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Microarray analysis of distinct gene transcription profiles in non-eosinophilic chronic sinusitis with nasal polyps

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Abstract

Background—Recent literature has indicated the feasibility of microarray analysis in the characterization of chronic sinusitis. We hypothesized that previously unexplored inflammatory mechanisms would be involved in the pathophysiology of noneosinophilic chronic rhinosinusitis with nasal polyps (NE-CRSwNP) and that this technology could be used to identify the gene expression of these novel and previously known mediators.

Methods—Patients with CRSwNP failing medical therapy were prospectively enrolled and NP tissue was removed at time of surgery. NE-CRSwNP was diagnosed based on clinical parameters including absence of allergic disease and confirmed with histopathology showing lack of eosinophilic infiltration. Messenger RNA (mRNA) transcripts extracted from study and control patients were then subjected to microarray analysis using Affymatrix based chips. Validation of findings was then confirmed *via* quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Results—Microarray analysis revealed activation of pathways involved in antigen presentation, cellular movement, hematopoiesis, carcinogenesis, apoptosis, and cell signaling. Previously unexplored genes of interest were identified and their differential regulation was validated *via* qRT-PCR. Our data showed up-regulation of innate inflammation genes (IL-6, IL-8, and monocyte chemoattractant protein 1), hypoxia-induced inflammation 1α, and fibrosis (tenascin) and lack of up-regulation of genes associated with allergic, eosinophilic inflammation (IL-4 and IL-13). Additionally, the genes for CXCL1 and autocrine motility factor receptor were novelly identified to be up-regulated

Conclusion—This study explores the utility of gene microarray technology in identifying unexplored targets of immune dysregulation in NE-CRSwNP. Furthermore, the data characterize the immunologic profile of NE-CRSwNP as it differs from other forms of CRSwNP, in particular, those known to be associated with eosinophilic inflammation.

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Keywords

CRSwNP; CXCL1; gene expression; IL-4; IL-6; IL-8; microarray; noneosinophilic; polyps; RT-PCR

Chronic rhinosinusitis (CRS) is a chronic inflammatory condition affecting the paranasal sinuses and nasal cavities, which has a significant adverse impact on the quality of life and daily functioning of its sufferers.[1–3] This single term, however, most likely represents multiple different diseases with or without various stages of severity along their spectra. A summary statement comprising expert opinion within this field divides CRS into two easily distinguishable subsets: CRS without nasal polyps (CRSsNP), characterized by a chronic inflammatory infiltrate and the absence of NP; and CRS with NPs (CRSwNP).[2,3] Research into these two subtypes has indicated a significant association between the presence of eosinophilia and nasal polyposis[4,5] but other work has also indicated that the level of this eosinophilia has been variable[6,7] and that certain forms of CRSwNP have a higher degree of eosinophilia such as allergic fungal sinusitis[8] and aspirin exacerbated respiratory disease.[9] However, a certain subset of NP specimens does not show significant eosinophilia and microscopic evaluation of them has indicated other significant differences between their histopathological appearance and that of polyps with eosinophilia.[10] These noneosinophilic (NE) polyps tend to show characteristics not seen in their eosinophilic counterparts including profound glandular hypertrophy, mononuclear cell infiltrate, fibrosis, and the presence of mast cells.[10] This noneosinophilic CRSwNP (NE-CRSwNP) would appear to represent a different subset of CRSwNP for which the elucidation of its specific cytochemical and inflammatory mediators is required to better understand and treat the disease.

Microarray analysis of complementary DNA (cDNA) has become a powerful tool in the analysis of gene expression profiles. By assessing the relative amount of messenger RNA (mRNA) in a sample compared with a control, this technique can quickly indicate which genes have been activated in the disease state and that may help explain its pathophysiology. This relatively new and revolutionary technique has recently been used in the characterization of human NP tissue.[11–17] However, none of these studies have specifically investigated NE polyps in the nonallergic patient. The purpose of this study, therefore, was to use cDNA microarray technology to identify the gene expression profile in polyp samples from patients with NE-CRSwNP when compared with control tissue.

MATERIALS AND METHODS

Ethics

Study approval was obtained from the Institutional Review Board of the University of Virginia.

Subjects

NP Tissue—Patients presenting to the University of Virginia Health System who met the diagnostic criteria for CRSwNP[18] and who failed medical therapy were eligible for the study. Patients without a history of inhalant allergies who chose to undergo endoscopic sinus surgery were then enrolled in the study. Additional exclusion criteria included the presence of cystic fibrosis, sinonasal tumor, or immunodeficiency. Preoperative oral steroids were not used.

Control Tissue—Control tissue was harvested from the ethmoid or sphenoid sinuses of patients undergoing nonsinusitis-related surgery (*e.g.*, orbital decompression or cerebrospinal fluid leak repair). At the time of surgery, sinus tissue was harvested and placed in sterile normal saline. Initial gene array studies were performed on two control and two NECRSwNP subjects.

Subsequent studies were performed on separate cohorts of six additional subjects for each the control and polyp samples.

Histological Evaluation

A portion of each polyp was placed in 4% paraformaldehyde (Sigma, St. Louis, MO) overnight at 4°C. The next day the polyps were washed in phosphate-buffered saline and stored in 70% ethanol until paraffin embedding. Paraffin embedding, tissue sectioning, and H&E staining were performed by the University of Virginia Histology Core. Samples were examined by microscopy. Inclusion of tissue sample required four or less eosinophils in each of 10 high-powered (400×) fields.

Gene Chip Hybridization and Analysis—For gene chip analysis, an adjacent portion of two of the polyp samples were frozen in liquid nitrogen. RNA extraction and gene chip analysis were performed by the UVA Biomolecular Research Facility using the Affymetrix (Santa Clara, CA) HgU133 plus 2.0 gene chip (which analyzes 38,500 genes) as previously described.[19] Gene ontology data mining was then performed using the Expression Analysis Systematic Explorer downloaded from the Database for Annotation, Visualization, and Integrated Discovery 2.0 program (www.david.niaid.nih.gov).[20] Gene expression data sets containing the information of gene identifier, fold change, and *p* values were submitted to the Expression Analysis Systematic Explorer for analysis. In these studies, a fold change cutoff of 1.5 and value of $p < 0.05$ was set for inclusion in the data set. Several genes were then selected for verification and additional study *via* reverse-transcription and quantitative polymerase chain reaction (qRT-PCR).

Reverse-Transcription Polymerase Chain Reaction—Total RNA was extracted from tissue using RNA-Bee isolation solvent (Tel-Test, Inc., Friendswood, TX). Conversion of the mRNA to cDNA was performed using a Taqman Reverse Transcription kit (Roche, Branchburg, NJ) according to manufacturer's instructions. Specific mRNA levels were quantified using realtime PCR. Primer pairs for the genes analyzed were sequenced and obtained from Integrated DNA Technologies (Coralville, IA) or ordered from SuperArray Biosciences Corp. (Frederick, MD). Information regarding these is listed in Table 1. Relative gene expression was calculated using the comparative crossing threshold (C_T) method as described elsewhere[21] with β-actin as a reference housekeeping gene. This method was similarly used to compare gene expression between the control and polyp tissue such that fold difference was calculated *via* the formula $2^{-\Delta\Delta Ct}$.[19]

Cytokine Protein Determination—IL-6, IL-8, and CXCL1 levels were measured in the supernatants of protein extracted from NP or control samples. Tissue samples were minced and added to 500 μL of cell lysis buffer (Bio-plex cell lysis kit; Bio-Rad, Hercules, CA) and dounce homogenized. Samples were sonicated and centrifuged at 3800 × *g* for 4 minutes. Supernatants were collected and any pelleted debris was discarded. IL-8 and IL-6 levels were determined using a Bio-Plex bead-suspension assay (Bio-Rad) and CXCL1 was measured using a commercial ELISA kit (R & D Systems, Minneapolis, MN). The sensitivity of the assay for CXCL1 was 10 pg/mL, for IL-6 was 2 pg/mL, and for IL-8 was 1.4 pg/mL. Cytokine concentrations were normalized to total protein to allow comparisons between different samples.

Immunohistochemistry—Samples were deparaffinized and hydrated to distilled water. Endogenous peroxidase activity was eliminated by incubating sections for 30 minutes at room temperature in 200 mL of methanol with 3 mL of 30% hydrogen peroxide. Epitope unmasking was performed by heating sections for 20 minutes in unmasking solution (Vector Laboratories, Burlingame, CA). Specific staining for CXCL1 was performed using a 1:250 dilution of rabbit

polyclonal antibody ab9804 (AbCam, Cambridge, MA). Isotype control rabbit IgG (Southern Biotech, Birmingham, AL) was used at the same concentration as the CXCL1 antibody. Blocking and signal amplification were performed using the RTU Vectastain Universal Elite ABC Kit (Vector Laboratories) according to manufactures directions. Samples were developed for 3 minutes with diaminobenzidine (Dako Cytomation, Carpinteria, CA) and counterstained 1 minute with Mayer's hematoxylin (Vector Laboratories). Samples were washed in distilled water and dehydrated through alcohol and xylenes and mounted.

RESULTS

Gene Array

Two polyps each from both the study and the control populations were submitted for microarray analysis. This small sample was chosen because this was an exploratory study and initial analysis between the two samples showed a high correlation (data not presented). A total of 120 genes were identified, which showed both an absolute fold change of ≥1.5 and a value of $p < 0.05$. Of these, 58 genes were up-regulated, and 62 were down-regulated. A breakdown of the various functional groups represented by these genes is presented in Fig. 1. An exhaustive list of the significantly differentially regulated genes is provided in Table 2. Many of the traditionally identified inflammatory modulators and genes associated with CRS were differentially expressed, although not significantly so. These included those associated with Th2 or allergic inflammation (IL-4, fold change 1.28; IL-13, −2.79) and those involved in innate immunity (IL-6, 5.13; IL-8, 2.3).

Quantitative Polymerase Chain Reaction

We next analyzed several genes by qRT-PCR on separate larger cohorts of samples. Despite a lack of significant fold change of the previously known inflammatory mediators on microarray analysis, 12 of these genes were selected for RT-PCR investigation to determine their involvement in this subset of disease. These included characteristically Th2-mediated genes (IL-4, IL-13, thymus and activation-regulated chemokine, RANTES, and thymic stromal lymphoprotein), Th1 mediated genes (interferon [IFN] γ), and proinflammatory or fibrosis-associated genes (stem cell factor [SCF], IL-6, IL-8, IL-11, tenascin-C, and hypoxia-inducible inflammation [HIF] 1α). The microarray data were also further evaluated for novel genes of interest including representative genes thought to be important in cell trafficking, inflammatory cell recruitment, neoplasia, and inflammation. Five previously unexplored genes were selected for RT-PCR analysis. The selection was based on either a significant fold change or a substantially high absolute fold change even if this change was not statistically significant. Information regarding the selected genes is presented in Table 3 and the fold change results are represented graphically in Fig. 2. Of the previously known modulators/mediators of inflammation, the analysis shows a likely clinically significant fourfold or greater up-regulation for SCF, IL-8, tenascin-C, and HIF1-α ($p < 0.01$). Of the novel genes, a fourfold up-regulation is seen with CXCL1 ($p < 0.05$), ATP-binding cassette B11 (ABCB11), and autocrine motility factor receptor (AMFR). IL-4, IL-13, and IFN-γ were substantially down-regulated compared with control tissue, whereas IL-11 and CLCA2 showed a general equivalency of expression between control and NE-CRSwNP.

Protein Expression

Using either Bioplex or ELISAs for IL-6, IL-8, and CXCL1 (Gro-α) on protein extracted from control and NE-CRSwNP tissue, we verified that the increased gene expression observed by qRT-PCR corresponded to increased protein production (Fig. 3).

Immunohistochemistry

Staining of the tissue indicated that the up-regulation of CXCL1 was noted to occur within the epithelial layers of the polyp and its glandular structures (Fig. 4).

DISCUSSION

CRS is a general term for what appears to be numerous distinct pathways to inflammation of the paranasal sinuses for which the distinction between the presence or lack of polyps can be of significant therapeutic benefit.[22] However, even within CRSwNP, multiple subsets of disease with respectively different treatments exist including allergic fungal sinusitis and aspirin-exacerbated respiratory disease. Nevertheless, polyps characterized by eosinophilia are only seen ~80% of the time in western cultures[23] and perhaps substantially less in certain Asian populations.[24,25] The remaining portion of patients has NPs that are significantly lacking in an eosinophilic infiltration, but this entity has not been subject to the same level of scrutiny as the eosinophilic variety as we sought to provide with the current study and microarray analysis.

Several studies using microarray technology in the evaluation of NPs have been published in recent years. Benson and colleagues have published several articles regarding their initial study on the effects of corticosteroids on the gene profile of NPs.[14] Later, Fritz and colleagues[15] and Figuerido *et al.*[12] compared polyp tissue with adjacent inflamed mucosa and showed significant differences in the gene expression profiles. Liu *et al.*[26] and Wang *et al.*[16] also found differences comparing polyps with sphenoid sinus mucosa and inferior turbinate mucosa, respectively. However, these several studies all evaluated CRSwNP in general, without specific attention to any subset therein. Orlandi *et al.*,[11] on the other hand, did evaluate subsets of CRSwNP, but unlike our study, theirs focused on two variants of eosinophilic CRSwNP (E-CRSwNP). As such, no study has yet specifically explored NE-CRSwNP through either microarray technology or extensive RT-PCR of genetic transcripts.

In this study, we found 120 genes that were either significantly differentially expressed in polyp tissue when compared with normal sinus tissue controls, but the various inflammatory cytokines typically seen in CRS were not found to have a significant change in regulation. This may reflect the inherent variability of gene expression and the heterogeneity of this disorder in addition to the low power analysis of the small sample size used for this screening technique. These problems seem to be common to microarray analysis and other authors have indicated that this can lead to both spurious data as well as underrecognizing important differences.[27] Pooling of specimens may have been able to compensate for this problem, but this method has also been discouraged elsewhere.[28]

Some trends were, however, observed with these cytokines and several of these were selected for further study with RT-PCR. Although not every prediction was confirmed, the data were of importance. Most compelling was the lack of evidence for expression of genes associated with allergic inflammation including Th2-associated cytokines (*e.g.*, IL-4 and IL-13), consistent with our belief that this is a unique disorder and the down-regulation of genes associated with eosinophilic inflammation (eotaxin and eosinophil cationic protein) on the gene array. In contrast, we did see a fourfold elevation of SCF (KITLG). As a stimulator of the differentiation and proliferation of mast cells, its up-regulation in the polyp tissue is consistent with our previous histological findings of the presence of mast cells in this tissue. The fibrosis in these polyps may well be mediated through this type of mast cell process, which has been implicated in other fibrotic diseases such as rheumatoid arthritis, scleroderma, and idiopathic pulmonary fibrosis.[28] Both transcripts and protein levels for IL-8 (CXCL8) and CXCL1 were also noted to be significantly elevated in the polyp tissue. These chemokines are both attractants for neutrophils indicating a role for this immune cell in the disease. Although neutrophils were not seen in our histological samples, it may be that polyp formation in this disease develops in

the setting of chronic infection, biofilm formation, and purulent exudates that are remote from the polyp.

As discussed, our previous studies indicated an increased fibrotic stroma in these types of polyps and the nearly 10-fold increase in tenascin-C in the qRT-PCR is consistent with this observation. Tenascin-C is often transiently expressed in acute tissue injury and inflammation where it regulates fibroblast migration.[29] Its presence in a chronic disease such as NECRSwNP implies a "persistent acute" injury to the tissue as a part of the overall disease process resulting in a more fibrotic appearing polyp.

The role that hypoxia might play in upper airway inflammatory disease is a potentially important one.[30] HIF-1α is an inducible transcription factor expressed in hypoxic conditions that is involved in activation of glycolytic and inflammatory pathways.[31] NP-derived fibroblasts have been specifically shown to increase their production of HIF-1α in response to hypoxic conditions[32] and a corresponding *in vivo* up-regulation was significantly shown in this study, consistent with the reported hypoxic milieu observed in CRS.[33] Although the increased expression of HIF-1α may simply be a stress response, the implication can not be ignored that hypoxia may play a significant role in the pathogenesis of NE-CRSwNP. Improved aeration with a secondary reduction of HIF-1α could contribute to the therapeutic benefit observed after surgery.

Thymus and activation-regulated chemokine and thymic stromal lymphoprotein were not found to be significantly up-regulated and IL-4, IL-13, and IFN-γ, the hallmark cytokines for Th2- and Th1-mediated disease, respectively, were found to be down-regulated. This correlates well with a recent study by Kim *et al*.[23] where on immunohistochemical study of NE polyps, 0/20 expressed only $CCR5^+$ (Th1) cells, 6/20 expressed only $CCR3^+$ (Th2) cells, 4/20 expressed both, and 10/20 (50%) expressed neither Th1 or Th2 cells.

Our investigation also found the expression of two other genes to be substantially increased on both microarray and RT-PCR data, ABCB11 and AMFR. The former is a bile salt exporter linked to drug resistance and for which studies investigating its presence in hepatic inflammation and stimulation with IL-6 have actually shown a down-regulation.[34] It is not immediately clear why this gene was found to be up-regulated in sinus tissues, especially under a condition of IL-6 up-regulation, and as such, this deserves additional evaluation in future studies.

AMFR, on the other hand, is a gene that codes for the receptor for autocrine motility factor, a cytokine seen to be produced by tumor cells,[35] and its up-regulation in our study correlates well with other studies on NPs indicating up-regulation of carcinogenic genes.[14,15] Although a large differential expression of SPINK7 and CLCA2 was seen during microarray analysis, this was not confirmed by RT-PCR.

When viewed in the context of the RT-PCR results, the predicted direction of change for the studied genes was confirmed in 11/16 (68.8%). It is uncertain as to why a greater correlation was not seen. Perhaps this relates to the inability for population estimates to be made based on a very limited sample size, especially in what may be a heterogeneous entity. Nevertheless, the microarray was able to provide some very useful confirmable data regarding some of the novel genes identified.

CONCLUSIONS

NE-CRSwNP is a separate histologically identifiable subset of CRSwNP. This article represents the first dedicated study investigating its gene expression profile through microarray analysis. Although not all findings were validated, the expression of SCF, IL-6, IL-8, tenascin-

C, and CXCL1 and lack of up-regulation of IL-4, IL-13, and IFN-γ indicate that the pathophysiology of NE-CRSwNP may be more related to an innate immune response as opposed to a directly Th1- or Th2-mediated process. The identification of novel genes in this process, ABCB11 and AMFR, deserve additional study to determine their exact role in this disease.

Acknowledgments

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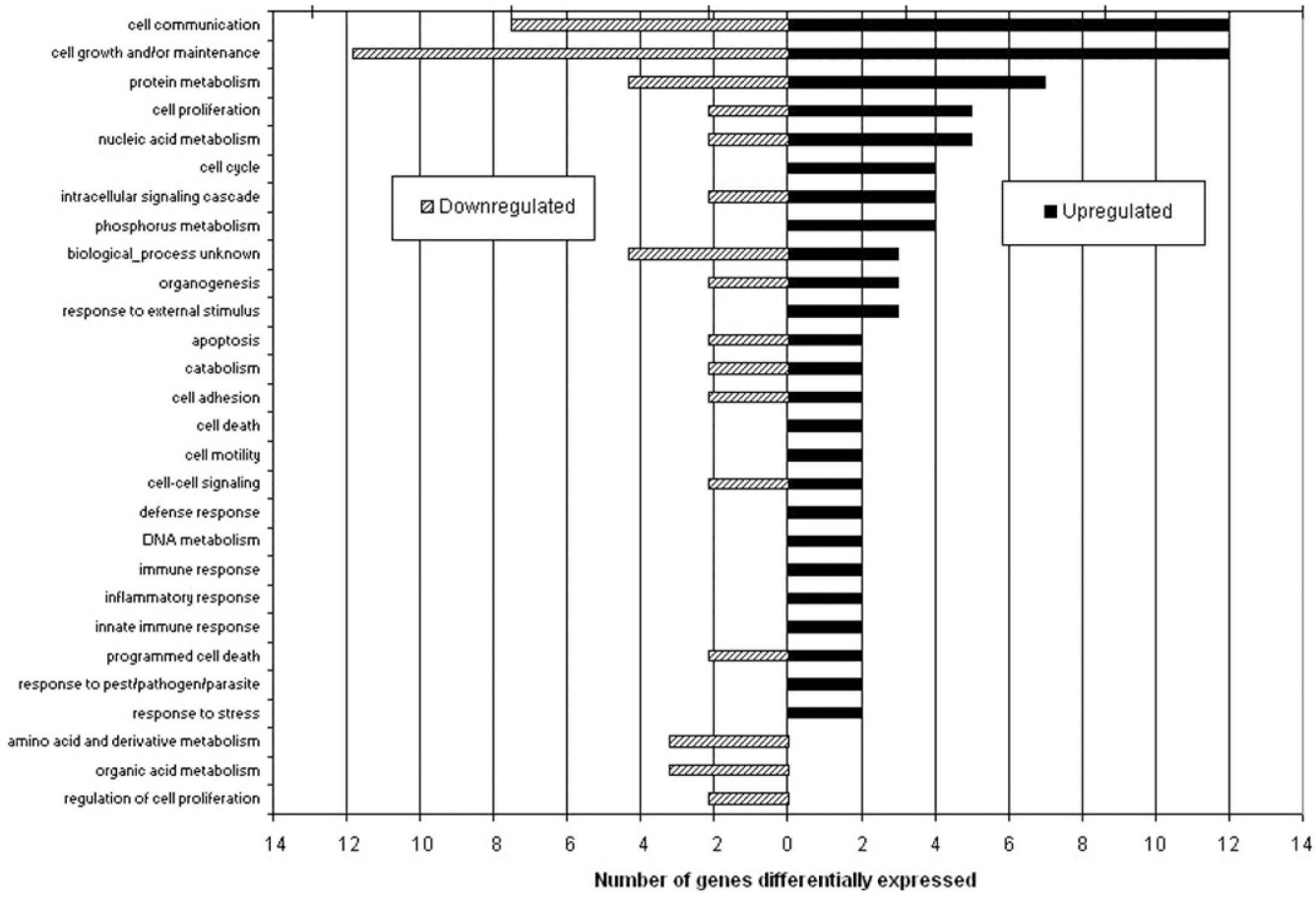

Figure 1.
Gene ontology (GO) family distribution of differentially expressed genes with absolute fold change of >1.5 and a value of $p < 0.05$.

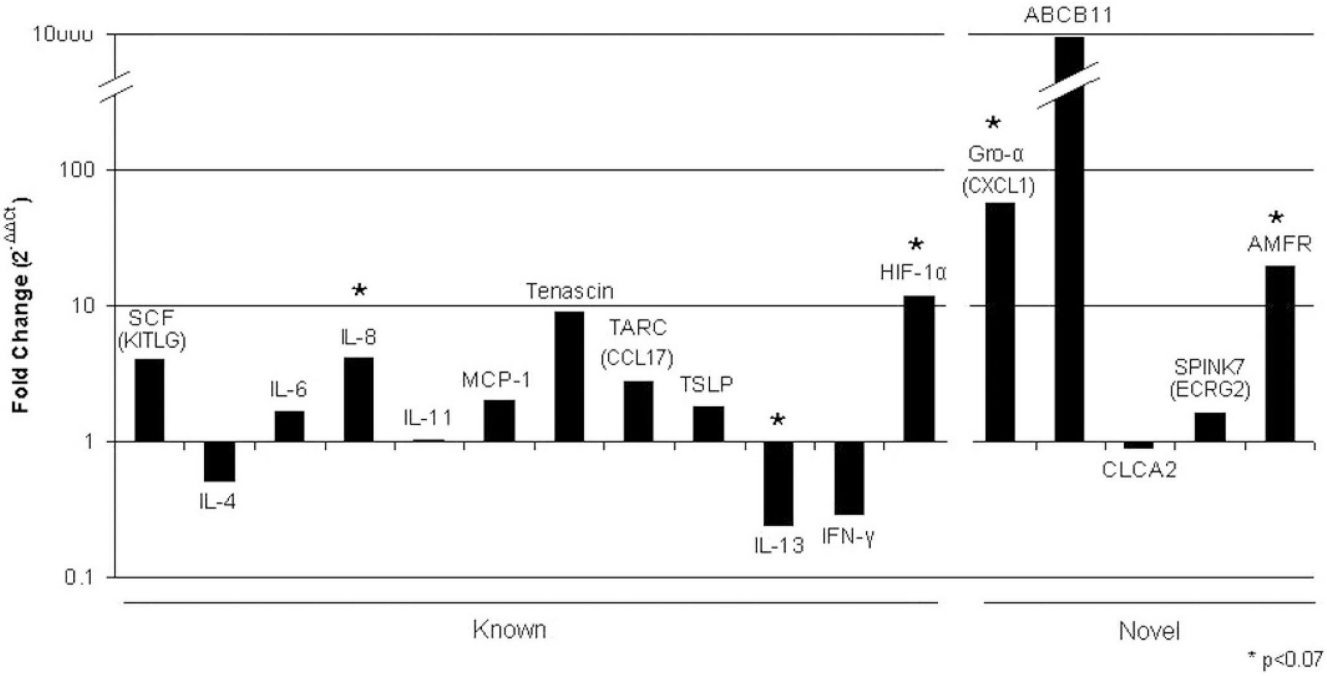

Figure 2.

Reverse-transcription polymerase chain reaction fold change of selected genes represented $2^{-\Delta\Delta Ct}$ method used as described in the text to linearize the fold change. Because of significantly greater up-regulation of novel genes ABCB11 and CXCL1, the y-axis is a 10 base logarithmic scale. Fold change values <1 represent a down-regulation of the gene, as what would be represented by a negative fold change in the microarray data. Studied genes are broken down into those that have been previously known to be involved in nasal inflammation and polyps and those novel genes identified through this study.

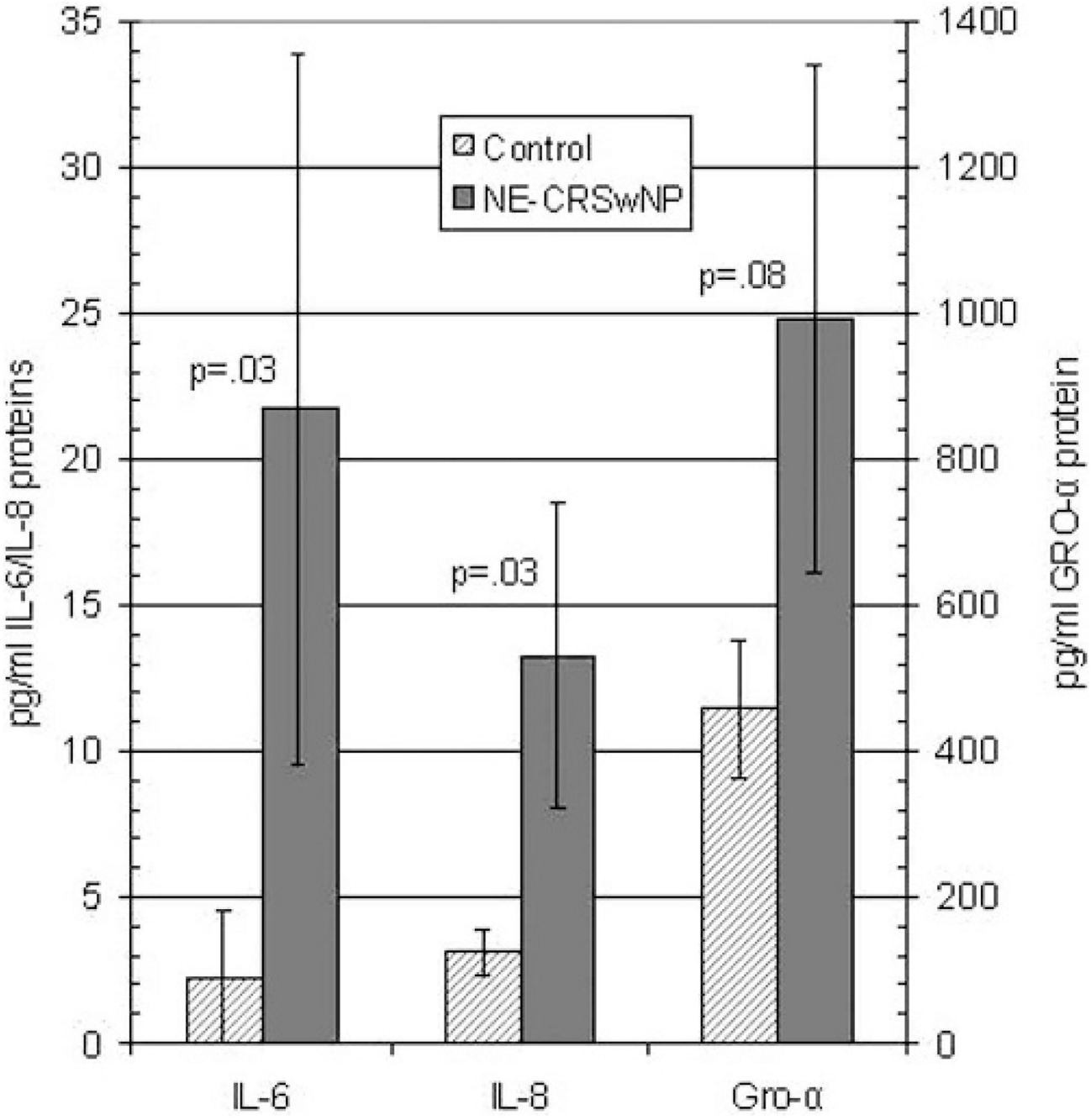

Figure 3.

Comparison of protein expression in control and noneosinophilic chronic rhinosinusitis with nasal polyp (NE-CRSwNP) tissue. After surgical removal, tissue was homogenized in cell lysis buffer to extract proteins. Protein levels for the various cytokines/chemokines were determined by ELISA or Bio-plex. A further correction was made by correcting for total protein in each sample to allow comparison between control (n = 9) and NE-CRSwNP samples (n = 23).

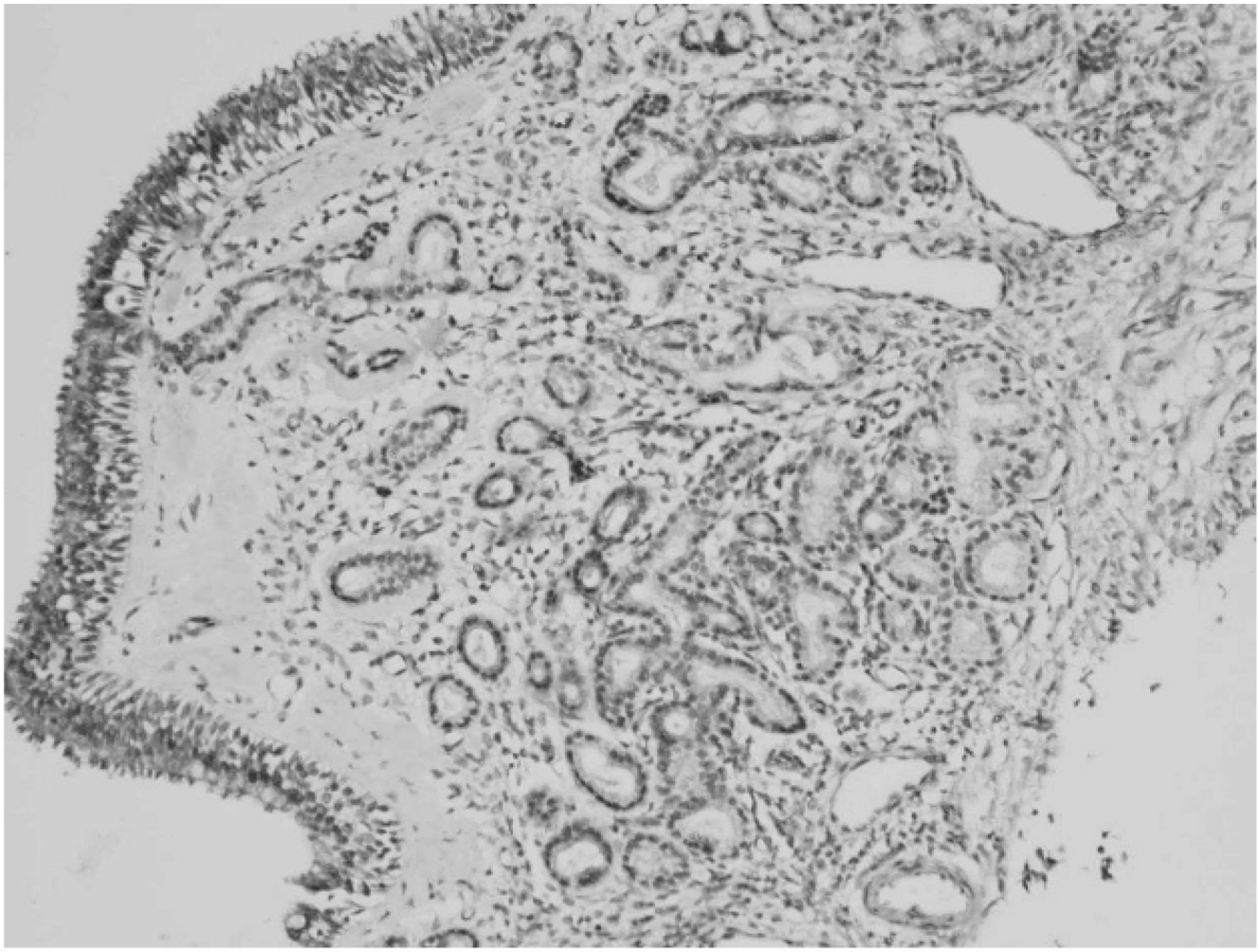

Figure 4.
Sinonasal polyp immunostained with antibodies against CXCL1 showing localization of the protein to the epithelial surface of the polyp and its glandular structures.

Table 1

Polymerase chain reaction primer pairs for qRT-PCR

Primer	Sequence
β-Actin	F5'-TGCGTGACATTAAGGAGAAG-3'
	R5'-GCTCGTAGCTCTTCTCCA-3'
SCF (KITLG)	F5'-CCCTTAGGAATGACAGCAGTAGCA-3'
	R5'-GCCCTTGTAAGACTTGGCTGTCTC-3'
IL-4	F5'-GAACAGCCTCACAGAGCAG-3'
	R5'-AGCACAGTCGCAGCC-3'
IL-6	F5'-ATGAACTCCTTCTCCACAAG-3'
	R5'-CGTCGAGGATGTACCG-3'
IL-8	F5'-ATGACTTCCAAGCTGGCCGTGGCT-3'
	R5'-TCTCAGCCCTCTTCAAAAACTTCTC-3'
IL-11	R5'-ATGAACTGTGTTTGCCGC-3'
	R5'-AGCCAGCTGCCGCGT-3'
MCP-1	F5'-GTAGAACTGTGGTTCAAGAGG-3'
	R5'-AGCCACCTTCATTCCCCAAG-3'
Tenascin	F5'-TGAACAAAATCACAGCCCA-3'
	R5'-CAGTGGAACCAGTTAACGCC-3'
TARC (CCL17)	F5'-CACGCAGCTCGAGGGACCAAGTG-3'
	R5'-TCAAGACCTCTCAAGGCTTTGCAGG-3'
TSLP	F5'-CAGAGCCCTAACCTTCAATC-3'
	R5'-CTTCTTCATTGCCTGAGTAG-3'
IL-13	F5'-TCATTGAGGAGCTGGTCA-3'
	R5'-CAGGGATTCCAGGGCT-3'
IFN-γ	F5'-AAAGATGACCAGAGCATCCA-3'
	R5'-TTGCGTTGGACATTCAAGTC-3'
HIF-1α	F5'-GAGGCTTACCATCAGCTATTTGC-3'
	R5'-ATCTGTGCTTTCATGTCATCTTCAATAT-3'
CXCL1	*SuperArray PPH00696B; RP 505–527, exon 4
ABCB11	*SuperArray PPH01516B; RP 706–725, exon 5
CACL2	*SuperArray PPH14870A; RP 2736–2756, exon 14
SPINK7	*SuperArray PPH14907A; RP194–215, exon 3
AMFR	*SuperArray PPH16329A; RP1865–1883, exon 13

*Primer sequence proprietary, reference position (RP) and exon location provided.

Table 2

Genes differentially expressed in polyps from patients with NE-CRSwNP

Affymetrix Probe Set ID	Representative Public ID	Gene Name	Fold Change	*p* Value
235193_at	BG036618	*Homo sapiens* transcribed sequences	8.24	0.0350
202203_s_at	NM_001144	Autocrine motility factor receptor	5.78	0.0126
222847_s_at	AI378406	egl nine homolog 3 (*C. elegans*)	5.47	0.0305
201939_at	NM_006622	Serum-inducible kinase	4.64	0.0189
223278_at	M86849	Gap junction protein, β 2, 26 kDa (connexin 26)	4.19	0.0452
232762_at	AU146385	Hypothetical protein DKFZp761L0424	4.1	0.0451
1556026_at	AI692623	Iduronate 2-sulfatase (Hunter syndrome)	3.79	0.0419
232058_at	AU158358	*Homo sapiens* cDNA FLJ13694 fis, clone PLACE2000115.	3.55	0.0360
232165_at	AL137725	Epiplakin 1	3.18	0.0224
224374_s_at	AF270513	Elastin microfibril interfacer 2 /// elastin microfibril interfacer 2	3.08	0.0261
205157_s_at	NM_000422	Keratin 17	2.95	0.0213
215322_at	AL080190	*Homo sapiens* mRNA; cDNA DKFZp434A202 (from clone DKFZp434A202)	2.71	0.0123
241990_at	BE547917	ras homolog gene family, member V	2.67	0.0377
228143_at	AI684991	Ceruloplasmin (ferroxidase)	2.61	0.0223
204470_at	NM_001511	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, α)	2.6	0.0303
228738_at	AI927858	Hypothetical protein MGC25181	2.38	0.0305
205443_at	NM_003082	Small nuclear RNA activating complex, polypeptide 1, 43 kDa	2.35	0.0194
218810_at	NM_025079	Hypothetical protein FLJ23231	2.34	0.0478
205870_at	NM_000623	Bradykinin receptor B2	2.33	0.0361
225265_at	AI580100	*Homo sapiens* mRNA; cDNA DKFZp564H0764 (from clone DKFZp564H0764)	2.31	0.0473
202464_s_at	NM_004566	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	2.25	0.0315
226736_at	BE568660	Chromosome 14 open reading frame 52	2.23	0.0493
219284_at	NM_024610	HSPB (heat shock 27 kDa)-associated protein 1	2.07	0.0299
230624_at	AW779950	Solute carrier family 25, member 27	2.07	0.0322
205214_at	NM_004226	Serine/threonine kinase 17b (apoptosis-inducing)	2.03	0.0208
206918_s_at	NM_003915	Copine I	2.01	0.0307
213679_at	AL049329	Hypothetical protein FLJ13946	1.98	0.0152
204094_s_at	NM_014779	KIAA0669 gene product	1.89	0.0174
204455_at	NM_001723	Bullous pemphigoid antigen 1, 230/240 kDa	1.89	0.0121
225344_at	AL035689	Nuclear receptor coactivator 7	1.88	0.0114
230398_at	AA158731	C-Terminal tensin-like	1.88	0.0278
227379_at	AI734993	*O*-acyltransferase (membrane bound) domain containing 1	1.85	0.0080
224572_s_at	BG485163	Interferon regulatory factor 2 binding protein 2	1.84	0.0462
225786_at	AI440495	Hypothetical protein LOC116228	1.82	0.0464
1555962_at	CA503291	UDP-GlcNAc:betaGal β-1,3-*N*-acetylglucosaminyltransferase 7	1.8	0.0460
219505_at	NM_017424	Cat eye syndrome chromosome region, candidate 1	1.79	0.0498

Affymetrix Probe Set ID	Representative Public ID	Gene Name	Fold Change	*p* Value
243539_at	AI560205	*Homo sapiens* transcribed sequence with weak similarity to protein ref:NP_060312.1 (*H.sapiens*) hypothetical protein FLJ20489 [*Homo sapiens*]	1.77	0.0326
46665_at	AI949392	Sema domain, immunoglobulin domain, transmembrane domain, and short cytoplasmic domain, (semaphorin) 4C	1.74	0.0308
1555963_x_at	CA503291	UDP-GlcNAc:betaGal β-1,3-*N*-acetylglucosaminyltransferase 7	1.72	0.0180
219039_at	NM_017789	Sema domain, immunoglobulin domain, transmembrane domain, and short cytoplasmic domain, (semaphorin) 4C	1.71	0.0377
204431_at	NM_003260	Transducin-like enhancer of split 2 (E(sp1) homolog, *Drosophila*)	1.7	0.0495
218599_at	NM_005132	REC8-like 1 (yeast)	1.65	0.0145
200919_at	NM_004427	Polyhomeotic-like 2 (*Drosophila*)	1.64	0.0382
212297_at	BF218804	ATPase family homolog up-regulated in senescence cells	1.64	0.0340
212689_s_at	AA524505	Jumonji domain containing 1	1.63	0.0267
205906_at	NM_001454	Forkhead box J1	1.58	0.0247
229302_at	AA058832	Hypothetical protein MGC33926	1.58	0.0352
201997_s_at	NM_015001	SMART/HDAC1-associated repressor protein	1.57	0.0251
218157_x_at	NM_020239	Small protein effector 1 of Cdc42	1.56	0.0483
218566_s_at	NM_012124	Cysteine and histidine-rich domain (CHORD)-containing, zinc binding protein 1	1.53	0.0250
202761_s_at	NM_015180	Spectrin repeat containing, nuclear envelope 2	1.52	0.0253
218611_at	NM_016545	Immediate early response 5	1.51	0.0250
229411_at	AI986390	Similar to calcium/calmodulin-dependent protein kinase 1, β	5.74	0.0194
1552639_at	NM_138433	Hypothetical protein BC009980	3.52	0.0402
210254_at	L35848	Membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)	2.33	0.0461
230578_at	AL042523	Zinc finger protein 471	1.98	0.0215
228538_at	BE671164	*Homo sapiens* cDNA FLJ46840 fis, clone UTERU2037423, moderately similar to Zinc finger protein 135	1.86	0.0185
240824_at	AI076185	*Homo sapiens* transcribed sequences	1.81	0.0401
225095_at	W81119	Serine palmitoyltransferase, long chain base subunit 2	−1.5	0.0436
227682_at	BE645154	*Homo sapiens* transcribed sequence with weak similarity to protein pir:A45973 (*H. sapiens*) A45973 trichohyalin—human	−1.5	0.0368
212310_at	D87742	C219-reactive peptide	−1.51	0.0421
202903_at	AU153477	LSM5 homolog, U6 small nuclear RNA associated (*S. cerevisiae*)	−1.52	0.0389
214512_s_at	NM_006713	Activated RNA polymerase II transcription cofactor 4	−1.52	0.0411
218528_s_at	NM_022781	Ring finger protein 38	−1.52	0.0231
229410_at	AI659219	*Homo sapiens* cDNA FLJ36689 fis, clone UTERU2008653, highly similar to GLYCODELIN PRECURSOR.	−1.52	0.0488
217761_at	NM_018269	SIPL protein	−1.55	0.0196
232899_at	R17536	*Homo sapiens* transcribed sequences	−1.55	0.0256
223054_at	BC001144	DnaJ (Hsp40) homolog, subfamily B, member 11	−1.57	0.0368
209511_at	BC003582	Polymerase (RNA) II (DNA directed) polypeptide F	−1.58	0.0410
218289_s_at	NM_024818	Ubiquitin-activating enzyme E1-domain containing 1	−1.58	0.0233
222446_s_at	AF178532	β-site APP-cleaving enzyme 2	−1.65	0.0158
218568_at	NM_018238	Hypothetical protein FLJ10842	−1.68	0.0421

Affymetrix Probe Set ID	Representative Public ID	Gene Name	Fold Change	*p* Value
226810_at	BE500942	*Homo sapiens* mRNA; cDNA DKFZp761M0111 (from clone DKFZp761M0111)	−1.7	0.0499
1569110_x_at	BC020552	Programmed cell death 6	−1.7	0.0240
223441_at	AK026921	Solute carrier family 17 (anion/sugar transporter), member 5	−1.74	0.0460
222877_at	AK024680	Neuropilin 2	−1.75	0.0265
225923_at	AW291083	Vesicle-associated membrane protein-associated protein B and C	−1.82	0.0461
200889_s_at	AI016620	Signal sequence receptor, α (transloconassociated protein α)	−1.87	0.0295
225435_at	BF679286	*Homo sapiens* cDNA FLJ14232 fis, clone NT2RP4000035.	−1.93	0.0341
226018_at	W73230	Hypothetical protein Ells1	−2.01	0.0488
1568764_x_at	AI692169	Programmed cell death 6	−2.03	0.0078
213611_at	BF726531	Aquaporin 5	−2.05	0.0250
225853_at	BE789346	Glucosamine-phosphate *N*-acetyltransferase 1	−2.05	0.0335
1554018_at	BC011595	Glycoprotein (transmembrane) nmb	−2.06	0.0475
214126_at	N39314	Mitochondrial carrier triple repeat 1	−2.15	0.0401
203582_s_at	NM_004578	RAB4A, member RAS oncogene family	−2.19	0.0467
210078_s_at	L39833	Potassium voltage-gated channel, *shaker*-related subfamily, β member 1	−2.26	0.0327
219582_at	NM_024576	Opioid growth factor receptor-like 1	−2.36	0.0246
203392_s_at	NM_001328	C-terminal binding protein 1	−2.38	0.0360
204437_s_at	NM_016725	Folate receptor 1 (adult)	−2.42	0.0186
1555812_a_at	AF498927	ρ GDP dissociation inhibitor β	−2.42	0.0217
203186_s_at	NM_002961	S100 Calcium binding protein A4 (calcium protein, calvasculin, metastasin, murine placental homolog)	−2.45	0.0264
219600_s_at	NM_006134	Chromosome 21 open reading frame	−2.54	0.0437
201633_s_at	AW235051	Cytochrome b5 outer mitochondrial membrane precursor	−2.64	0.0270
202730_s_at	NM_014456	Programmed cell death 4 (neoplastic transformation inhibitor)	−2.67	0.0170
210999_s_at	U66065	Growth factor receptor–bound protein 10	−2.7	0.0351
205110_s_at	NM_004114	Fibroblast growth factor 13	−2.82	0.0401
218858_at	NM_022783	Hypothetical protein FLJ12428	−2.94	0.0191
207717_s_at	NM_004572	Plakophilin 2	−3.19	0.0076
222385_x_at	AF346602	Sec61 α 1 subunit (*S. cerevisiae*)	−3.44	0.0476
216733_s_at	X86401	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	−4.09	0.0067
206628_at	NM_000343	Solute carrier family 5 (sodium/glucose cotransporter), member 1	−4.29	0.0314
201860_s_at	NM_000930	Plasminogen activator, tissue	−4.54	0.0383
207001_x_at	NM_004089	δ-Sleep inducing peptide, immunoreactor	−6.6	0.0070
1556114_a_at	BC036906	*Homo sapiens* mRNA; cDNA DKFZp451A211 (from clone DKFZp451A211)	−7.99	0.0414
205009_at	NM_003225	Trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in)	−9.01	0.0100
230319_at	AI222435	*Homo sapiens* cDNA FLJ36413 fis, clone THYMU2010816	−10.04	0.0341
207430_s_at	NM_002443	Microseminoprotein, β-	−10.28	0.0101
204607_at	NM_005518	3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	−13.81	0.0133

Affymetrix Probe Set ID	Representative Public ID	Gene Name	Fold Change	*p* Value
1552559_a_at	NM_139158	Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 7	−1.68	0.0435
238647_at	AA496213	Chromosome 14 open reading frame 28	−1.98	0.0339
1562219_at	BC040854	*Homo sapiens* cDNA FLJ41649 fis, clone FEBRA2024343	−2.13	0.0314
1570314_at	BC020913	*Homo sapiens*, clone IMAGE:4733200, mRNA	−2.21	0.0463
201559_s_at	AF109196	Chloride intracellular channel 4	−2.63	0.0308
237664_at	AV745243	Human clone JkA5 mRNA-induced upon T-cell activation, 3’ end.	−2.85	0.0361
219778_at	NM_012082	Zinc finger protein, multitype 2	−4.22	0.0458
209613_s_at	M21692	Alcohol dehydrogenase IB (class I), β polypeptide	−4.68	0.0191
37512_at	U89281	3-Hydroxysteroid epimerase	−6.12	0.0441
1567035_at	U63828	Chromosome 20 open reading frame 181	−6.69	0.0103
214839_at	AF052108	*Homo sapiens* clone 23687 mRNA sequence	−15.54	0.0319

Table 3

Genes selected for evaluation by RT-PCR

Gene Name	GeneID		Micrarray Fold Change	qRT-PCR Fold Difference*	*p*	Function
NM_000899	KITLG	KIT ligand (aka stem cell factor; SCF)	−2.48	4.00		Stimulates the differentiation and proliferation of mast cells. Augments the proliferation of myeloid and lymphoid hematopoietic progenitors in bone marrow culture. Mediates cell-cell adhesion. Acts synergistically with other cytokines[#]
NM_000589	IL-4	Interleukin-4	1.28	0.52		IL-4 is a pleiotropic cytokine produced by activated T cells which induces differentiation of Th0 to Th2 cells. The IL-4 receptor also binds to IL-13, which may contribute to many overlapping functions of this cytokine and IL13. It is a hallmark cytokine of Th2-mediated immune response.
NM_000584	IL-8	Interleukin-8	2.3	4.11	0.07	Chemotactic factor for neutrophils, basophils, and T cells, but not monocytes. Also involved in neutrophil activation.[#]
NM_000641	IL-11	Interleukin-11	16.35	1.04		Directly stimulates the proliferation of hematopoietic stem cells and megakaryocyte progenitor cells and induces increased platelet production. Important in fibrosis.[#]
NM_000600	IL-6	Interleukin-6	5.13	1.67		Plays an essential role in the final differentiation of B cells into Ig-secreting cells. Induces myeloma and plasmacytoma growth, nerve cells differentiation, and, in hepatocytes, acute phase reactants. Mediates Th17 lymphocyte differentiation.[#]
S69738	CCL2	Chemokine (C-C Motif) ligand 2 (aka monocyte chemotractant protein 1; MCP-1)	17.47	1.99		Chemotactic factor for monocytes and basophils but not neutrophils or eosinophils. Augments monocyte antitumor activity. Implicated in diseases characterized by monocytic infiltrates, including psoriasis, rheumatoid arthritis and atherosclerosis.[#]
BF434846	TNC	Tenascin-C	3.83	8.82		Expressed during wound healing, nerve regeneration, and tissue involution, and in pathological states including vascular disease, tumorigenesis, and metastasis.[#]
NM_002987	CCL17	Chemokine (C-C motif) ligand 17, (aka thymus and activation-regulated chemokine; TARC)	−2.01	2.77		Chemotactic factor for T lymphocytes (especially Th2-like cells) but not monocytes or granulocytes. May play a role in T cell development in thymus and in trafficking and activation of mature T cells. Binds to CCR4.[#]
AW118681	TSLP	Thymic stromal lymphopoetin	2.81	1.82		Impacts myeloid cells to induces the release of T cell–attracting chemokines and enhances the maturation of dendritic cells. Activates dendritic cells to promote their differentiation of Th2-like cells—important mediator of allergic inflammation.[#]

Gene Name	GeneID		Micrarray Fold Change	qRT-PCR Fold Difference*	*p*	Function
NM_002188	IL-13	Interleukin-13	−2.79	0.24	0.07	Primarily produced by Th2 cells. Is involved in several stages of B-cell maturation and differentiation. Up-regulates CD23 and MHC class II expression, and promotes IgE isotype switch of B cells. Down-regulates macrophage activity, inhibiting the production of proinflammatory cytokines and chemokines. Critical to the pathogenesis of allergic inflammatory disorders including asthma.§
M29383	IFN-G	Interferon-γ	3.03	0.29		Activates antigen-presenting cells and promotes Th1 differentiation by up-regulating the transcription factor T-bet. The hallmark cytokine of Th1 cells (Th2 cells produce IL-4). NK cells and CD8+ cytotoxic T cells also produce IFN-γ.[16]
AA913703	HIF-1A	Hypoxia inducible factor 1α	5.46	11.71	0.007	A transcription factor found under reduced oxygen tension that plays an essential role in cellular and systemic homeostatic responses to hypoxia. Also produced in normoxic conditions in response to tissue injury.§
NM_001511	CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, α), aka Gro-α	2.6	56.10	0.05	Chemotactic for neutrophils. Plays a role in inflammation and exerts effects on endothelial cells in an autocrine fashion.#
AF136523	ABCB11	ATP-binding cassette, subfamily B (MDR/TAP), member 11	184.3	1375.59		Member of the MDR/TAP subfamily of proteins that are involved in multidrug resistance. Is the major bile salt transporter. Mutations cause a form of progressive familial intrahepatic cholestases.§
NM_006536	CLCA2	Chloride channel, calcium activated, family member 2	−19.96	0.91		Member of the calcium sensitive chloride conductance protein family. Expressed predominantly in trachea and lung, where it is suggested to play a role in cystic fibrosis. May serve as adhesion molecule for lung metastatic cancer cells, mediating vascular arrest and colonization. Implicated as a tumor suppressor gene for breast cancer.§
AF268198	SPINK7	Serine peptidase inhibitor, kazal type 7 (aka esophageal cancer–related gene 2; ECRG2)	−342.79	1.62		SPINK7/ECRG2 is thought to inhibit aggressiveness of cancer cells, possibly through the down-regulation of uPA/plasmin activity.
NM_001144	AMFR	Autocrine motility factor receptor	5.78	19.84	0.03	Autocrine motility factor is a tumor motility-stimulating protein secreted by tumor cells. The protein encoded by this gene is a glycosylated transmembrane protein and its receptor. Shows sequence similarity to tumor protein p53, is localized to the leading and trailing edges of carcinoma cells.§

*$2^{-\Delta\Delta Ct}$ method; because of the different manner by which fold change is calculated for microarray and RT-PCR data, a negative fold change as reported on the microarray is equivalent to a fractional (*i.e.*, <1) fold difference on RT-PCR analysis.

#Function data as noted on NIAID DAVID Bioinformatics Resources (Available online at www.david.abcc.ncifcrf.gov; last accessed April 1, 2008).

[§] Function data as noted in summary data for EntrezGene record (Available online at www.ncbi.nlm.nih.gov/sites/entrez?Db=gene; last accessed April 1, 2008).

RT-PCR = reverse-transcription polymerase chain reaction.