Expression Profiling of Inflammatory Mediators in Pediatric Sinus Mucosa

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Objective: To evaluate gene expression by microarray analyses of inflammatory mediators in the sinus mucosa of children with and without chronic rhinosinusitis (CRS).

Design: Prospective molecular genetics analysis.

Setting: Children’s National Medical Center, Washington, DC.

Subjects: Eleven patients with CRS who underwent endoscopic sinus surgery and 10 control children who underwent craniofacial resection or neurosurgical procedures.

Main Outcome Measures: Gene expression levels of sinus tissue from 6 patients with CRS and 6 controls and messenger RNA expression levels of upregulated inflammatory/immune response genes, as well as cytokines of interest, determined by quantitative reverse transcription–polymerase chain reaction.

Results: Gene expression using the Plier algorithm yielded the most consistent grouping of samples: 96 genes were significantly upregulated more than 2-fold, and 123 genes were downregulated by at least 50% in the CRS sinus tissues compared with controls (P < .05). Gene-Spring analysis demonstrated significant changes in several ontology categories in the CRS samples, including inflammatory/immune response genes. The chemokines CXCL13 and CXCL5, serum amyloid A, serpin B4, and defensin β1 were highly upregulated (≥3-fold). Increased expression of these genes was validated by quantitative reverse transcription–polymerase chain reaction in an independent set of tissues. Expression levels of interleukins 5, 6, and 8 were similar in both cohorts; these results were validated by reverse transcription–polymerase chain reaction.

Conclusions: Microarray analyses of sinus mucosa in children with CRS showed an increased expression of inflammatory genes involved in innate and adaptive immune systems. This technology can be successfully used to identify genes implicated in the pathogenesis of pediatric CRS.


The sinus mucosa has developed both innate and adaptive immune mechanisms to protect it from pathogens and environmental irritants. Alterations in either system may make patients susceptible to chronic rhinosinusitis (CRS), which is defined as sinonasal inflammation of the paranasal sinuses manifesting with rhinorrhea, nasal congestion, facial pain, and headache of more than 12 weeks’ duration and refractory to medical management.1,2 The cause of CRS is not well understood; however, it is generally accepted that various proinflammatory mediators and immunoreactive products play a significant role in initiating and sustaining the inflammatory response seen in these patients. Cytokines are proinflammatory mediators that function as part of the adaptive immune system via complex intercellular signals. The cytokines granulocyte-macrophage colony-stimulating factor and interleukin (IL) 3 have been implicated in adults with nonallergic CRS.3 These cytokines, in addition to IL-4 and IL-5, have been observed at high levels in adult CRS sinus tissues, and IL-8 levels appear to correlate with the degree of disease severity.3,6 Levels of IL-6, another proinflamm-
matory cytokine, are also elevated in adults with CRS, as are those of RANTES, a chemotactic cytokine.

The innate immune system, another line of defense for respiratory epithelium, includes the paranasal sinus mucosa, which traps particles and pathogens and removes them from the sinus tissues through mucociliary clearance. Other mechanisms used by the innate immune system include the secretion of broad-spectrum antimicrobial peptide products such as β-defensins and the production of acute-phase proteins such as serum amyloid A (SAA), which is expressed in adult sinonasal tissues. Properdin, complement 3, and toll-like receptors have also been identified in human sinus mucosa of adults with CRS and control patients. However, little information is available on inflammatory mediators and innate immune response agents involved in the pathophysiologic mechanism of pediatric CRS, and these profiles may be different between adults and children.

Genome-wide expression array analysis is a relatively new technology in which simultaneous analysis of messenger RNA (mRNA) expression of the more than 30,000 genes in the human genome can be determined. Identification of differentially expressed genes between control and diseased tissues, followed by bioinformatics analyses and integration into pathway analyses, has led to increased understanding of pathways and mechanisms wherein inflammation leads to pathological changes. This has been shown for several systems and diseases, including muscle and allergy. Furthermore, adult sinus and nasal mucosa has been evaluated by this technique. Genes associated with innate host responses, inflammation, cell activation, signal transduction, and cellular proliferation were differentially expressed when the nasal polyps of 10 patients with CRS (3 with allergy, 5 with asthma, and 2 aspirin-sensitive patients) were compared with sphenoid sinus mucosa from 4 control patients undergoing pituitary surgery. In another study, the inflammatory genes for IL-6, IL-12A, IL-13, and tumor necrosis factor-α (TNF-α) were upregulated when the anterior ethmoid mucosa of 14 adult patients with CRS and the nasal mucosa from 4 adult control patients were compared. However, these mRNA alterations were not validated by reverse transcription–polymerase chain reaction (RT-PCR) in an independent set of sinus tissues. Because gene microarray analyses have the potential to direct attention to new genes of interest as well as to identify new associations between established inflammatory and immune response genes that may be involved in CRS, we used this technique on sinus mucosa of children and adolescents with and without CRS. We evaluated the gene expression of mediators in the adaptive and innate immune system to determine whether age affects the inflammatory and immune mediator profile in CRS.

### TISSUE SAMPLES

Sinus tissues from patients who underwent craniofacial and/or neurosurgical procedures for abnormalities other than sinusitis served as controls. For the control group, 10 patients (4 boys and 6 girls), aged 152 to 222 months, were studied. Exclusion criteria for the control population included a history of sinonasal surgery, current sinonasal infection, sinonasal or allergic symptoms within the previous 3 months, and/or treatment with topical nasal corticosteroids within 1 month or antihistamines within 3 months before surgery. A combination of computed tomographic (CT) scans and/or magnetic resonance images with sinonasal sections was obtained. There was no radiographic evidence of sinusitis at the time of surgery for children in the control group. One patient had a meningioma encroaching on the right frontal sinus, but it did not violate the mucosa.

Sinus tissue was obtained from patients with CRS who underwent functional endoscopic sinus surgery for CRS refractory to medical management. The clinical data of 11 patients (8 boys and 3 girls) with CRS, aged 30 to 215 months, are shown in Table 1. Chronic rhinosinusitis was defined as persistent symptoms for more than 3 months, despite antimicrobial and topical nasal corticosteroid therapies. Patient criteria for entry into the study were identical to those of patients recruited for earlier studies. Patients with sinusitis had at least 2 of the major signs and symptoms of chronic sinusitis: nasal congestion, rhinorrhea, headache, facial pain/pressure, or change in olfaction. In addition, all of these children had at least 1 of the following minor signs or symptoms in conjunction with a minimum of 2 major signs and symptoms: fever, halitosis, cough, or irritability. All patients with CRS were receiving antibiotics and/or topical nasal corticosteroids at the time of surgery. Patients with cystic fibrosis, ciliary dyskinesias, and craniofacial abnormalities were excluded. In all patients with CRS, CT scans of the sinuses were obtained, evaluated, and scored by means of the Lund-Mackay system. All CT scans received a minimum score of 8.

All patients were entered consecutively into the study after appropriate surgical and research consents (and assents when applicable) were obtained. This study was reviewed and approved by the institutional review board of Children’s National Medical Center.

### CLINICAL DATA

Patient age (months), sex, atopy, presence of reactive airway disease, immunodeficiency, and use of systemic corticosteroids were recorded. Atopy was categorized as present (yes) or absent (no) and was based on results of prick puncture or in-
tradermal skin testing whenever possible. Immunologic status was determined from quantitative analyses of total immunoglobulins and IgG subclasses in serum, as well as antibody responses to pneumococcal vaccine challenge.\textsuperscript{11} The CT scans of sinuses were also reviewed.

COLLECTION OF SPECIMENS

With the use of standard endoscopic instruments, mucosa from the right and left paranasal sinuses was collected separately at the time of surgery. Specimens ranged from 0.2 to 1.3 cm\textsuperscript{3}. In control patients, the mucosa of the paranasal sinuses, entered as a result of the craniofacial and/or neurosurgical procedure performed, was collected in an identical manner to that of the sinusitis population. Specimens were immediately frozen in liquid nitrogen for RNA isolation.

GENE EXPRESSION PROFILING AND MICROARRAY DATA ANALYSIS

Sample preparation and microarray analyses were performed in the Microarray Core at Children's National Medical Center according to established protocols\textsuperscript{12} based on standard methodology from the manufacturer of the analysis equipment (Affymetrix Inc; Santa Clara, California; http://www.affymetrix.com). Total RNA from frozen mucosal samples was isolated by means of a reagent (Trizol; Invitrogen, Carlsbad, California) and purified with RNeasy MinElute Cleanup Kit (QIAGEN Inc, Valencia, California) according to the manufacturer's protocols. The RNA was quantified on a spectrophotometer (ND-1000; NanoDrop Technologies, Wilmington, Delaware). Purity and integrity were assessed by means of an analyzer (Agilent 2100 Bioanalyzer; Agilent Technologies, Santa Clara, California). All samples showed intact 28S and 18S ribosomal RNA bands in an approximate ratio of 2:1. Five micrograms of total RNA was used to initiate the complementary DNA–complementary RNA (cDNA-cRNA) cycle. Both cDNA synthesis and in vitro transcription were performed with a kit (GeneChip Expression 3Amplication One-Cycle Target Labeling and Control Reagent kit; Affymetrix Inc) according to the manufacturer's instructions. Twenty micrograms of biotin-labeled cRNA from each sample was fragmented and hybridized to Affymetrix Human Genome U133_Plus2.0 Arrays (Affymetrix Inc) for 16 hours, followed by standard washing-scanning protocols on the Affymetrix Fluidics Station 400 (Affymetrix Inc) and incubation with phycoerythrin-streptavidin to detect bound cRNA. The signal intensity was amplified by means of biotin-labeled antistreptavidin antibody. Fluorescent images were captured by means of a gene array scanner (Hewlett-Packard G2500A; Palo Alto, California).

All data were analyzed by means of Affymetrix Microarray Analysis Software version 5.0 (MAS 5.0), dChip, and Plier algorithms. Only probe sets that were statistically significant with 1-way analysis of variance and a \( P < .05 \) were used. The data set from each algorithm was loaded into a data mining program (GeneSpring GX, version 7.3.1; Agilent Technologies) for future analysis. The results were visualized by means of the gene tree cluster feature of the program, which rearranges the order of the probe sets and groups them on the basis of the similarity (Pearson correlation) of their expression dynamics.\textsuperscript{18} Hierarchical clustering analysis of all 3 algorithm data sets was performed with the Hierarchical Clustering Explorer 3.5 program (http://www.cs.umd.edu/hcil/hce/).\textsuperscript{19} Similarity and distance measures were assessed by Pearson correlation coefficient. Multivariate analyses of the expression array data set of immune and inflammatory mediator genes were performed for the controls and patients with CRS according to age.

REAL-TIME RT-PCR ANALYSIS

Sinus mucosa was homogenized in reagent (Trizol). RNA was isolated per standard techniques, followed by cleaning with the RNeasy MinElute Cleanup Kit. Total RNA yields were measured on a spectrophotometer (NanoDrop), and RNA quality was visualized by means of agarose gel electrophoresis. Four micrograms of RNA, treated with DNaSe I (Invitrogen) for 15 minutes at room temperature, was used to synthesize first-strand cDNA with a reverse transcription kit (SuperScript III; Invitrogen). Real-time PCR was performed with a PCR reagent kit (Power SYBR Green; Applied Biosystems, Foster City, California) in a sequence detection system (ABI Prism\textsuperscript{®} 7900HT; Applied Biosystems). The mRNA for target genes and the housekeeping gene \( \beta \)-actin were amplified and quantified in the sample reaction plate. Primers were designed with Primer3 software (http://frodo.wi.mit.edu/) to cross the intron-exon gap so as to prevent amplification of any genomic DNA contamination. The melting temperature was between 59°C and 61°C. All procedures were performed according to the manufacturer’s instructions. Standard curves were generated on the basis of five 5-fold serial dilutions of a starting concentration of a control cDNA (150 pg/µL). The PCR reactions were incubated at 50°C (2 minutes), 95°C (2 minutes), and then 40 cycles of 95°C (15 seconds) and 60°C (30 seconds). Threshold cycle and baseline were automatically calculated by means of the SDS 2.2 software (Applied Biosystems). A standard curve plotting CT values against the logarithm of template cDNA input was constructed for each gene. The numbers of copies in the experimental samples were calculated from the equation of the straight line for the standard curve. The final quality of each gene was normalized with housekeeping gene expression. The statistical significance of differences was determined by 1-tailed, unpaired \( t \) test. Data were expressed as mean values and standard errors. Differences were considered statistically significant at \( P < .05 \).

RESULTS

CLINICAL DATA

Twenty-one patients ranging in age from 30 to 220 months were studied. The median age of the pediatric patients with CRS was 89 months (range, 30-215 months), compared with the median age of 204.5 months (range, 152-222 months) in the control subjects. Clinical profiles of the patients with CRS are summarized in Table 1. Patient 8 had an immunoglobulin subclass deficiency. Patients with CRS had a score of 8 or more on the CT scan by Lund-Mackay staging.\textsuperscript{16}

EXPRESSION ARRAY ANALYSES OF SINUS MUCOSA FROM CONTROLS AND PATIENTS WITH CRS

To identify the differential expression of genes in the sinus mucosa of controls and patients with CRS, a genome-wide microarray analysis of the sinus mucosa from 12 individuals (6 patients with CRS and 6 controls) was performed with Affymetrix Human Genome U133_2.0 Arrays. Data interpretation is often profoundly affected by the type of algorithm used.\textsuperscript{20} Thus, to obtain the most accurate data set of differentially expressed genes, data were analyzed separately by MAS 5.0, dChip, and Plier

(ADDITIONAL INFORMATION TO MANUSCRIPT)
Figure 1. Gene tree analyses (Plier) of sinus tissues. Differential expression levels of each probe set are shown vertically for each patient sample. Red represents increased and blue represents decreased expression compared with control. CRS indicates chronic rhinosinusitis.

Figure 2. Venn diagram showing the number of statistically significantly altered probe sets in 3 algorithms for patients with chronic rhinosinusitis vs controls. Microarray Analysis Software version 5.0 (MAS 5.0; n=1652), dChip (n=1703), and Plier (n=2034). The white area in the center denotes the 576 common significantly changed probe sets in all 3 algorithms (P<.05).

algorithms, then loaded into GeneSpring G, where the data were normalized and filtered. Hierarchical cluster analysis showed that the Plier algorithm yielded the most consistent grouping of samples in the 3 groups. The overall F-measure score, which can range between 0 and 1, with higher scores reflecting better clustering results in biological samples, showed that the Plier algorithm at F = 0.68 had the highest F-measure score. Therefore, the Plier software program was used for data presentation of each probe set in each of the 12 sinus mucosa samples for the control and CRS cohorts (Figure 1). By means of a Venn diagram, the statistically significant genes (P<.05) from each algorithm were overlapped to yield a list of differentially expressed genes common to all algorithms (Figure 2). The data showed that 576 of 54 000 probe sets (47 000 transcripts) were significantly differentially expressed in CRS samples (P<.05). Ninety-six genes were significantly upregulated more than 2-fold, and 123 genes were downregulated by at least 50% (P<.05), compared with control samples. We focused our initial analyses on inflammatory/immune response genes, which have been implicated in adult CRS.

MICROARRAY ANALYSES OF INFLAMMATORY/IMMUNE RESPONSE GENES

Twelve genes related to inflammatory/immune response were significantly increased (P<.05) (Table 2). Five of these genes, including chemokine (C-X-C motif) ligand 13 (CXCL13) (GenBank NM_006419) and ligand 5 (CXCL5) (GenBank AK026546), serum amyloid A1/A2 (SAA1/SAA2) (GenBank NM_030754), S100 calcium-binding protein A8 (S100A8) (GenBank AW238654), and serpin peptidase inhibitor, member 4 (SERPINC4) (GenBank AB040400), were increased more than 5-fold. β-Defensin 1 (DEFB1) (GenBank U73945), arachidonate 5-lipoxygenase (ALOX5) (GenBank NM_000698), and 2 IgG affinity receptors were increased more than 2-fold.

VALIDATION OF INFLAMMATORY/IMMUNE RESPONSE GENE MRNA EXPRESSION LEVELS BY REAL-TIME PCR

The expression levels of 7 inflammatory/immune response genes (CXCL3, CXCL13, SAA1/SAA2, SERPINB4, S100A8, DEFB1, and ALOX5; Table 2) were evaluated by real-time PCR in an independent set of sinus tissue from 5 patients with CRS (Table 1) and 4 control patients. Data showed that mRNA levels for CXCL13, CXCL5, SAA1/SAA2, SERPINB4, and DEFB1 were increased in the sinus mucosa of patients with CRS compared with the sinus mucosa of control patients (Figure 3). These results are consistent with the microarray analyses (Table 2), although the rank order and magnitude of gene expression levels differed between the 2 methods. The mRNA levels of S100A8 and ALOX5, which were significantly increased in CRS tissues by microarray analyses, were, however, not significantly increased when analyzed by real-time RT-PCR (Figure 3).

ANALYSES OF CYTOKINES IN THE SINUS MUCOSA OF CRS AND CONTROL SAMPLES

The expression levels of most of the cytokines of interest in the CRS literature are compared in Table 3. Expression levels between the control and CRS cohorts were either similar or not statistically different for IL-4, IL-5, IL-13, granulocyte-macrophage colony-stimulating factor, and various members of the TNF superfamily (SF), eg, TNFSF11, TNFSF4, and TNFSF8. The IL-3 expression was increased in CRS, but the increase was not statistically significant. However, expression levels of IL-6, IL-8, IL-12A, TNFSF7, TNFSF9, and TNFSF12 were significantly decreased in CRS sinus mucosa. Real-time PCR analyses of IL-5, IL-6, and IL-8 were performed with the
Table 2. Upregulated Inflammatory and Immune Response Gene List (P<.05) Identified by Microarray Expression Profiling of Sinus Mucosa From 6 Patients With CRS and 6 Control Patients

<table>
<thead>
<tr>
<th>ID No.</th>
<th>Gene</th>
<th>CRS Fold Changes</th>
<th>P Value</th>
<th>Gene Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>208607_s_at</td>
<td>SAA1; SAA2*</td>
<td>12.70</td>
<td>.04</td>
<td>Serum amyloid A1; serum amyloid A2</td>
</tr>
<tr>
<td>214974_x_at</td>
<td>CXCL5*</td>
<td>12.57</td>
<td>.04</td>
<td>Chemokine (C-X-C motif) ligand 5</td>
</tr>
<tr>
<td>205242_at</td>
<td>CXCL13*</td>
<td>9.57</td>
<td>.02</td>
<td>Chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)</td>
</tr>
<tr>
<td>211906_s_at</td>
<td>SERPINB4*</td>
<td>9.10</td>
<td>.02</td>
<td>Serpin peptidase inhibitor, clade B (ovalbumin), member 4</td>
</tr>
<tr>
<td>214370_at</td>
<td>S100A8*</td>
<td>5.83</td>
<td>.003</td>
<td>S100 calcium-binding protein A9 (calgranulin A)</td>
</tr>
<tr>
<td>210397_at</td>
<td>DEFBI*</td>
<td>4.98</td>
<td>.03</td>
<td>Defensin β-1</td>
</tr>
<tr>
<td>216950_s_at</td>
<td>FCGR1A*</td>
<td>2.78</td>
<td>.03</td>
<td>Fc fragment of IgG, high-affinity Ia, receptor (CD64)</td>
</tr>
<tr>
<td>204466_s_at</td>
<td>ALOX5*</td>
<td>2.15</td>
<td>.02</td>
<td>Arachidonate 5-lipoxygenase</td>
</tr>
<tr>
<td>215121_x_at</td>
<td>IGLC1-2, IGLV2-25*</td>
<td>1.84</td>
<td>.049</td>
<td>Immunoglobulin λ locus; immunoglobulin λ constant 1 (Mcg marker); immunoglobulin λ constant 2 (Kern-Oz marker); immunoglobulin λ variable 2-25</td>
</tr>
<tr>
<td>231829_at</td>
<td>KIAA1271</td>
<td>1.71</td>
<td>.002</td>
<td>KIAA1271 protein</td>
</tr>
<tr>
<td>204864_s_at</td>
<td>IL6ST*</td>
<td>1.35</td>
<td>.02</td>
<td>Interleukin 6 signal transducer</td>
</tr>
</tbody>
</table>

Abbreviations: CRS, chronic rhinosinusitis; Fc, fragment crystallizable region; ID, Affymetrix identifier.

*Expression levels of these genes were also analyzed by real-time reverse transcription–polymerase chain reaction.

Figure 3. Quantitative reverse transcription–polymerase chain reaction analyses of 7 upregulated genes from microarray study. Data show fold change (with standard errors) in transcript levels of tissues from controls (n=4) and patients with chronic rhinosinusitis (CRS) (n=5). *Statistically significant difference (P<.05) between the 2 groups.
independent set of sinus mucosa used for validation in Figure 3. Data showed that the expression levels of these cytokines were variable, but were not significantly altered in CRS tissues (Figure 4).

**ANALYSIS OF AGE AND INFLAMMATORY GENE EXPRESSION**

Multivariate analyses of the expression array data sets of the immune and inflammatory mediator genes shown in Table 3 were performed for the control and CRS groups according to age. The age of the control patients was significantly higher in both the microarray group (P = .006) and real-time RT-PCR group (P = .03) compared with the diseased cohort. Age was not a significant factor when adjusted for disease status. Statistical analyses adjusted for age were inconclusive (data not shown).

Gene microarrays are now a standard experimental platform for investigating gene expression in different biological tissues and to identify differential gene expression in control and diseased tissues, including adult CRS tissues. This, to our knowledge, the first study to use gene microarray technology to begin to analyze the inflammatory/immune response gene products that may be involved in the pathophysiologic mechanism of pediatric CRS. However, owing to the variations in clinical profiles of the disease cohort, including the differences in age, medical management, length of medical management, duration of the disease, and presence of allergy and reactive airway disease, the results have to be interpreted somewhat cautiously because of the inability to eliminate confounding factors in the patient population. Nevertheless, the stratification of the CRS and non-CRS cohorts shown in Figure 1 is encouraging.

In this preliminary analysis, 5 inflammatory/immune response gene products were shown by microarray technology and real-time RT-PCR to be highly and significantly upregulated in pediatric CRS sinus mucosa. Two of these gene products were the cytokines CXCL5, a neutrophil chemoattractant, and CXCL13, a potent chemoattractant of B lymphocytes, cells which are not routinely associated with pediatric CRS. Cytokines are typically small proteins that are part of the adaptive immune system and thought to play an integral role in the pathophysiologic mechanism of CRS. They include interleukins, chemokines, interferons, colony-stimulating factors, and tumor necrosis factors. The deregulation of cytokines is believed, in large part, to be responsible for the excessive inflammation seen in the sinus mucosa of patients with CRS. In the sinus mucosa of adults, elevated levels of the cytokines granulocyte-macrophage colony-stimulating factor, IL-3, IL-4, IL-5, IL-6, RANTES, and IL-8 have been reported. Levels of IL-6, IL-12A, IL-13, and TNF-α have been shown by microarray analyses to be increased in the sinus mucosa of patients with nonallergic CRS without nasal polyps. Interestingly, none of these cytokines exhibited increased expression in our microarray analyses of sinus mucosa from pediatric patients with CRS and control patients. In fact, levels of IL-6, IL-8, and IL-12A and several TNF factors were significantly decreased in the microarray data sets (Table 3). In addition, IL-5, IL-6, and IL-8 levels were not significantly altered when evaluated by real-time RT-PCR in an independent set of sinus mucosa tissues (Figure 4).

**Table 3. Comparison of mRNA Expression Levels of Differentially Expressed Cytokines Identified by Microarray Expression Profiling in the Sinus Mucosa of Patients With CRS and in Control Patients**

<table>
<thead>
<tr>
<th>ID No.</th>
<th>Gene</th>
<th>CRS Fold Changes</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>207906_at</td>
<td>IL3</td>
<td>1.74</td>
<td>.07</td>
</tr>
<tr>
<td>207538_at</td>
<td>IL4</td>
<td>1.04</td>
<td>.75</td>
</tr>
<tr>
<td>207952_at</td>
<td>IL6</td>
<td>0.66</td>
<td>.61</td>
</tr>
<tr>
<td>205207_at</td>
<td>IL6a</td>
<td>0.38</td>
<td>.001</td>
</tr>
<tr>
<td>211506_s_at</td>
<td>IL8</td>
<td>0.46</td>
<td>.006</td>
</tr>
<tr>
<td>207160_at</td>
<td>IL12A</td>
<td>0.55</td>
<td>.001</td>
</tr>
<tr>
<td>207844_at</td>
<td>IL13</td>
<td>1.03</td>
<td>.63</td>
</tr>
<tr>
<td>210643_at</td>
<td>TNFSF11</td>
<td>1.77</td>
<td>.41</td>
</tr>
<tr>
<td>208508_at</td>
<td>TNFSF7</td>
<td>1.35</td>
<td>.02</td>
</tr>
<tr>
<td>207426_s_at</td>
<td>TNFSF4</td>
<td>1.26</td>
<td>.57</td>
</tr>
<tr>
<td>206907_at</td>
<td>TNFSF9</td>
<td>0.88</td>
<td>.04</td>
</tr>
<tr>
<td>235735_at</td>
<td>TNFSF9</td>
<td>0.87</td>
<td>.26</td>
</tr>
<tr>
<td>205611_at</td>
<td>TNFSF12</td>
<td>0.67</td>
<td>.007</td>
</tr>
<tr>
<td>210228_at</td>
<td>GM-CSF</td>
<td>1.00</td>
<td>.99</td>
</tr>
</tbody>
</table>

Abbreviations: CRS, chronic rhinosinusitis; ID, Affymetrix identifier; mRNA, messenger RNA.

*Expression levels of these genes were also analyzed by real-time reverse transcription–polymerase chain reaction.

![Figure 4](image-url). Quantitative reverse transcription–polymerase chain reaction validation of interleukin (IL) 5, IL-6, and IL-8 in pediatric sinus mucosa. Data were obtained and are presented as described in Figure 3. No statistically significant differences in expression between the control and chronic rhinosinusitis (CRS) groups were observed.
The other 3 genes that exhibited markedly increased upregulation in this study were SAA1/SAA2, SERPINB4, and DEFB1, which genes encode for proteins that are involved in the innate immune system. SAA1/SAA2 are acute-phase proteins, DEFB1 is a broad-spectrum antimicrobial protein, and SERPINB4 neutralizes serine or cysteine proteinases. Serum amyloid A has previously been shown to be expressed in sinonasal tissue of controls and patients with CRS.22 DEFB1 has been identified in the lung.7 SERPINB4 has been detected in the sinonasal squamous epithelium.23 Other innate immunity mediators, such as properdin, complement 3, and toll-like receptors, have also been shown to be expressed in the sinus mucosa of controls and patients with CRS.8,22 Altered expression of toll-like receptor 2 (TLR2) was not observed in our studies, although it was reported to be increased in one study22 but not in another.8

The microarray gene profile generated by this study indicates that the sinus mucosa in children with CRS is immunocompetent and that both arms of the immune system are involved. Innate immunity is evolutionarily conserved and normally acts in concert with the adaptive arm of the immune system. Both adults and children are expected to use these functional mechanisms, and upregulation of components of the innate immune system in our study is not unexpected. Although there are similarities to adult patients with respect to the innate immune system, especially with regard to SAA, the cytokine gene expression pattern is different, suggesting that pediatric CRS is not the same entity as adult CRS. This is not a surprising conclusion and has been supported in the literature with respect to other variables. Chan et al20 demonstrated that the sinus mucosa of children with CRS has less eosinophilic inflammation, basement membrane thickening, and mucus gland hyperplasia than that of adults with CRS.

Other investigators have shown that age can affect the composition and magnitude of the inflammatory mediators.23 We considered whether the variation in the age of the patients studied affected the identification of immune and inflammatory mediator genes with microarray technique. Statistical analyses adjusted for age were inconclusive because of the small sample size and large variations in expression levels of these gene products. Therefore, no definite conclusion can be made with respect to the influence age has on the inflammatory and immune mediators identified in this investigation.

In conclusion, this study shows that gene array profiling is a powerful technique that can be used successfully to identify the inflammatory and immune mediator profiles in children with CRS and that different mediator profiles are seen in pediatric CRS than in adult CRS. Validation of specific differentially expressed genes by an independent method and in an independent set of sinus mucosal tissues demonstrated that specific genes in both arms of the immune system are markedly upregulated in the sinus mucosa of pediatric patients with CRS.

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Author Contributions: Drs Wu, Rose, and Peña had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Wu, Aujla, Rose, and Peña. Acquisition of data: Wu and Ghimbovschi. Analysis and interpretation of data: Wu, Ghimbovschi, Rose, and Peña. Drafting of the manuscript: Wu, Ghimbovschi, Rose, and Peña. Critical revision of the manuscript for important intellectual content: Wu, Aujla, Rose, and Peña. Statistical analysis: Ghimbovschi. Obtained funding: Rose and Peña. Administrative, technical, and material support: Wu, Ghimbovschi, Aujla, Rose, and Peña. Study supervision: Rose.

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