

Gene expression profiles in chronic idiopathic (spontaneous) urticaria

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ABSTRACT

Background: The pathophysiology of chronic idiopathic (spontaneous) urticaria (CIU) is poorly understood.

Objective: We hypothesized that a study of gene expression in active lesions from patients with CIU would uncover unexpected associations.

Methods: We enrolled eight patients with CIU and six healthy controls, and obtained 4 mm punch biopsy specimens of active lesions and unaffected skin of patients with CIU and of skin from normal controls. Routine histologic evaluation was performed, RNA was isolated, and gene expression data were assessed. Due to technical reasons, the final evaluation included six samples of lesional skin, six samples of nonlesional skin, and five samples of normal skin.

Results: As expected, lesional skin had more inflammatory cells per high-powered field (mean $\pm$ SE, 96 ± 6) than did samples from nonlesional skin of the subjects with CIU (17 ± 2) ($p < 0.01$). Lesions of CIU showed significant upregulation of 506 genes and reduced expression of 51 genes. Those most upregulated were predominantly involved in cell adhesion (e.g., selectin E [SELE]), cell activation (e.g., CD69), and chemotaxis (e.g., CCL2). Twelve independent canonical pathways with $p \leq 0.001$ were identified (including intracellular kinase pathways (RAS-related nuclear protein [RAN] and Janus activated kinase/interferon), cytokine signaling pathways (IL-9, IL10, and IFN), a strong inflammatory response (interferon, IL-9, IL-10, inducible nitric oxide synthase and glucocorticoid pathways) and increased cell proliferation (RAN signaling, cell cycle control, and tRNA charging).

Conclusions: This preliminary study describes a method to study gene activation in urticarial lesions and demonstrated a strong inflammatory response with a large variety of activated genes that are distinct from those reported with other dermatologic conditions.

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BACKGROUND

Chronic idiopathic (spontaneous) urticaria (CIU) is a difficult-to-treat illness of uncertain etiology.^{1–3} CIU is characterized clinically by the frequent occurrence of pruritic and erythematous wheals with surrounding erythema and, histologically, by a dense perivascular infiltrate composed of basophils, eosinophils, neutrophils, and CD4 and CD8 T-lymphocytes.^{4–7} Increased expression of Th1 and Th2 cytokines, TNF α , IL-3, CCL2, and CXCL8 has been reported (for brief descriptions of all genes and gene products mentioned, see www.genenames.org).^{8–10} Th17 cells are reported to be decreased.¹¹ Polymorphisms of the high affinity receptor for Immunoglob-

ulin E (IgE), Fc ϵ RI, and histamine N-methyltransferase have been described in patients with aspirin intolerant urticaria and have been proposed to be important in CIU.^{12–14}

METHODS

Subjects

We enrolled eight patients with CIU, two men and six women (mean $\pm$ SD, 40 ± 14 years old), based on clinical history and typical urticarial lesions, and six healthy controls, one man and five women (48 ± 12 years old) ($p = \text{n.s.}$). This study was approved by the University of Colorado Institutional Review Board. All the subjects signed informed consent forms. Standard doses of antihistamines had failed for each patient; patients had not received steroids or immunomodulatory drugs for 1 month and had stopped all medications except for “as needed” diphenhydramine for 5 days before the biopsy. As shown in Table 1, the subjects with CIU were 40 ± 14 years old (compared with 48 ± 12 years old for the controls), the disease had been present for 12.8 ± 12.4 months (range, 4–35 months), had urticaria symptom scores of 14.2 ± 6.5 (range, 11–24), and had lesion scores of 18 to >100 at the time of the biopsy (each, as described by Breneman *et al.*¹⁵).

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Table 1 Demographics

Subject No.	Urticaria (U) or Control (C)	Age, y (sex)	Duration of Urticaria, mo	Urticaria Activity Score	Lesion Score	ASST Wheal/Erythema, mm*
1	U	39 (F)	4	14	111	6/8
2	U	20 (M)	7	13	42	10/17
3	U	57 (M)	7	11	>100	8/12
4	U	51 (F)	30	14	>100	0/3
5	U	25 (F)	5	13	33	4/5
6	U	48 (F)	9	12	31	0/0
7	U	31 (F)	5	23	19	5/25
8	U	53 (F)	35	24	18	ND
9	C	54 (F)	na	na	na	na
10	C	49 (F)	na	na	na	na
11	C	56 (M)	na	na	na	na
12	C	44 (F)	na	na	na	na
13	C	26 (F)	na	na	na	na
14	C	56 (F)	na	na	na	na

ASST = autologous serum skin test; na = not applicable; ND = not done.

*mm greater than the saline solution control.

Five of eight subjects had evidence of autoimmunity based on an autologous serum skin test performed as described by Grattan.¹⁶ The night before the biopsy, the lesions were circled in black, and, in the morning, new lesions were marked with red. Although the exact age of the lesions was not known, the lesions were likely between 4 and 12 hours old.

Biopsies

Four millimeter punch biopsy specimens of new lesions were obtained within 4–6 hours after awakening, and, immediately, half of each sample was immersed in optimum cutting temperature compound (OCT) and flash frozen. The other half was immersed in RNAlater (Life Technologies, Grand Island, NY) and stored at -70°C for 6 years.

Gene Expression Analysis

RNA of sufficient quantity and quality was recovered from urticarial lesions of seven subjects, nonlesional skin of eight subjects, and normal skin of five controls. RNA was isolated, and gene expression data were determined by using an Affymetrix Human Gene 1.0 ST Array.¹⁷ The chip interrogated 28,869 well-annotated genes with 764,885 distinct probes, including 19,734 gene-level probe sets with full length, which provided comprehensive coverage of 99% of the National Center for Biotechnical Information Reference Sequence collection. Data from two patients with urticaria were eliminated from the array study due to quality control concerns (one had poor-quality RNA, and one was a clear outlier, which showed no significant upregulation of message). We analyzed the non-

lesional samples only from those subjects with CIU for whom we had an evaluable lesional sample. For the final analysis, we studied six samples of lesional skin, six samples of nonlesional skin, and five samples of normal skin. All RNA was used for the gene chip analysis, so there was insufficient RNA to verify gene expression by specific polymerase chain reaction analysis.

Microarray data were processed by using a robust multiarray average normalization corrected for GC content as implemented by Partek Genomics Suite v6.6 software.¹⁸ All normalized data, along with all of the raw Affymetrix Cel files used to generate the data set, has been deposited in the Gene Expression Omnibus.¹⁹

Statistics

The Partek Genomics Suite was also used for all statistical analysis of gene expression data (analysis of variance test for significance with a false discovery rate <0.001 to control for multiple testing) and used to visualize the data by using principle components analysis, an unsupervised analysis of data clustering. Data are expressed as mean (standard deviation) for age, duration of illness, and symptom scores, and are mean $\pm$ standard error (SE) for cell counts. A two-tailed Mann-Whitney test was used to compare cell numbers between affected and unaffected skin (GraphPad Prism 5.0c, Macintosh; GraphPad Software Inc., La Jolla, CA). Ingenuity pathway analysis was used to identify overrepresented biologic functions and pathways that contained genes different between lesions and control skin, and based on the direction of expression of the affected genes used to predict their activity.²⁰

Table 2 Histology												
Subject No.	Affected Skin						Unaffected Skin					
	Cells/ hpf*	MNC/ hpf*	EOS/ hpf#	PMN/ hpf*	Interstitial Inflam Cells/hpf*	Perivascular Cells/cap*	Cells/hpf	MNC/ hpf	EOS/hpf	PMN/hpf	Interstitial Inflam Cells/hpf	Perivasc Cells/cap
1	na	na	na	na	na	na	na	na	na	na	na	na
2	92	92	0	0	21	18	13	13	0	0	5	3
3	98	79	2	17	22	19	14	14	0	0	3	2
4	114	84	5	25	31	22	na	na	na	na	na	na
5	84	82	9	3	24	13	na	na	na	na	na	na
6	94	81	9	3	24	13	19	19	0	0	4	3
7	122	119	0	3	15	13	20	20	0	0	4	3
8	67	61	4	1	20	15	na	na	na	na	na	na
Mean ± SE	96 ± 7	86 ± 7	3 ± 1	7 ± 4	21 ± 2	16 ± 2	17 ± 2	17 ± 2	0 ± 0	0 ± 0	4 ± 0	3 ± 0
hpf = high powered field; MNC = mononuclear cell; EOS = eosinophil; PMN = neutrophil; cap = capillary; na = not applicable; SE = standard error. All compared with unaffected skin. *p ≤ 0.01. #p = 0.03.												

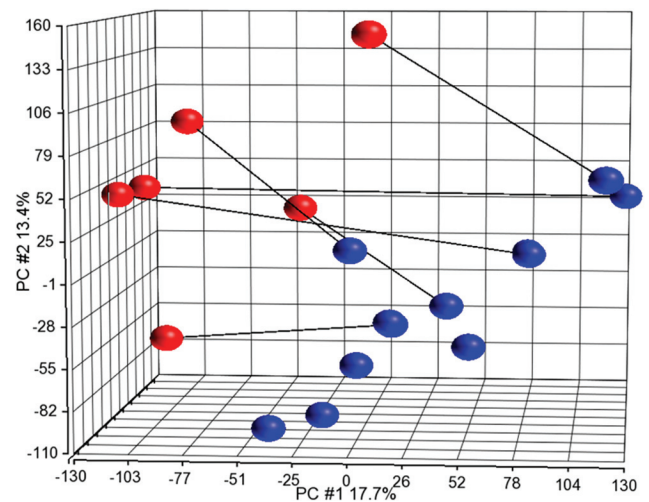


Figure 1. Principle component analysis of gene expression data. Urticarial lesions (red) and control skin biopsy specimens (blue) are represented in three-dimensional space based on the global gene expression patterns of each sample. Lesional and control biopsy specimens obtained from the same individual are connected by a line. The biopsy specimens from healthy controls are not associated with lines.

RESULTS

Lesions Had Typical Histology

As expected, lesional skin had more inflammatory cells per high-powered field (mean ± SE, 96 ± 6) than did samples from nonlesional skin of the subjects with CIU (17 ± 2 ; $p < 0.01$). These were predominantly mononuclear cells, although both eosinophils and neutrophils were present in most biopsy specimens and were more than that seen in nonlesional skin ($p < 0.05$) (Table 2).

Gene Expression Was Highly Consistent

Principle components analysis of gene expression data revealed distinct consistency among samples. In Fig. 1, the axes are arbitrary values that summarize the variability in these data points in each of three independent analyses and account for 18% (x-axis), 13% (y-axis), and 9% (z-axis) of the variability among the samples. Samples from lesional skin (Fig. 1, red spheres) grouped together and clustered on the graph in an area that is distinct compared with samples from the nonlesional skin of subjects with CIU (Fig. 1, blue spheres with lines that connect to matched samples from lesional skin) and from the skin of normal controls (Fig. 1, blue spheres without lines). Although the distribution of the control data indicated that the gene expression profiles from nonlesional skin may be somewhat different from that of skin from normal controls due to the limited sample size, the data from normal skin and from nonlesional skin of patients with

Table 3 In rank order, the 12 most differentially expressed genes (A), most differentially downregulated genes (B), and genes with the largest fold change (C)

Symbol	Entrez Gene Name	Entrez Gene ID no. (human)	p Value	Fold Change	Affymatrix No.
A: Most differentially expressed genes					
(by p value)					
SELE	Selectin E	6401	7.17E-12	20	7922229
CLEC4A	C-type lectin domain family 4, member A	50856	3.80E-11	5.4	7953723
CH25H	Cholesterol 25-hydroxylase	9023	5.64E-11	6.8	7934916
CCL2	Chemokine (C-C motif) ligand 2	6347	7.62E-11	18	8006433
MT2A	Metallothionein 2A	4502	9.60E-11	5.8	7995783
CD69	CD69 molecule	969	1.54E-10	3.4	7961075
BYSL	Bystin-like	705	4.67E-10	2.1	8119492
ADAMTS9	ADAM metalloproteinase with thrombospondin type 1 motif, 9	56999	6.98E-10	7.0	8088560
OSMR	Oncostatin M receptor	9180	1.02E-09	5.0	8105040
MT1M	Metallothionein 1M	4499	1.06E-09	7.1	7995787
Mir-21	MicroRNA 21	406991	1.15E-09	10	8008885
PNO1	Partner of NOB1 homolog (<i>Saccharomyces cerevisiae</i>)	56902	1.56E-09	2.6	8042381
B: Most differentially downregulated genes (by p value)					
STARD9	StAR-related lipid transfer domain containing 9	57519	3.10E-08	-1.7	7983132
KLHL24	Kelch-like 24 (<i>Drosophila</i>)	54800	1.19E-07	-1.8	8084219
ING4	Inhibitor of growth family, member 4	51147	2.48E-07	-1.5	7960654
FBXL20	F-box and leucine-rich repeat protein 20	84961	3.34E-07	-1.5	8014825
DCAF6	DDB1 and CUL4 associated factor 6	55827	3.54E-07	-1.3	7907104
SLC25A27	Solute carrier family 25, member 27	9481	5.37E-07	-1.8	8120067
MCEE	Methylmalonyl CoA epimerase	84693	5.62E-07	-1.3	8052934
EZH1	Enhancer of zeste homolog 1 (<i>Drosophila</i>)	2145	5.74E-07	-1.6	8015685
CCNG2	Cyclin G2	901	5.93E-07	-1.8	8095870
YPEL2	Yippee-like 2 (<i>Drosophila</i>)	388403	1.32E-06	-1.5	8008819
NPR2	Natriuretic peptide receptor B/guanylate cyclase B (atriatriuretic peptide receptor B)	4882	1.52E-06	-1.3	8155121
OPHN1	Oligophrenin 1	4983	1.65E-06	-1.5	8173310

Table 3 Continued	Symbol	Entrez Gene Name	Entrez Gene ID no. (human)	p Value	Fold Change	Affymatrix No.
C. Genes with the largest fold-change						
	TNFAIP6	TNF α -induced protein 6	7130	7.1E-08	28	8045688
	SELE	Selectin E	6401	7.2E-12	20	7922229
	CCL2	Chemokine (C-C motif) ligand 2	6347	7.6E-11	18	8006433
	THBS1	Thrombospondin 1	7057	1.05E-08	13	7982597
	S100A8	S100 calcium binding protein A8	6279	1.67E-06	13	7920244
	Mir-21	MicroRNA 21	406991	1.2E-09	10	8008885
	STEAP4	STEAP family member 4	79689	8.6E-09	9.3	8140840
	TNC	Tenascin C	3371	2.6E-06	8.6	8163637
	CTSL1	Cathepsin 1	1514	1.1E-08	8.4	8156228
	UAP1	UDP-N-acetylglucosamine pyrophosphorylase 1	6675	6.0E-08	7.9	7906863
	MMP12	Matrix metalloproteinase 12	4321	1.2E-08	7.6	7951297
	SRGN	Serglycin	5552	7.7E-08	7.4	7927964
For specific names of genes and their products, please see www.genenames.org .						

CIU were not statistically different and were grouped together for the subsequent analysis.

Multiple Genes Were Upregulated

Overall, compared with biopsy specimens of normal controls and nonlesional skin combined, lesions of CIU showed significant upregulation of 506 genes and reduced expression of 51 genes (data not shown; see (<http://www.ncbi.nlm.nih.gov/geo/>)). The 12 genes most upregulated (by *p* value), the 12 genes most differentially downregulated (by *p* value), and the 12 genes with the largest fold change are listed in Table 3 (sections A–C, respectively). The genes most differentially upregulated (by *p* value) (Table 3, section A) and those with the largest fold change (Table 3, section C) are predominantly involved in cell adhesion (*e.g.*, SELE), cell activation (*e.g.*, CD69), and chemotaxis (*e.g.*, CCL2). Of particular note, upregulation of message for CCL2 is consistent with the report by Santos *et al.*¹⁰ Those genes that were significantly downregulated (Table 3, section B) were not dramatically downregulated and did not share functions that were easy to discern.

Two pathways thought to be important in the pathophysiology of chronic urticaria are those associated with mast cells and with the complement system.¹ Multiple genes reported to be upregulated by mast cells after activation through the high affinity receptor for IgE were detected, including CSF1 (1.6-fold), IL1R1 (2.5-fold), CCL4 (1.4-fold), CD69 (3.4-fold), TNFAIP6 (28-fold), NFKB1 (2-fold), MYC (2.9-fold), and MAP3K14 (1.3-fold) (Table 3, section A and data not shown).^{21,22} However, most mast cell-related genes that are upregulated after activation tend to revert to baseline levels within 12 hours so that upregulation of other mast cell-related genes may have been missed.²¹ The only genes associated with the complement system²³ that were found to be upregulated were C1QBP (2.01-fold) and ITGAX (1.96-fold) (data not shown).

Canonical and Cellular Function Pathways

We next determined which canonical pathways contained genes that are overrepresented (Table 4) and which cellular functions include these pathways (Table 5). Twelve independent pathways with *p* $\leq$ 0.001 were identified (including intracellular kinase pathways (RAN and JAK/interferon signaling pathways), cytokine signaling pathways (IL-9, IL10, and IFN), a strong inflammatory response (interferon, IL-9, IL-10, iNOS, and glucocorticoid pathways), and increased cell proliferation (RAN signaling, cell-cycle control, and tRNA charging) (Table 4). The two cellular functions that are most dramatically upregulated are those associated with infection (activation *z*-score = 6.023; *p* = 1.64×10^{-6}) and more specifi-

Table 4 In rank order, 12 most activated canonical pathways (by *p* value)

Ingenuity Canonical Pathway	<i>p</i> Value*	Genes Upregulated	Percentage#†
RAN signaling	7.2×10^{-9}	KPNB1, KPNA4, KPNA2, RANBP2, RAN, RANBP1, KPNA1, IPO5	33
JAK1, JAK2, and TYK2 in interferon signaling	5.1×10^{-6}	PTPN2, IFNGR2, IFNGR1, STAT3, STAT1, IFNAR, NFKB1	26
Hepatic fibrosis and/or hepatic stellate cell activation	2.2×10^{-5}	IL4R, VCAM1, ICAM1, TGFBR1, IL1RL1, TNFRSF1A, IFNGR2, IFNGR1, IL1R1, IFNAR2, NFKB1, BCL2, CCL2, CSF1 (includes EG:12977), STAT1	10
Type I DM signaling	2.5×10^{-5}	SOCS3, MAP3K14, MYD88, TNFRSF1A, SOCS2, IFNGR2, IFNGR1, IL1R1, HSPD1, NFKB1, IRF1 (includes EG:16362), BCL2, STAT1	11
tRNA charging	3.8×10^{-5}	LARS, WARS, YARS, MARS2, GARS, TARS, MARS, IARS, FARSB	11
Glucocorticoid receptor signaling	4.2×10^{-5}	MAP3K14, VCAM1, SRA1, SELE (includes EG:20339), TGFBR1, ICAM1, SGK1, HSPA9, STAT3, NFKB1, HSPA5, BCL2, HSPA8, CCL13, CCL2, HSP90AB1, SMARCA2, CDKN1A, HSP90AA1, STAT1, JAK3	7
IL-9 signaling	6.2×10^{-5}	SOCS3, CISH, SOCS2, STAT3, STAT1, NFKB1, JAK3	18
Interferon signaling	6.2×10^{-5}	PTPN2, IFNGR2, IFNGR1, STAT1, IFNAR2, IRF1 (includes EG:16362), BCL2	19
IL-10 signaling	2.1×10^{-4}	CCR1, SOCS3, MAP3K14, IL4R, FCGR2A, IL1RL1, STAT3, IL1R1, NFKB1	12
iNOS signaling	3.4×10^{-4}	MYD88, IFNGR2, IFNGR1, STAT1, NFKB1, JAK3, IRF1 (includes EG:16362)	14
Cell cycle: G1/S checkpoint regulation	5×10^{-4}	MYC, E2F4, HDAC2, PA2G4, PAK1IP1, CDKN1A, GNL3, ATR	12

*The probability (*p* value) of obtaining genes associated with the given pathways by random chance.

#Percentage of genes in the named pathway that were found to be differentially expressed in this study.

†For specific names of genes and their products, please see www.genenames.org.

cally viral infections (activation z-score = 5.1; $p = 4.97 \times 10^{-10}$) (Table 5). Not unexpectedly, genes associated with the cellular functions of growth and proliferation are also highly upregulated (activation z-score = 4.9; $p = 3.8 \times 10^{-12}$) (Table 5).

DISCUSSION

We undertook this study based on the hypothesis that a study of gene expression in active lesions from patients with CIU would uncover unexpected associations and stimulate new thinking about this challenging condition. Limitations of this study include the small number of subjects, lack of information regarding cell specificity associated with altered gene expression, the single time point sampled, and the lack of verification of gene expression by real-time polymerase chain reaction assays (all of the RNA was used for the gene chip analysis). In that CIU may represent a heterogeneous group of disorders, it is unclear if this small sample of patients with CIU is truly representa-

tive of the pathophysiology of this disorder. For example, 62% of our patients with chronic urticaria had evidence of autoimmunity compared with the usual finding of ~40%. These patients were not routinely checked for thyroid autoimmunity or for antinuclear antibodies.²⁴ Finally, in that only one time point was analyzed, we are unable to make inferences if the observed changes in gene expression may play specific roles in the development or perpetuation of chronic urticaria.

The strengths of this study are the methodology to obtain lesions with highly consistent patterns of gene activation (Fig. 1) and the novel nature of the findings. As expected, samples of lesional skin from patients with chronic urticaria demonstrated high levels of up-regulation of multiple genes related to the influx of inflammatory cells into the lesions, and these genes include those seen after activation of mast cells. Up-regulation of genes associated with glucocorticoid signaling (including NFKB) was unexpected in that these

Table 5 In rank order, 12 most upregulated cellular functions (by z-score)

Category	Functions Annotation	p Value	Activation z-Score	Genes*
Infectious disease	Infection of cells	1.64E-06	6.023	AREG/AREGB, C1QBP, CCR1, CCT2, CD44, CTSL1, DCAF13, DDX10, DDX55, DHX33, DIMT1, ETF1, ETS2, G3BP1, GABPB1, HEATR1, HNRNPF, HNRNPU, HNRPD1, HSPA5, HSPA9, ICAM1, IRF1 (includes EG:16362), KLHDC2, KPNA4, KPNB1, LARS, LCP2, MAP3K14, MAT2A, MDN1, MT2A, MYD88, NDUFAF2, NFKB1, NOP56, NUP153, NUP98, PARP9, PICALM, POLR1E, PPP1R15B, PSMD12, RAD23A, RANBP1, RANBP2, SLC20A1, SLCO2A1, SRSF2, STAT1, STIP1, TRIM44, TRMT6, TWIF1 (includes EG:19230), UAP1, UTP11L, UTP6 (includes EG:216987), WDR46, ZYX
Infectious disease	Viral infection	4.97E-10	5.143	ABCE1, ACTB, ADAR, AGFG1, ALOX5, APOL1, AREG/AREGB, ATP6V1B2, ATR, BCL2, CCL13, CCL2, CCL4, CCL8, CCR1, CCT2, CD44, CD69, CEBPD, CH25H, CSF1 (includes EG:12977), CTSL1, DCAF13, DDX10, DDX55, DHX33, DIMT1, DNAA1, EIF4A3, ETF1, ETS2, G3BP1, G3BP2, GABPB1, GLUL, GYG1, HCK, HDAC2, HEATR1, HNRNPF, HNRNPU, HNRPD1, HSP90AA1, HSP90AB1, HSPA5, HSPA9, HSPD1, ICAM1, IFNAR2, IFNGR1, IL4R, INSR, IRF1 (includes EG:16362), KLHDC2, KPNA4, KPNB1, LARS, LCP2, MAP3K14, MAT2A, MDN1, MT2A, MYC, MYD88, NCL, NDUFAF2, NFKB1, NOP56, NUP153, NUP98, PANX1, PARP9, PICALM, PLAUR, POLR1E, PPP1R15B, PRPS1, PSMD12, PTPRC, RAB31, RAD23A, RANBP1, RANBP2, RELB, S100A8, SAMS1, SGK1, SLAMF1, SLC2A3, SLCO2A1, SMARCA2, SOCS2, SOCS3, SPI1 (includes EG:20375), SRSF2, STAT1, STAT3, STIP1, TGFB1, TMEM173, TNFRSF1A, TRAF3, TRIM44, TRMT6, TWIF1 (includes EG:19230), UAP1, UTP11L, UTP6 (includes EG:216987), WDR46, ZYX
Cellular growth and proliferation	Proliferation of cells	3.80E-12	4.9	AATF, ABL2, ACSL4, ACTB, ACTG1, ADAMTS1, ADAMTS8, ADAR, AIMP2, ALOX5, AMD1, AREG/AREGB, ARHGDIA, ATF6, ATP2A2, ATR, BCL2, BYSL, C1QBP, CAPRIN1, CBFB, CCL13, CCL19, CCL2, CCL4, CCNG2, CCR1, CCT2, CCT3, CCT5, CD180, CD44, CD69, CDC123, CDKN1A, CEBPD, CHST11, CISH, COPS3, CSF1 (includes EG:12977), CTSL1, CYR61, CYTIP, DDX20, DDX21, DKC1, DNAA1, DNAAJ2, DNM1L, E2F4, EBNA1BP2, EIF3B, EIF4A1, EIF4G1, ERO1L, ETS2, EXOSC2, FXN, GADD45B, GFM1, GLUL, GNL3, GPR183, GREM1, GTPBP4, HAS2, HCK, HDAC2, HDGFRP3, HNRNPA0, HNRNPAB, HNRNPF, HNRNPR, HNRNPU, HSPA5, HSPA8, HSPD1, ICAM1, IFI16, IFNAR2, IFNGR1, IL1R1, IL1RL1, IL4R, ILF2, ING4, INSR, IRF1 (includes EG:16362), ITGAX, JAK3, JUNB, KPNA2, LCP2, LDHA, LITAF, LMNB1, LTBR, MAPKAPK2, MINA, mir-21, MIP12, MT1A, MT2A, MYC, MYD88, NAMPT, NBN, NCL, NDUFAF2, NDUFAF4, NFKB1, NME2, NNMT, NOC3L, NOL8, NOLC1, NOP2, NOP58, NR1D1, NUBP1, NUP98, ODC1, OSMR, PA2G4, PAK1IP1, PES1, PIK3IP1, PLAUR, PLSCR1, PNP,

Table 5 Continued

Category	Functions Annotation	p Value	Activation z-Score	Genes*
Hematologic system development and function	Adhesion of immune cells	2.25E-07	4.803	PNPT1, PPRC1, PRMT1, PRRC2C, PSME3, PTPN2, PTPRC, RAC2, RALA, RAN, RELB, RNF2, S100A8, SAMSN1, SELE (includes EG:20339), SELP, SEMA3A, SERPINE2, SET, SF3A3, SGK1, SLAMF1, SLC1A3, SLC20A1, SLC25A27, SMARCA2, SOCS2, SOCS3, SOD2, SPI1 (includes EG:20375), SPRED1, SRA1, SRF, SRGN, SRSF2, SRSF3, STAT1, STAT3, STEAP2, TAF1D, TEAD4, TGFB1, THBS1, TNC C (includes EG:116640), TNFAIP6, TNFRSF1A, TP53INP1, TPM3, TRAF3, TRIB1, UTP20, UTP6 (includes EG:216987), VASP, VCAM1, VMP1, WARS, WDR12, XRN2, YWHAG, ZNF259, ZNF267, ZYX
				CCL13, CCL19, CCL2, CCL4, CCR1, CD44, CD69, CSF1 (includes EG:12977), FCGR2 A, HCK, ICAM1, IFNGR1, ITGAX, LTBR, MAP3K14, MSN, MYC, MYD88, PANX1, PLAUR, PTPRC, RAC2, RELB, S100A8, SELE (includes EG:20339), SELP, SPI1 (includes EG:20375), THBS1, TNFRSF1A, VASP, VCAM1
Immune cell trafficking	Adhesion of immune cells	2.25E-07	4.803	CCL13, CCL19, CCL2, CCL4, CCR1, CD44, CD69, CSF1 (includes EG:12977), FCGR2A, HCK, ICAM1, IFNGR1, ITGAX, LTBR, MAP3K14, MSN, MYC, MYD88, PANX1, PLAUR, PTPRC, RAC2, RELB, S100A8, SELE (includes EG:20339), SELP, SPI1 (includes EG:20375), THBS1, TNFRSF1A, VASP, VCAM1
				CCL13, CCL19, CCL2, CCL4, CCR1, CD44, CD69, CSF1 (includes EG:12977), FCGR2A, HCK, ICAM1, IFNGR1, ITGAX, LTBR, MAP3K14, MSN, MYC, MYD88, PANX1, PLAUR, PTPRC, RAC2, RELB, S100A8, SELE (includes EG:20339), SELP, SPI1 (includes EG:20375), THBS1, TNFRSF1A, VASP, VCAM1
Cell-to-cell signaling and interaction	Adhesion of immune cells	2.25E-07	4.803	CCL13, CCL19, CCL2, CCL4, CCR1, CD44, CD69, CSF1 (includes EG:12977), FCGR2A, HCK, ICAM1, IFNGR1, ITGAX, LTBR, MAP3K14, MSN, MYC, MYD88, PANX1, PLAUR, PTPRC, RAC2, RELB, S100A8, SELE (includes EG:20339), SELP, SPI1 (includes EG:20375), THBS1, TNFRSF1A, VASP, VCAM1
				CCL13, CCL19, CCL2, CCL4, CCR1, CD44, CD69, CSF1 (includes EG:12977), FCGR2A, HCK, ICAM1, IFNGR1, ITGAX, LTBR, MAP3K14, MSN, MYC, MYD88, PANX1, PLAUR, PTPRC, RAC2, RELB, S100A8, SELE (includes EG:20339), SELP, SPI1 (includes EG:20375), THBS1, TNFRSF1A, VASP, VCAM1
Tissue development	Adhesion of immune cells	2.25E-07 ⁷	4.803	CCL13, CCL19, CCL2, CCL4, CCR1, CD44, CD69, CSF1 (includes EG:12977), FCGR2A, HCK, ICAM1, IFNGR1, ITGAX, LTBR, MAP3K14, MSN, MYC, MYD88, PANX1, PLAUR, PTPRC, RAC2, RELB, S100A8, SELE (includes EG:20339), SELP, SPI1 (includes EG:20375), THBS1, TNFRSF1A, VASP, VCAM1
				CCL13, CCL19, CCL2, CCL4, CCR1, CD44, CD69, CSF1 (includes EG:12977), FCGR2A, HCK, ICAM1, IFNGR1, ITGAX, LTBR, MAP3K14, MSN, MYC, MYD88, PANX1, PLAUR, PTPRC, RAC2, RELB, S100A8, SELE (includes EG:20339), SELP, SPI1 (includes EG:20375), THBS1, TNFRSF1A, VASP, VCAM1
Cellular movement	Chemotaxis	1.82E-06 ⁶	4.391	ALOX5, CCL13, CCL19, CCL2, CCL4, CCL8, CCR1, CD44, CD69, CDKN1A, CSF1 (includes EG:12977), CYR61, FCGR2A, GREM1, HCK, HSPD1, ICAM1, IL1R1, IL4R, MAP3K14, MAPKAPK2, MYD88, PLAUR, PTPRC, RAC2, RALA, RELB, S100A8, SELE (includes EG:20339), SELP, SEMA3A, SERPINB1, SOCS3, SPI1 (includes EG:20375), TGFB1, THBS1, TNFRSF1A, TRIB1, YARS
				ALOX5, CCL13, CCL19, CCL2, CCL4, CCL8, CCR1, CD44, CD69, CDKN1A, CSF1 (includes EG:12977), CYR61, FCGR2A, GREM1, HCK, HSPD1, ICAM1, IL1R1, IL4R, MAP3K14, MAPKAPK2, MYD88, PLAUR, PTPRC, RAC2, RELB, S100A8, SELE (includes EG:20339), SELP, SEMA3A, SERPINB1, SOCS3, SPI1 (includes EG:20375), TGFB1, THBS1, TNFRSF1A, TRIB1, YARS
Cellular movement	Chemotaxis of cells	1.72E-06	4.372	ALOX5, CCL13, CCL19, CCL2, CCL4, CCL8, CCR1, CD44, CD69, CDKN1A, CSF1 (includes EG:12977), CYR61, FCGR2A, GREM1, HCK, HSPD1, ICAM1, IL1R1, IL4R, MAP3K14, MAPKAPK2, MYD88, PLAUR, PTPRC, RAC2, RELB, S100A8, SELE (includes EG:20339), SELP, SEMA3A, SERPINB1, SOCS3, SPI1 (includes EG:20375), TGFB1, THBS1, TNFRSF1A, TRIB1, YARS

Table 5 Continued

Category	Functions Annotation	p Value	Activation z-Score	Genes*
Hematologic system development and function	Chemotaxis of myeloid cells	4.27E-06	4.32	ALOX5, CCL13, CCL2, CCL4, CCL8, CCR1, CD69, CDKN1A, CSF1 (includes EG:12977), FCGR2A, HCK, ICAM1, IL1R1, IL4R, MAPKAPK2, MYD88, PLAUR, PTPRC, RAC2, S100A8, SELE (includes EG:20339), SELP, SERPINB1, SPI1 (includes EG:20375), THBS1, TNFRSF1A, YARS
Inflammatory response	Chemotaxis of myeloid cells	4.27E-06	4.32	ALOX5, CCL13, CCL2, CCL4, CCL8, CCR1, CD69, CDKN1A, CSF1 (includes EG:12977), FCGR2A, HCK, ICAM1, IL1R1, IL4R, MAPKAPK2, MYD88, PLAUR, PTPRC, RAC2, S100A8, SELE (includes EG:20339), SELP, SERPINB1, SPI1 (includes EG:20375), THBS1, TNFRSF1A, YARS
Cellular Movement	Chemotaxis of myeloid cells	4.27E-06	4.32	ALOX5, CCL13, CCL2, CCL4, CCL8, CCR1, CD69, CDKN1A, CSF1 (includes EG:12977), FCGR2A, HCK, ICAM1, IL1R1, IL4R, MAPKAPK2, MYD88, PLAUR, PTPRC, RAC2, S100A8, SELE (includes EG:20339), SELP, SERPINB1, SPI1 (includes EG:20375), THBS1, TNFRSF1A, YARS

*For specific names of genes and their products, please see www.genenames.org.

patients had not received glucocorticoids for at least 1 month before biopsy. Furthermore, the pattern of gene expression seen in this pilot study is different from that reported for other skin diseases (e.g., atopic dermatitis and psoriasis^{25,26}). Unexpectedly, the patterns of gene expression seen are most consistent with those seen in interferon signaling (Table 4).^{27,28} This approach could be used to determine if there are any temporal variations in the expression profiles or are correlated with comorbidities among patients with chronic urticaria.

CONCLUSIONS

This preliminary study described a method to study gene activation in urticarial lesions and demonstrated a strong inflammatory response with a large variety of activated genes and distinct from that reported with other dermatologic conditions.

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