNA from patch-tested skin sites as well as the potential for differentiating ACD skin from ICD and normal skin through gene transcript profiling. The current gold standard of ACD diagnosis relies on patch testing done in a specialized clinic; this test method, however, may not be sufficient for unequivocal differentiation between ACD and ICD, which have different implications for the patient. Our study identified specific genes whose transcript abundance was linked to ACD and could help us differentiate between the two diagnoses in the setting of patch testing.

Molecular marker-based analysis of skin biopsies hold potential for improving diagnostic accuracy in dermatology, but routine sampling of skin tissue through an invasive biopsy is unrealistic and undesirable with regards to patch testing and the diagnosis of ACD. The goal of this study was to establish a workflow for non-invasive isolation of RNA from patch test sites and assess the feasibility of profiling gene transcripts using the isolated RNA. We found that tape strip-isolated skin RNA contained gene transcripts expressed in cells that not only constituted the superficial skin layer but also resided in deeper epidermal and dermal

areas. This observation does not necessarily indicate that cells situated in the basal epidermis and dermis as such can be sampled by TS. It is more likely that RNA released from or trapped in dead or dying cells during epidermal and dermal turnover is transported toward the desquamating end of the epidermis and becomes accessible to TS. The RNA cargo carried by extracellular vesicles such as exosomes shed from living epidermal and dermal cells¹¹ may disperse toward the skin surface and serve as an alternative source of tape strip-isolated RNA. These possibilities need to be substantiated by further research. In the analysis involving within-patient sample pairing, we identified *LOR* (cornified envelop protein), *KRT6A* (hyperproliferative/activated epidermal keratin), and *MMP13* (matrix metalloproteinase) as potential markers that could differentiate ACD skin from CON skin based on their transcript abundance in tape strip-isolated RNA. The potential of *LOR* as an ACD-associated marker was also revealed in the unpaired comparison of transcript abundance. Moreover, *CD274* (negative regulator of T-cell immunity), *KRT14* (basal epidermal keratin), and *LCE5A* (cornified envelop protein) have emerged as candidates for further investigation into their usefulness in ACD versus ICD discrimination: their transcript abundance showed a trend of difference in our paired and unpaired comparison without reaching a statistical significance. Our observation of diminished *LOR* transcript abundance in tape strip-isolated RNA from ACD skin is also in line with the recent report of epidermal barrier disruption and decreased *LOR* expression in skin exposed to *p*-phenylenediamine, a potent contact allergen.¹⁰ It should be noted, however, that certain contact allergens, including those examined in this study (Table 1), can induce an irritant reaction as well. Therefore, we do not rule out the possibility that such an irritant-like effect of allergens might have contributed to the ACD-associated transcript changes observed in our study. Besides being candidates for molecular markers of ACD reactions in patch tests, the genes found to be associated with ACD skin in our study suggest altered activities of keratinocytes in skin reactive to ACD allergens.

Limitations of our current study include the heterogeneous patch test reactions and degree of positivity in our cohort. In addition, transcriptome-wide analysis, for example by RNA sequencing, was not performed in this pilot stage. Furthermore, our sampling by TS was confined to the superficial epidermal layer and did not directly access deeper skin areas where many immune-related gene products are formed and exert their activity. It should be noted that all patients received topical immunosuppressive agents (eg. corticosteroids and/or calcineurin inhibitors) prior to patch testing; this might have affected transcript profiles in our study. Nevertheless, our findings demonstrate that TS sampling offers a non-invasive approach to isolating and analysing RNA derived from diverse skin cell types. It remains unclear whether TS achieves comparable sampling of CON, ICD and ICD skin, which likely have dissimilar structural properties and biochemical features. These factors can influence the efficiency of RNA isolation by TS and should be controlled by improved methodologies.

5. CONCLUSIONS

The identification of molecular markers that can differentiate ACD from CON and ICD reactions in patch tests can devise strategies for improving the diagnosis of ACD in patients. Furthermore, these markers, if mechanistically linked to ACD pathogenesis, may reveal as-yet-undiscovered molecular events underlying the disease; such knowledge will help develop

topical or systemic therapeutics aimed at treating ACD in patients who cannot avoid the allergenic trigger due to lifestyle or occupation. As we adopted a targeted molecular profiling based on selected candidate genes that had known implications in the development of ACD, additional markers remain to be determined. More comprehensive gene transcript profiling such as involving RNA sequencing should be able to elucidate such markers. It is also of interest to seek markers that are not just diagnostic for ACD but also associated with skin reactions to specific allergens. Future efforts will be focused on stratifying patient reactions according to allergens and expanding sample analysis for appropriately powered studies to identify allergen-specific markers. Findings from these efforts will advance the current diagnostic methods for patients suffering with ACD and have significant implications for the development of targeted therapeutics for these patients.

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Abbreviations:

ACD	allergic contact dermatitis
CON	vehicle-control skin
D	day
ICD	irritant contact dermatitis
PCA	principal component analysis
qPCR	quantitative real-time PCR
SLS	sodium lauryl sulfate
TS	tape stripping

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Figure 1. Characterization of RNA isolated from skin tape strips

(A) RNA isolated from cultured primary keratinocytes, peripheral blood mononuclear cells, and skin tape strips (Kc, PBMC, and TS, respectively) was analysed by TapeStation electrophoresis. Gel images (left) and electropherograms (right) from this analysis are shown with the positions of size markers and 28S/18S rRNA indicated on the left and top, respectively.

(B) Images of immunohistochemistry of human skin sections (top) and gene expression data from qPCR analysis of the indicated samples (bottom) are shown. The immunohistochemistry images were from the Human Protein Atlas. Individual qPCR data values (open circle) are plotted together with their medians (line).

Figure 2. Paired analysis of gene transcript abundance in tape strip samples from the CON, ICD and ACD skin sites within individual patients

Tape strip-isolated RNA from CON, ICD and ACD skin was analysed by qPCR using a panel of genes representing various cell types in the skin and their distinct differentiation and functional states. qPCR data from the CON, ICD and ACD samples of each patient were matched for within-patient paired comparison.

(A) Differential abundance of the indicate gene transcripts is shown as P values obtained by the paired t -test. Black and gray dotted lines denote $P = 0.05$ and 0.1 , respectively. Arrows indicate gene transcripts whose abundance is significantly different ($P < 0.05$; black arrows) or shows a trend of difference ($P < 0.1$; gray arrows) in between the CON, ICD and ACD sites of each patient.

(B) Gene transcript abundance in individual sample pairs (circle) is shown with P values obtained by the paired t -test.

Figure 3. Unpaired analysis of gene transcript abundance in tape strip samples between the CON, ICD and ACD groups

Tape strip-isolated RNA from CON, ICD and ACD skin was analysed by qPCR as in Figure 2. qPCR data in each of the CON, ICD and ACD groups were pooled for between-group unpaired comparison.

(A) Differential abundance of the indicate gene transcripts is shown as P values obtained by the unpaired t -test. Black and gray dotted lines denote $P=0.05$ and 0.1 , respectively. Arrows indicate gene transcripts whose abundance is significantly different ($P<0.05$; black arrows) or shows a trend of difference ($P<0.1$; gray arrows) in between the CON, ICD and ACD groups.

(B) Gene transcript abundance in individual samples is shown with P values obtained by the unpaired t -test. Individual data values (triangle) are shown together with their median (line).

Figure 4. Heterogeneity in the pattern of gene transcript abundance among ACD skin samples
 (A) qPCR data from individual ACD tape strip samples were subjected to hierarchical clustering. Dendrograms on the top and left of the heat map indicate their cluster hierarchies.
 (B) Principal component analysis was performed on ACD tape strip samples based on the qPCR data for the entire gene set.

Table 1.

Patient Characteristics and Patch Test Results

Patient	Age	Sex	ICD (SLS%)	Allergen, patch test reaction	Strength of Reaction
1	31	F	2%	Methylisothiazolinone	+
2	45	F	2%	Quaternium-15	+
3	25	F	2%	Nickel	++
4	36	M	2%	Hydroperoxides of linalool	+/-
5	29	M	2%, 4%	Thiuram mix	+
				Fragrance mix I	+
				Cinnamal	+
6	24	F	4%	4-tert-butylphenolformaldehyde resin	+
				Nickel	+
7	53	F	4%	Potassium dichromate	+
				Methylisothiazolinone	+
8	32	F	4%	Nickel	+
9	72	F	4%	Shellac	+

SLS sodium lauryl sulfate, +/- questionable/weak positive, + positive, ++ strong positive, +++ extreme positive

Table 2.

Oligonucleotide primers used in the study

Gene	Forward (5' to 3')	Reverse (5' to 3')
<i>18S rRNA</i>	gcttaattgactcaacacggga	agctatcaatctgtcaatcctgtc
<i>ALOX12</i>	ccagaagcatcgagagaagg	agggtggcccagcagtagat
<i>CCL20</i>	tttattgtgggttcacacg	gcattgatgtcacagccttc
<i>CD14</i>	ttgtgagctggacgatgaag	ctgagggtcggagaagtgc
<i>CD1A</i>	tggctgagtgatttcagac	gaaaacgatgggtcgtggaat
<i>CD1C</i>	cctcaagcccatttctga	ggtcaagggaagatcgttggga
<i>CD274</i>	tatgggtgtgccgactaaa	ttgtttgatggggcattga
<i>CD68</i>	tggcctgtaatcccagctac	tcctgggtcaagcagttct
<i>CDH1</i>	gaacagcacgtacacagccct	gcagaagtgtccctgttccag
<i>CXCL10</i>	aaaccagaggggagcaaat	cctctgtgtgtccatcctt
<i>CXCL2</i>	aatggcaatccaactgacc	cttcaggaacagccaccatt
<i>CXCL3</i>	atccccatggttcagaaa	gggtgtccccctgtttagta
<i>CXCL5</i>	gcaaggagttcatccaaaa	ctatggcgaaactgtgcaga
<i>CXCL8</i>	atgactccaagctggcgtggct	tctcagccctcttcaaaaactctc
<i>CXCL9</i>	tttctcttgggcatcac	tactgggttccctgtcactc
<i>FCGR3A</i>	caaccagaagcacacaggaa	ggccaagaaagcattttcac
<i>FLG</i>	tgcagatgaagcttgccac	cagcagacagctccagacac
<i>IFIT1</i>	gcccagacttacctggacaa	agggatttgaagcttcttgc
<i>IFNB1</i>	tgggaggattctgcattacc	ctatgggtccaggcacagtgc
<i>IL1A</i>	aatgacgccctcaatcaaa	agcagccgtgaggtactgat
<i>ITGAM</i>	gggaagtggcaaggaatgta	cttgccttcaccacctgat
<i>KRT1</i>	agcggacaaatgcagagaat	gcaccatccacatccttctt
<i>KRT10</i>	ttgaacaatccctggaagc	tgcacacagtagcgaccttc
<i>KRT14</i>	ggcctgctgagatcaagac	tcctcaggtcctcaatgtgc
<i>KRT15</i>	gctgacctggaggtgaagat	gttgggtctgtcttctggta
<i>KRT16</i>	aggtagcatgcagaacctc	gcacctgtccaggtaggag
<i>KRT17</i>	caccatgcagaacctcaatg	gcacctgtccaggtaggag
<i>KRT6A</i>	aggtagcctgaaccagagt	gatcatttgcaggttgagg
<i>LCE5A</i>	ctctatttccatggaagg	ggcaggataaaggagggaac
<i>LOR</i>	acctggccgtccaatagat	aaacacctccaactccttcg
<i>MMP13</i>	gctccgagaaatgcagtctt	tcgtcaagtttgccagtcac
<i>MX1</i>	ggcaagggtcagttaccagga	cctctgaagcatccgaaatc
<i>PTGS2</i>	caagatggcaaaatgctgaa	tgggaagatgcattggaaca
<i>RSAD2</i>	ttcaggtggagagccatttc	caccaactgcccagggtatt
<i>S100A8</i>	gccaaagcctaaccgtataa	aagagacatgcagggtctgag
<i>S100A9</i>	gacctggacacaaatgcaga	ccatcagcatgatgaactcc
<i>SCD1</i>	agcaggagctcatcgtctgt	gcagccgagcttctgaagag
<i>SDC4</i>	cccgttgaaagagatgagga	cagtgtctggacattgacacc
<i>THBD</i>	ccctcagtgccctcatttta	gcatttgcagtggttctgag

Gene	Forward (5' to 3')	Reverse (5' to 3')
<i>TSLP</i>	ctctggagcatcaggagac	cccttattcacccatgctgt

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