



Distinct epithelial gene expression phenotypes in childhood respiratory allergy

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ABSTRACT: Epithelial cell contribution to the natural history of childhood allergic respiratory disease remains poorly understood. Our aims were to identify epithelial pathways that are dysregulated in different phenotypes of respiratory allergy.

We established gene expression signatures of nasal brushings from children with dust mite-allergic rhinitis, associated or not associated with controlled or uncontrolled asthma. Supervised learning and unsupervised clustering were used to predict the different subgroups of patients and define altered signalling pathways. These profiles were compared with those of primary cultures of human nasal epithelial cells stimulated with either interleukin (IL)-4, IL-13, interferon (IFN)- α , IFN- β or IFN- γ , or during *in vitro* differentiation.

A supervised method discriminated children with allergic rhinitis from healthy controls (prediction accuracy 91%), based on 61 transcripts, including 21 T-helper cell (Th) type 2-responsive genes. This method was then applied to predict children with controlled or uncontrolled asthma (prediction accuracy 75%), based on 41 transcripts: nine of them, which were down-regulated in uncontrolled asthma, are directly linked to IFN. This group also included *GSDML*, which is genetically associated with asthma.

Our data revealed a Th2-driven epithelial phenotype common to all children with dust mite allergic rhinitis. It highlights the influence of epithelially expressed molecules on the control of asthma, in association with atopy and impaired viral response.

KEYWORDS: Allergic rhinitis, asthma, epithelium, microarrays, paediatric asthma, phenotypes of asthma

Asthma is a heterogeneous and complex disease that frequently co-occurs with allergic rhinitis. Classical pathogenic models characterise asthma and allergic rhinitis as inflammatory diseases associated with an increased T-helper cell (Th) type 2 response. Despite chronic inflammatory processes, several additional defects intrinsic to the airway epithelium may contribute to the pathogenesis. The airway remodelling observed in asthma can appear several years before any clear clinical diagnosis, and even affect preschool asthmatic children [1]. Asthma patients appear more susceptible to infections by respiratory viruses [2]. Epithelial cells derived from asthma patients exhibit a reduced capacity to secrete fibronectin, a well-known contributor to repair in damaged airway epithelium, together with defective antioxidant pathways and tight junctions [3–5].

Some studies have attempted to associate specific gene expression profiles with asthma or allergic rhinitis, mostly in adults [3, 6–10]. Information has also been gathered by genome-wide association studies. Single-nucleotide polymorphisms (SNPs) in the *GSDML* locus (near *ORMDL3*), a protein detected in epithelial tissues, are strongly associated with childhood-onset disease [11]. Based on these findings, our rationale was that defects in the airway epithelium could correspond to primary defects or early secondary events, which could favour chronic inflammation and lead to asthma and/or allergic rhinitis. Thus, we determined the transcriptional signatures of nasal brushings collected from paediatric patients with different clinical phenotypes of dust mite respiratory allergy. Data analysis revealed altered behaviour of several signalling pathways that were further analysed in

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an *in vitro* model of differentiated human nasal epithelial cells (HNECs).

MATERIALS AND METHODS

Subjects and samples

Patients were aged 6–17 yrs ($n=45$). 33 were being followed up for a dust mite-allergic rhinitis. 14 were also asthmatic, with either controlled asthma (group “a”; $n=7$) or uncontrolled asthma (group “A”; $n=7$). 19 had rhinitis without asthma (group “R”). The control group (group “C”) comprised 12 healthy children. Clinical diagnoses and asthma control staging corresponded to the Allergic Rhinitis and its Impact on Asthma and the paediatric US National Asthma Education and Prevention Program Expert Report 3 criteria (see online supplementary file 1 for details). The project received the approval of the Nice University Hospital Ethics Committee (Nice, France) and all of the volunteers’ parents gave written, informed consent.

Nasal respiratory epithelium sampling was performed by brushing the inferior turbinates.

Viral respiratory tract infection detection by real-time quantitative PCR (qPCR) was performed using the RealAccurate™ Respiratory Kit according to the manufacturer’s instructions (PathoFinder, Maastricht, the Netherlands). Seven children (one asthmatic, five rhinitics without asthma and one control) positive for respiratory viruses were not included in further analyses. The characteristics of the patients and samples are summarised in table 1 and online supplementary table E1.

Isolation and culture of primary HNECs

Primary cultures of HNECs derived from the nasal mucosa of inferior turbinates were performed as described previously [12]. Each experiment was performed using three different donors.

TABLE 1 Subject characteristics

	Group			
	a	A	R	C
Subjects	7	6	14	11
Dust mite-allergic rhinitis	Yes	Yes	Yes	No
Asthma	Controlled	Uncontrolled	No	No
Age yrs	11.5±3.2	9.1±0.6	11.3±2.8	11.5±3.1
Males/females	2/5	4/2	7/7	7/4
Epithelial cells %	85.8±10.4	86.5±6.5	86.9±8.7	86.9±8.5
PMNs %	6.9±6.1	5±3.2	7.2±7.8	6.8±6.1
Lymphocytes %	7.3±10.7	8.4±6	5.5±7.3	6.3±5.6
FEV₁	97.6±13.2	78.2±7.7*	99±9.4	NA
FEV₁/FVC	89.3±5.7	76.5±3.2*	90.4±5.2	NA
FEF_{25–75%}	103.6±17.9	56.2±9.6*	107.3±18.2	NA

Data are presented as n or mean±SD, unless otherwise stated. Statistical comparisons were performed in allergic (groups a, A and R) versus healthy control subjects (group C) and in uncontrolled (group A) versus controlled (group a) asthmatics using unpaired t-tests and the Chi-squared test. PMN: polymorphonuclear cell; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF_{25–75%}: forced expiratory flow at 25–75% of FVC; NA: not available. *: $p<0.005$ for group A versus group a by unpaired t-test.

Before treatment with cytokines, HNECs were cultured for 4 weeks at an air–liquid interface to obtain a well-differentiated epithelium [12]. Tested agents (interleukin (IL)-4 (BD Pharmingen, San Diego, CA, USA) and IL-13 (PeproTech, Rocky Hill, NJ, USA), 10 ng·mL⁻¹ for 24 h; interferon (IFN)- α /D and IFN- β 1a (Sigma-Aldrich, Saint-Quentin Fallavier, France), 1,000 IU·mL⁻¹ for 6 h; and IFN- γ (BD Pharmingen), 100 ng·mL⁻¹ for 6 h) were added on the basolateral side.

Gene expression analyses

Microarray analyses were performed on the GeneChip Human Gene 1.0 ST Array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer’s instructions. Microarray data are archived in the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) under references GSE19190 and GSE22147, and on the MEDIANTE database developed in our laboratory [13, 14]. The qPCR procedure is detailed in online supplementary file 1, with the primers and probes listed in online supplementary table E2.

Biostatistics

Most statistical calculations and prediction analyses were performed using limma and Random Forest, two packages of the R statistical environment [15]. We used the Functional Annotation Clustering report available from the Database for Annotation, Visualization and Integrated Discovery (DAVID) [16]. Hierarchical clustering was performed with the MultiExperiment Viewer program, version 4.3 (TM4, Boston, MA, USA), using a Manhattan distance metric and average linkage [17]. Biological networks were built using Ingenuity Pathway Analysis™ software (Ingenuity Systems Inc., Redwood City, CA, USA). Gene Set Enrichment Analysis (GSEA) was used to determine whether a set of genes defined *a priori* can characterise differences between two biological states [18, 19]. ENCODE (Encyclopedia of DNA Elements) functional genomics data were used to determine potential binding sites for IFN regulatory factors.

Immunohistochemistry

Immunohistochemistry methods are described in online supplementary file 1.

ELISA assay

The level of MUC5AC released by HNECs in response to IL-13 stimulation was measured using ELISA according to the manufacturer’s instructions (EIAab and USCNLIFE; Wuhan EIAab Science Co. Ltd, Wuhan, China).

RESULTS

Genes differentially expressed in the nasal epithelial cells of children with dust mite allergy

The transcriptional signature of dust mite-allergic rhinitis children ($n=27$) was compared with that of the healthy children ($n=11$). We identified 169 differentially expressed transcripts (adjusted $p<0.05$) (data on request). Among them, 24 transcripts were modulated with an absolute value of $\log_2(\text{ratio}) >1$ (table 2). Functional annotation by DAVID is provided in online supplementary table E3. Variation of some genes (*CST1*, *POSTN*, *NTS*, *CD44*, *GSN* and *ALOX15*) was confirmed by qPCR (online supplementary fig. E1).

Supervised clustering was used to predict a status for each of the 38 patients (allergic or healthy). 35 out of the 38 patients were correctly predicted (prediction accuracy of 91%) (fig. 1a).

TABLE 2 Genes differentially expressed in nasal epithelial brushings from dust mite-allergic rhinitis patients versus healthy subjects (microarray analysis)

Probe set identifier	mRNA accession number	UniGene identifier	Gene	Gene product	Cytoband	log ₂ (signal)	log ₂ (allergic/healthy)	p-value
8065412	NM_001898	Hs.123114	CST1	Cystatin SN	20p11.21	9.65	7.15	4.95 × 10 ⁻¹²
7971077	NM_006475	Hs.136348/ Hs.664318	POSTN	Periostin, osteoblast-specific factor	13q13.3	7.12	3.56	2.66 × 10 ⁻³
8083260	NM_001870	Hs.646	CPA3	Carboxypeptidase A3 (mast cell)	3q21-q25	6.17	2.51	5.20 × 10 ⁻⁵
7921690	NM_017625	Hs.50813	ITLN1	Intelectin 1 (galactofuranose binding)	1q22-q23.5	5.83	2.45	3.87 × 10 ⁻²
8065416	NM_001322	Hs.669305	CST2	Cystatin SA	20p11.21	7.97	1.87	1.06 × 10 ⁻³
8084657	NM_014375	Hs.81073	FETUB	Fetuin B	3q27	5.78	1.77	3.63 × 10 ⁻³
8021635	NM_002575	Hs.594481	SERPINB2	Serpin peptidase inhibitor, clade B (ovalbumin), member 2	18q21.3	9.20	1.64	6.97 × 10 ⁻³
8056222	NM_001935	Hs.368912	DPP4	Dipeptidyl-peptidase 4 (CD26)	2q24.3	6.44	1.56	6.18 × 10 ⁻³
7921900	NM_053282	Hs.350581	SH2D1B	SH2 domain-containing 1B	1q21	5.91	1.55	3.05 × 10 ⁻⁷
8036755	NM_001828	Hs.889	CLC	Charcot-Leyden crystal protein	19q13.1	4.57	1.50	2.73 × 10 ⁻²
7984001	NM_004751	Hs.194710	GCNT3	Glucosaminyl (N-acetyl) transferase 3, mucin type	15q21.3	8.73	1.39	8.15 × 10 ⁻⁵
8063761	NM_177980	Hs.54973	CDH26	Cadherin-like 26	20q13.2-q13.33	8.67	1.38	6.84 × 10 ⁻³
8089568	NM_138806	Hs.309158	CD200R1	CD200 receptor 1	3q13.2	8.90	1.35	3.90 × 10 ⁻⁵
8112668	NM_016591	Hs.272404	GCNT4	Glucosaminyl (N-acetyl) transferase 4, core 2	5q12	5.85	1.29	4.05 × 10 ⁻²
8070567	NM_003226	Hs.82961	TFF3	(IL-1,6-N-acetylglucosaminyltransferase) Treffol factor 3 (intestinal)	21q22.3	9.86	1.26	2.11 × 10 ⁻²
7942135	NM_018043	Hs.503074	TMEM16A	Transmembrane protein 16A	11q13.3	7.41	1.23	6.48 × 10 ⁻³
8154233	NM_014143	Hs.521989	CD274	CD274 molecule	9p24	6.75	1.23	3.84 × 10 ⁻⁵
7957458	NM_006183	Hs.80962	NTS	Neurotensin	12q21	9.14	1.16	4.79 × 10 ⁻²
8023688	NM_002974	Hs.123035	SERPINB4	Serpin peptidase inhibitor, clade B (ovalbumin), member 4	18q21.3	8.67	1.15	3.40 × 10 ⁻³
8147132	NM_000067	Hs.155097	CA2	Carbonic anhydrase II	8q22	6.44	1.14	3.87 × 10 ⁻²
7909946	GENSCA-N00000026059	NA	NA	NA	NA	6.01	1.14	1.05 × 10 ⁻²
8156134	NM_006180	Hs.494312// Hs.653428	NTRK2	Neurotrophic tyrosine kinase receptor, type 2	9q22.1	5.63	1.10	5.93 × 10 ⁻³
8102050	NM_178833	Hs.546482	NHEDC2	Na ⁺ /H ⁺ exchanger domain-containing 2	4q24	6.02	1.02	6.84 × 10 ⁻³
8089851	NM_000187	Hs.368254	HGD	Homogentisate 1,2-dioxygenase (homogentisate oxidase)	3q13.33	5.71	-1.22	2.50 × 10 ⁻²

Genes were ranked according to decreasing log₂(allergic/healthy). Only genes with a log₂(ratio) > 1 and a significant p-value (p < 0.05) after a Benjamini-Hochberg correction for multiple tests were selected. Bold indicates genes induced *in vitro* with a value of log₂(ratio) > 1 by both interleukin (IL)-4 and IL-13 in human nasal epithelial cells. A more detailed table is provided as online supplementary table E3. NA: not available.

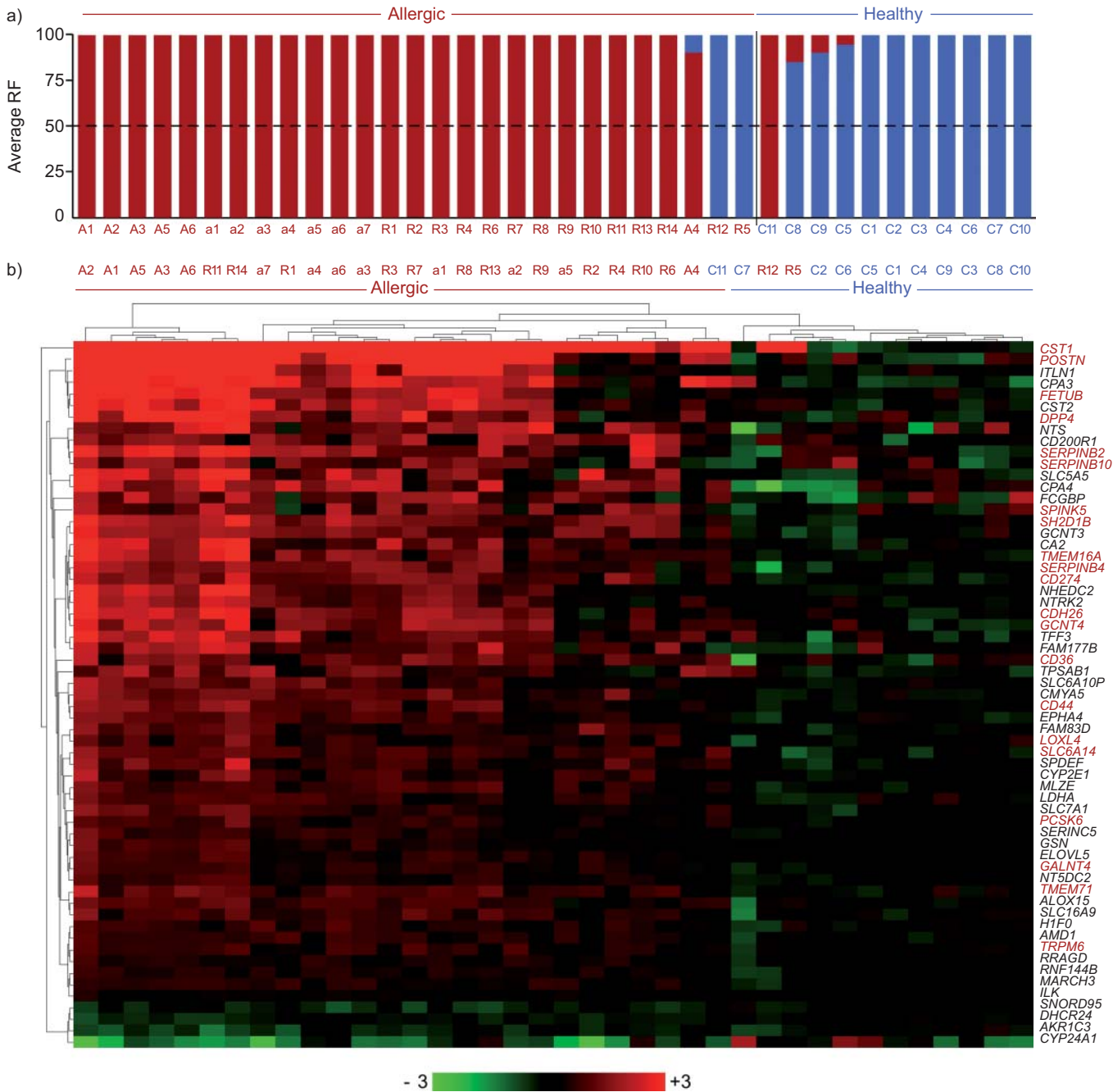


FIGURE 1. Discrimination by Random Forest (RF) of children with dust mite allergic rhinitis from healthy children and heat-map representation of the 61 most discriminant genes. a) Histogram showing, for each patient, the percentage of classification as a dust mite-allergic rhinitis (red) or healthy (blue) child. b) Nonsupervised, hierarchical clustering of the same patients using the set of 61 genes common to all Random Forest classifiers (see details in the Materials and methods section). Each square represents the expression level of a given gene in a given sample relative to the average expression level in controls. A red to green colour scale indicates gene expression levels above (red) or below (green) the average level of expression in healthy subjects for the same transcript. Clustering was performed using an average linkage method, using a Manhattan distance. The red colour on gene names (right) indicates genes induced *in vitro* by interleukin (IL)-4 and IL-13.

Specificity and sensitivity are summarised in a receiver operating characteristic (ROC) curve in online supplementary figure E2A, showing an area under the ROC curve of 0.88. The Random Forest approach that was used allowed the identification of the most influential genes for the prediction, leading to a set of 61 transcripts that was common to 20 independent cross-validations. Most genes were up-regulated in the allergic conditions (fig. 1b). 16 of

them had previously been linked to asthma or allergic rhinitis by gene expression profiling (online supplementary table E4) [3, 6–10]. The signature was linked to allergic status but independent of asthma status. Indeed, supervised clustering was unable to discriminate the 13 asthmatic patients from the 14 allergic rhinitis patients without asthma. No differential expression was detected between these two groups of patients (data not shown).

Epithelial Th2 signature discriminates allergic children from healthy controls

We observed that several transcripts dysregulated in patients allergic to dust mites were also linked in the literature to IL-4 or IL-13 [20]. We mimicked this situation *in vitro* by stimulation of primary HNECs with IL-4 or IL-13. With this model, we confirmed that IL-13 stimulation increased the expression (online supplementary fig. E3A) and secretion of MUC5AC, a goblet cell marker (online supplementary fig. E3B). We also observed, in parallel, the decreased expression of FoxJ1, a ciliated cell marker (online supplementary fig. E3A). 46 airway epithelial transcripts were selected, based on their high response to Th2 cytokines and their detection in highly differentiated airway epithelial cells (online supplementary table E5). 21 transcripts of this “Th2 signature” were among the 61 transcripts predicting allergic or healthy children (fig. 1). The importance of Th2 regulation was assessed by a normalised enrichment score >1 (1.61, with nominal *p*-value and false discovery rate *q*-value of <0.001), as determined by GSEA [18, 19]. Interestingly, we noticed that the transcripts associated with the Th2 signature displayed a larger $\log_2(\text{ratio})$ in uncontrolled (group A) than in controlled (group a) asthma (fig. 1). This led us to investigate the existence of a specific expression profile associated with uncontrolled asthma.

Control of asthma affects gene expression signature of nasal epithelial cells

The next analysis compared the two groups of subjects with uncontrolled or controlled asthma. Table 1 describes the characteristics of these patients. The expression of 160 genes differed significantly between the two groups (online supplementary table E6). Functional annotation is reported in online supplementary table E7. The Random Forest approach correctly predicted the class for 12 out of 14 samples, with a 75.4% prediction accuracy (fig. 2a). Specificity and sensitivity are summarised in a ROC curve in online supplementary figure E2B, showing an area under the ROC curve of 0.833. It also identified a minimal set of the 41 most influential genes for the prediction (fig. 2b). Up-regulation of *POSTN*, *FETUB* or *DPP4* (also belonging to Th2 signature) (online supplementary table E5) in the uncontrolled group is coherent with the larger $\log_2(\text{ratio})$ observed for Th2 responding transcripts in figures 1 and 2. We also noticed a down-regulation of several IFN-stimulated genes (ISGs), such as *CXCL10*, *MX2*, *IFIT2*, *DUOX2*, *GBP2* and *GBP5*, among a larger cluster of down-regulated genes (fig. 2). This was illustrated by Ingenuity Pathways Analyses (online supplementary fig. E4). Our cluster of down-regulated genes also included *GSDML*, a gene located in a region of chromosome 17q21, which has recently been linked to juvenile asthma in several genome-wide association studies [11]. The differential expression of *CXCL10*, *MX2*, *IFIT2*, *DUOX2* and *GSDML* was confirmed by qPCR (online supplementary fig. E1). *GSDML* and *DUOX2* proteins were immunodetected by histochemistry in nasal and bronchial airway epithelia (fig. 3).

IFN response analysis in patients and cultured HNECs

IFN regulation was explored *in vitro* by stimulating primary HNECs with IFN- α , IFN- β or IFN- γ . We selected IFN-responsive transcripts (online supplementary table E8). The GSEA approach revealed significant normalised enrichment scores for all subtypes of IFNs in the uncontrolled/controlled asthma comparison (1.7 for IFN- α , 1.64 for IFN- β and 1.56 for IFN- γ). The

capacity of IFNs to induce the expression of *MX1*, *ST8SIA4* and *GSDML* was confirmed by qPCR on primary cultures of HNECs. A peak of *GSDML* induction was observed after 12 h of IFN- α stimulation, while the peak of induction for *MX1* and *ST8SIA4* was reached at 6 h (fig. 4a). A survey of the genomic context for *MX2*, *IFIT2*, *CXCL10*, *GSDML*, *ST8SIA4* and *DUOX2* revealed the presence of several consensus binding sequences for IFN regulatory factors (full data on request) (online supplementary fig. E5). Altogether, these observations suggest a direct regulation of these different genes by IFN. Importantly, the transcript levels of IFN- β , IFN- $\lambda 1$, IFN- $\lambda 2/3$, IFN- $\alpha 1$ and IFN- $\alpha 2$ were decreased in uncontrolled asthmatics compared with either controlled asthmatics or healthy subjects (fig. 4b). Endogenous production of epithelial IFNs, rather than defective IFN signalling pathways, is therefore probably involved in these processes.

Airway epithelial regeneration and severity of allergic diseases

Impaired capacity of repair of the airway epithelium is a hallmark of allergic airway diseases [5]. We have recently established gene expression signatures during *in vitro* HNEC regeneration and differentiation [12]. The “terminal differentiation” signature was used to enrich the annotation derived from the expression profiles obtained with Th2 cytokines and IFN experiments. As expected, the classification of the patients was principally linked to the effects of Th2 cytokines and IFNs. However, several transcripts associated with the disease were also linked to the terminal differentiation signature (online supplementary table E9). A first group of genes, including *PLK4*, *CDC20B*, *GSDML* and *CYFIP2*, was clearly induced during differentiation while being down-regulated in patients with uncontrolled asthma. This group puts together important markers of multiciliogenesis (*PLK4* and *CDC20B*) [12] and molecules associated with asthma in genome-wide analyses (*GSDML* and *CYFIP2*). A second group, which includes *POSTN*, *SPINK5*, *CD44*, *TFF3*, *ITLN1*, *FOXA3* and *SPDEF*, was clearly associated with basal or mucous cells [21]. Most of them were decreased during *in vitro* differentiation while all were up-regulated in patients with uncontrolled asthma.

DISCUSSION

The airway epithelium is a complex physical barrier, playing an intrinsic role in innate and adaptive immunity, through the production of numerous cytokines and chemokines. Our work highlights the importance of IFN- and Th2-driven epithelial responses in the pathogenesis of allergic respiratory diseases. Our findings were based on biostatistical approaches, such as Random Forest, Ingenuity and GSEA, which take into account not only the number of patients, but also the size of gene clusters that can be regulated by these cytokines. Importantly, these approaches increase the statistical significance of our observations.

The choice to perform our investigations on upper, rather than on lower, airway epithelium was motivated by its noninvasive sampling, which is easily applicable in young children. It allows the recruitment of true controls exempt of pulmonary disease, a situation which is clearly not possible in studies using bronchoscopic sampling [1]. It also allows investigation of patients with severe or uncontrolled asthma phenotypes without stopping their long-term inhaled corticosteroid treatment for asthma, as nasal deposition can be considered as negligible with the devices used in this study [22]. Interestingly, we observed a good overlap

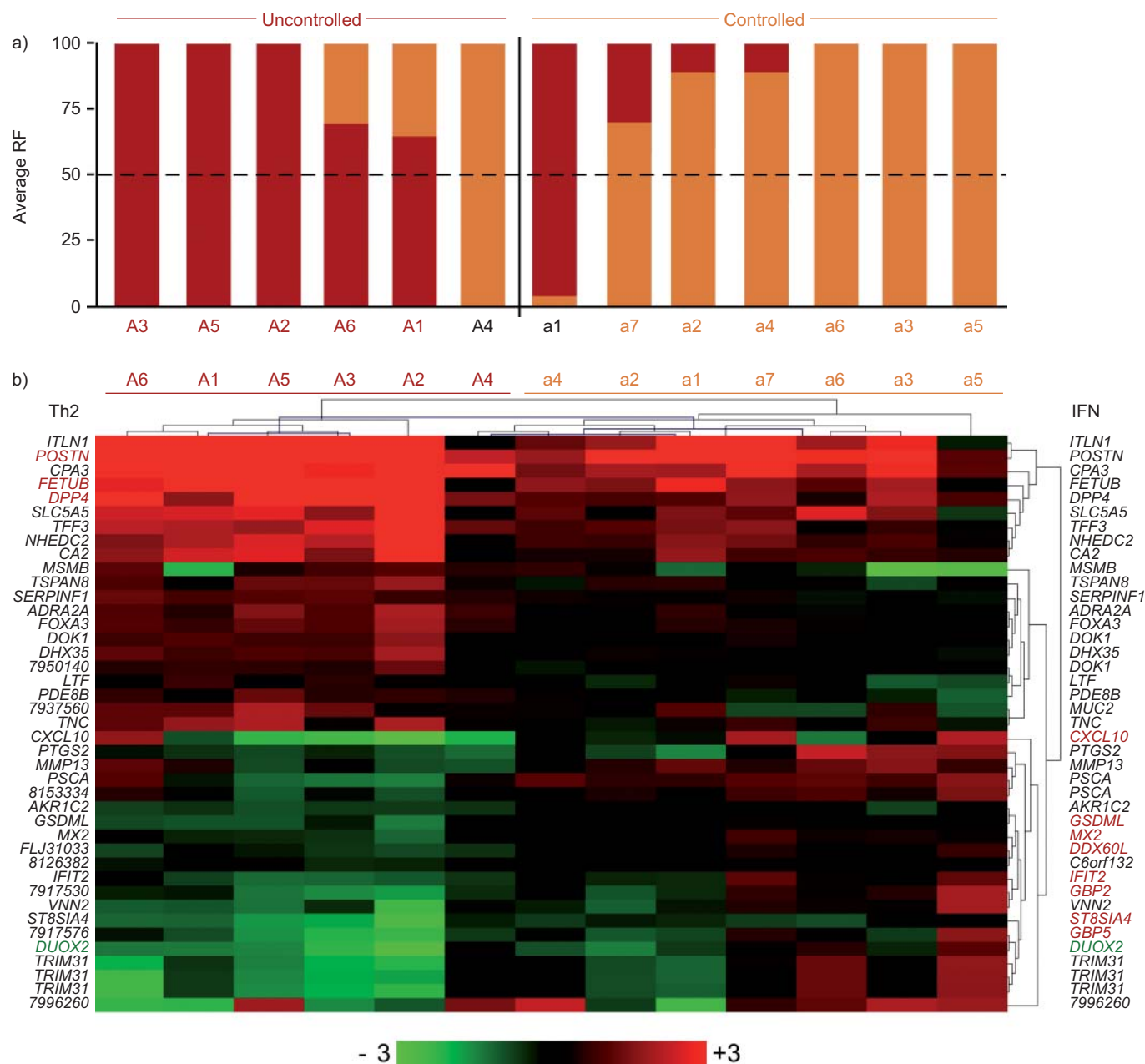


FIGURE 2. Discrimination by Random Forest (RF) of children with uncontrolled versus controlled asthma and heat-map representation of the 41 most discriminant genes. a) Histogram showing, for each patient, the percentage of classification as uncontrolled asthma (red) and controlled asthma (orange). b) Nonsupervised, hierarchical clustering of the same patients using the 41 probes common to all Random Forest classifiers (see Materials and methods section for details). Each square represents the expression level of a given gene in a given sample. A red to green colour scale indicates gene expression levels above (red) or below (green) the average level of the healthy controls for the same transcript. Clustering was performed using an average linkage method, using a Manhattan distance. Red-coloured gene names indicate transcripts induced by interleukin (IL)-4 and IL-13 (left) or by interferons (IFNs) (right) (corresponding to a $\log_2(\text{ratio}) > 1$ in human nasal epithelial cells (HNECs)). Gene names coloured in green indicate transcripts down-regulated by IL-4 and IL-13 (left) ($\log_2(\text{ratio}) > -1$ in HNECs).

between our “nasal epithelial” gene lists and several gene expression profiles in bronchial epithelium, notwithstanding differences between nasal and bronchial epithelial cells [23, 24], such as the near absence of remodelling in the nasal epithelium [25]. In this context, the use of alternative techniques to harvest lower airway cells during general anaesthesia could be worth investigating [26]. Our inability to identify *ad hoc* biomarkers for discriminating asthmatic and rhinitis patients could be due to the

choice of sampling material. If this is true, a comprehensive comparison of bronchial and nasal epithelial cells from the same patients would help explore this issue. This was unfortunately not planned in the present study. The relatively small size of the study (45 samples) could also be limiting. A third explanation could be that the major differences discriminating asthma from allergic rhinitis would, rather, depend on other cell types (dendritic or mesenchymal cells, *etc.*). From that perspective,

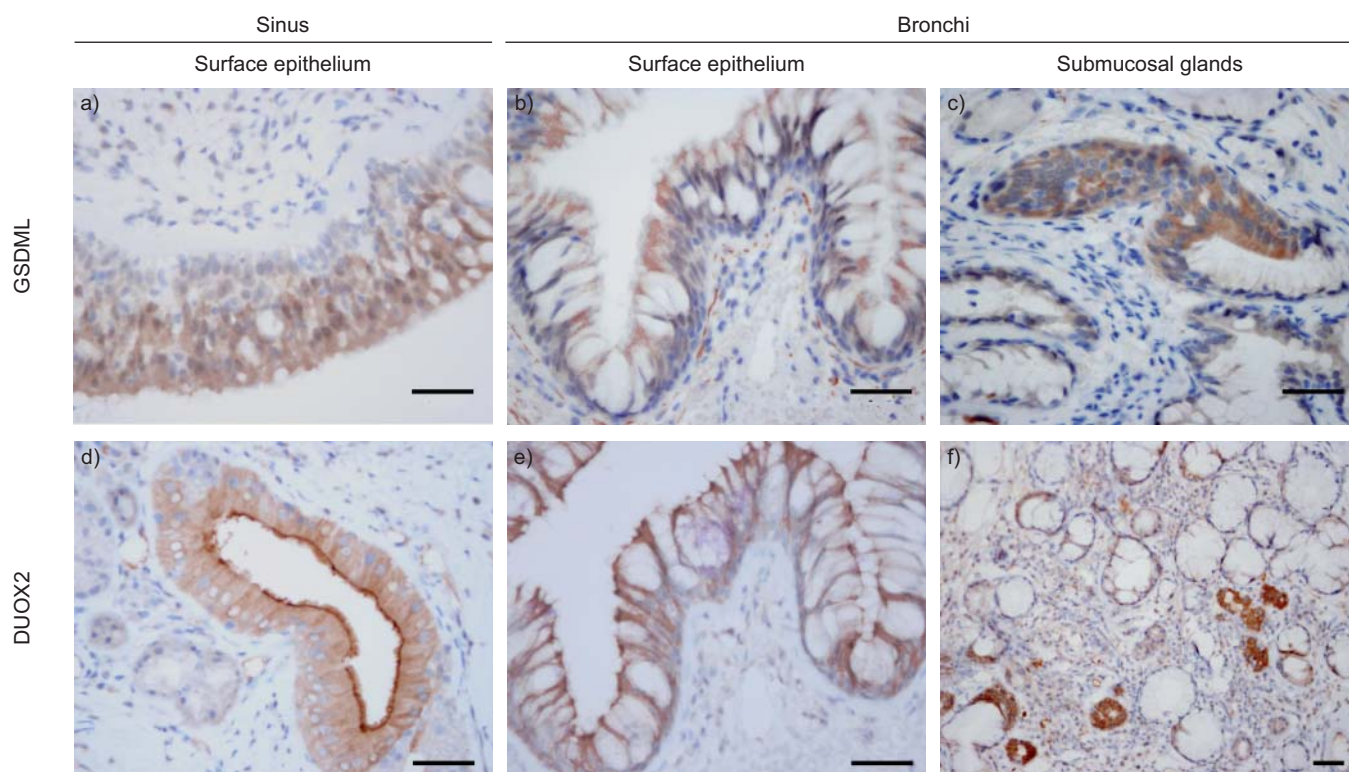


FIGURE 3. Immunohistochemical staining of sinus and bronchial biopsies. Representative immunostaining of a–c) GSDML and d–f) DUOX2 in surface epithelium and submucosal glands for healthy adults are shown. Positive immunostaining was observed for the two proteins in sinus and bronchial sections. Strong signals were also detected in submucosal glands for the different markers. Scale bars=100 μ m.

progression from allergic rhinitis to asthma could not be detected in the epithelium *per se*.

Our study establishes epithelial activation by Th2 cytokines as a central trait of airway allergic respiratory disease. This is in line with the observation that IL-13 can recapitulate most of the characteristics of asthma in several experimental models of the disease [27]. Our clinical and *in vitro* results clearly demonstrate the major impact of Th2 cytokines on allergic subjects, reflecting the strength of the atopic status. Several Th2-induced genes, such as *POSTN*, *ITLN1*, *ALOX15*, *CD44* and *SERPINB4*, have previously been linked to asthma. Others could represent additional relevant biomarkers, due to their large and consistent variations in the allergic group. For example, *CSTII*, which encodes a cysteine protease inhibitor, is already associated with pulmonary fibrosis in systemic sclerosis patients [28]. Another example is *NTS*, which has been implicated in wound healing in chronic colitis [29].

An enhanced up-regulation of Th2-responsive genes (characterised by larger $\log_2$ (ratio) values) was observed in uncontrolled asthma. In fact, asthma severity in children has already been associated with atopy in epidemiological studies [30]. This association seems specific for childhood asthma, as an inverse relationship has been reported in adults [31]. Interestingly, IL-13 has been shown to induce a profibrotic bronchial phenotype *via* a continuous secretion of transforming growth factor- β 2 [32]. Strikingly, the first gene ontology theme in the comparison between uncontrolled and controlled asthma corresponds to the term “structural constituents of extracellular matrix”, with enhanced expression of *POSTN*, *TNC*, *FBN2*, *FBLN2*, *FMOD*

and *COL4A6*. It is tempting to speculate that this dysregulation could be directly related to the structural modifications and remodelling of the epithelium particularly observed in severe asthma [33]. At the same time, *POSTN* has been reported to induce re-entry of differentiated mammalian cardiomyocytes into the cell cycle, in connection with tissue repair [34]. Mucus hypersecretion and goblet cell metaplasia are important traits of asthma, also linked with IL-13 dysregulation. We also noticed that *TFF3*, which is expressed in mucous cells and can promote the differentiation of ciliated human airway epithelial cells [21], was up-regulated in allergic patients. This illustrates the complexity of the mechanisms leading to abnormal repair and remodelling.

The major impact of Th2 cytokines probably masks weaker, intrinsic epithelial features. We believe that this represents a confounding factor when analysing epithelial susceptibility with the microarray technique. In order to dampen the impact of the Th2 response, we have compared subgroups of patients with uncontrolled or controlled asthma.

Another observation of our study is the existence of an altered IFN response in the nasal epithelium of uncontrolled asthmatic patients; this trait may be more directly related to an intrinsic epithelial susceptibility. The IFN response was reduced in the uncontrolled asthma group relative to the controlled asthma and healthy groups. We excluded the possibility that these effects were caused by viral infections: 1) a viral PCR assay, able to detect 90% of respiratory tract viral infections, eliminated cases of viral infection; and 2) a careful evaluation of CXCL10 transcript levels in “uninfected” patients identified additional infected samples.

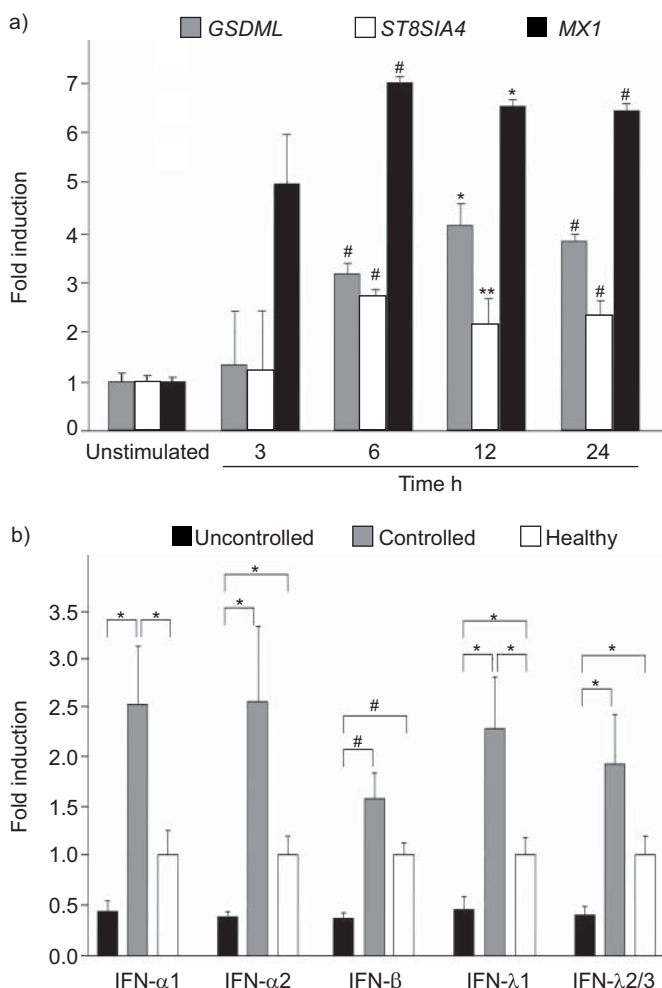


FIGURE 4. Characterisation of the interferon (IFN) response. a) Measurement by real-time quantitative PCR (qPCR) of the induction of *GSDML*, *ST8SIA4* and *MX1* by IFN- α in human nasal epithelial cells (HNECs). Levels of *GSDML*, *ST8SIA4* and *MX1* were assessed in differentiated HNECs 3, 6, 12 and 24 h after stimulation with IFN- α . Measurements were performed using SYBR Green. Results are expressed as fold-change relative to the unstimulated HNECs. b) Measurement of IFNs by qPCR in patients. Levels of IFN- α 1, IFN- α 2, IFN- β , IFN- λ 1 and IFN- λ 2/3 transcripts in patients with uncontrolled asthma, patients with controlled asthma and healthy controls. Data are expressed relative to an average of 11 healthy controls. Measurements were performed using Taqman probes. Results are expressed as fold-change relative to the healthy control group. Data are presented as mean $\pm$ SEM. #: $p < 0.005$ by unpaired t-test; *: $p < 0.05$ by unpaired t-test; **: $p < 0.01$ by unpaired t-test.

ISGs that are decreased in patients with uncontrolled asthma represent a quarter of the genes identified by our second supervised analysis. Unexpectedly, we identified *GSDML* in that cluster. Our study reports several additional observations suggesting a direct association of this gene with the abnormalities affecting the paediatric asthmatic epithelium: first, the existence of several response elements for IFN regulatory factors in its promoter region; secondly, its transcriptional induction by epithelial IFN in HNECs; and thirdly, its protein expression in airway epithelial cells. This is in line with the fact that one of its SNPs (rs7216389) has been associated with early onset of asthma,

acute severe exacerbations (80% of which are virus-induced in children) and asthma severity [11, 35, 36]. MOFFATT *et al.* [37] initially noted an association of childhood asthma risk with modified expression of *ORDML3*, located in the immediate vicinity of *GSDML*. The identification of a variant in a splice site for *GSDML* in strong linkage disequilibrium with *ORMDL3* has recently suggested that *GSDML* would be the causative gene associated with asthma [38].

Decreased levels of IFN- α , IFN- β , IFN- λ 1 and IFN- λ 2/3 paralleled the down-regulation of IFN-stimulated genes observed in patients with uncontrolled asthma. This confirms and extends findings by DE BLIC *et al.* [39] showing a correlation between asthma symptoms and the IFN response in a cohort of 28 children with difficult-to-treat asthma. In their study, lower levels of IFN- γ were associated with persistent symptoms [39]. Deficient epithelial cell IFN- β and IFN- λ production during rhinovirus-induced asthma exacerbations was associated with increased viral load, defective apoptosis and cell death bias [2]. Epidemiological studies have associated the onset of asthma with reduced IFN- γ production by peripheral blood mononuclear cells at 9 months of age, while a normalisation of IFN- γ production was observed in children showing a resolution of asthma symptoms [40, 41].

Regardless of the exact molecular mechanisms leading to impaired IFN production, from our results, one could anticipate some benefits of IFN treatments in patients with uncontrolled asthma, as suggested by recent *in vitro* experiments with IFN- β [42]. As the association between defective epithelial cell IFN pathways and the lack of asthma control in patients could be specific to paediatric asthma, careful evaluation of therapeutic options based on the development of innate immunity would require the organisation of clinical trials addressing this specific issue in a paediatric population. Finally, our work illustrates some specificities of severe paediatric asthma, which not only results from the development of T-cell-mediated biased adaptive responses, but also from an inefficient epithelial innate immunity.

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CLINICAL TRIAL

This study is registered as www.clinicaltrials.gov with identifier number NCT00569361.

STATEMENT OF INTEREST

A statement of interest for the study itself can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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Supplementary Table E1. Subject characteristics.

Patient	Sex	Age	% Epithelial cells	Asthma control (NHLBI/EPR3)	FEV1 (%predicted)	FEV1/FVC
a1	M	15y 11m	97.3	WC	119	86
a2	M	14y 3m	94.9	WC	112	87
a3	F	6y 8m	93.9	WC	92	100
a4	F	13y	73.0	WC	86	83
a5	F	9y 4m	87.6	WC	99	92
a6	F	11y 3m	81.6	WC	89	86
a7	F	9y 7m	72.5	WC	86	91
A1	M	9y	91.9	NWC	76	78
A2	F	8y 9m	89.6	VPC	67	73
A3	M	9y 9m	90.2	NWC	91	78
A4	M	9y 7m	85.8	VPC	77	72
A5	F	8y 11m	74.1	NWC	78	80
A6	M	8y 1m	87.2	NWC	80	78
R1	F	8y 7m	80.5		108	92
R2	M	7y	76.2		114	94
R3	M	7y 5m	72.6		103	87
R4	M	14y 5m	96.8		103	84
R5	F	12y 6m	88.2		79	82
R6	M	13y 10m	93.4		95	85
R7	F	9y 3m	89.7		103	90
R8	F	10y 11m	87.4		83	97
R9	M	13y 7m	97.6		100	87
R10	F	11y 8m	94.8		97	89
R11	F	8y 8m	88.2		96	95
R12	M	16y 1m	72.7		106	100
R13	F	13y 1m	96.0		94	93
R14	M	10y 1m	83.0		105	90
C1	M	6y 4m	91.1			
C2	F	14y 4m	91.3			
C3	M	14y 1m	98.1			
C4	M	7y 9m	84.6			
C5	M	14y 4m	83.1			
C6	M	12y 1m	96.1			
C7	M	11y 10m	84.7			
C8	F	14y	90.4			
C9	F	13y 11m	75.2			
C10	M	10y 2m	91.5			
C11	F	7y 6m	70.2			

Supplementary Table E2. Sequences of primers and probes designed with Primer Express®

Gene symbol	Sequence of primers and probes	
CST1	Forward	5' -GGT GGC ATC TAT AAC GCA GACC-3'
	Reverse	5' -TGT TGC CTG GCT CTT AGT ACC C-3'
POSTN	Forward	5' -TCA TTG AAG GTG GTG ATG GTC A-3'
	Reverse	5' -TTT CAC TGA GAA CGA CCT TCC C-3'
NTS	Forward	5' -GAG GAG CTT GTT GCA AGA AGG A-3'
	Reverse	5' -GCC CTG CTG TGA CAG ATT TTG T-3'
CD44	Forward	5' -TTG CAT TGC AGT CAA CAG TCG-3'
	Reverse	5' -CGT TGA GTC CAC TTG GCT TTC T-3'
ALOX15	Forward	5' -TTG ATG GCT GAG GTC ATT GTT G-3'
	Reverse	5' -GCC CGG ACG TTA ATT TCC A-3'
GSN	Forward	5' -AGC CTT GAC TTC TGC TAA GCG G-3'
	Reverse	5' -ATC ATC ATC CCA GCC AAG GAA C-3'
CXCL10	Forward	5' -CCA GAA TCG AAG GCC ATC AA-3'
	Reverse	5' -GAA GCA CTG CAT CGA TTT TGC-3'
MX2	Forward	5' -CGA GAG AAT GGT GAC TCC TTG C-3'
	Reverse	5' -TTG GTA GCG GTC TCA CTC TGC T-3'
IFIT2	Forward	5' -TCA CGG TAT GCT TGG AAC GAT T-3'
	Reverse	5' -ACA GGC AAA CAT TTT CCA ACC C-3'
DUOX2	Forward	5' -GTC CCT GCT TGT GAC AAG TGT G-3'
	Reverse	5' -ACC AAA GCA ACG CAT CGA TT-3'
GSDML	Forward	5' -CTG AAA AGC GAC CGG CAA TA-3'
	Reverse	5' -TCG ATA GCT CAG GAC CCG ATT-3'
ST8SIA4	Forward	5' -AGA GCA TTT GGA GGC TTT CGA-3'
	Reverse	5' -CAC GTG CTT CTC TCC TCC TTT G-3'
MX1	Forward	5' -TTT TCA GCC CTC AGA ATC GCT-3'
	Reverse	5' -CAC GGC ACT CAT GCT CCT AAA-3'
GAPD	Forward	5' -ACC ACA CTG AAT CTC CCC TCC T-3'
	Reverse	5' -ATG GTA CAT GAC AAG GTG CGG-3'
RPL13A	Forward	5' -GTC TCA AGG TGT TTG ACG GCA-3'
	Reverse	5' -GCC CCA GAT AGG CAA ACT TTC T-3'

Supplementary Table E3. Functional annotation clustering of the 169 genes regulated in allergic patients compared with healthy subjects.

Category	Term	Count	Pop Hits	p-value	Gene symbol
BP	nitrogen compound metabolic process	13	496	2.66E-04	AMD1, CAV1, GNE, KITLG, MAOA, SHMT2, SLC6A14, SLC7A1, SMOX, TGFB2 ASL, HGD, MTR
BP	amino acid and derivative metabolic process	11	376	4.23E-04	AMD1, KITLG, MAOA, SHMT2, SLC6A14, SLC7A1, SMOX, TGFB2 ASL, HGD, MTR
MF	racemase and epimerase activity, acting on carbohydrates and derivatives	3	9	2.12E-03	DSE, GALE, GNE
BP	glycoprotein metabolic process	7	164	1.27E-03	BACE2, GALNT7, ST3GAL4, ST6GAL1, ST6GALNAC4 B3GALT2, DHCR24
BP	metabolic process	83	9181	2.01E-03	8161632, 7937612, AACS, AADACL1, ADAM9, ALOX15, AMD1, ARNTL2, ATP8B1, BACE2, CA2, CAV1, CD55, CDC2L6, CLC, CLTB, CPA3, CREB3L4, CTSC, CYP2E1, DHCR24, DPP4, ELOVL5, ENOX1, F13A1, FKBP2, FN1, GALE, GALNT4, GALNT7, GNE, HK2, ILK, IVNS1ABP, KITLG, KLF4, LDHA, LTB4DH, MAOA, MARCH3, NEDD4, NRIP3, NTRK2, PCSK6, PDIA5, PTGS1, PTPRH, SAMD4A, SEC11C, SERPINB4, SHMT2, SLC6A14, SLC7A1, SLK, SMOX, SMURF2, SPDEF, ST3GAL4, ST6GAL1, ST6GALNAC4, TGFB2, TIMP1, TRPM6, UBE2Q2, VAT1, VDR, XBP1 ASL, B3GALT2, BRD8, C2, GCN5L2, HGD, HLF, MDFIC, MTR, NUDT4P1, PSPC1, QTRT1, SNORA67, TOP3B, TOX
BP	innate immune response	5	103	6.81E-03	CD55, IL1R1, IL1RL1 C2, CRTAM
MF	endopeptidase inhibitor activity	6	166	9.94E-03	CST1, FETUB, SERPINB2, SERPINB4, SLPI, TIMP1
CC	membrane	72	7262	2.15E-02	AADACL1, ADAM9, ALOX15, ATP8B1, BACE2, CAV1, CD200R1, CD274, CD44, CD55, CDH26, CLTB, CREB3L4, CYP2E1, DISP1, DPP4, DSE, EDEM3, ELOVL5, ENOX1, FKBP2, GALNT4, GALNT7, HK2, IL1R1, IL1RL1, ILK, ITLN1, KITLG, LOXL4, LRRC8A, LRRC8D, MAOA, MARCH3, MRC1, MRC1L1, NHEDC2, NTRK2, PCSK6, PHLDB2, PLEKHA1, PTGS1, PTPRH, RAB22A, RABAC1, RAP2C, RNF185, SEC11C, SERINC5, SHMT2, SLC16A9, SLC6A14, SLC7A1, SSFA2, ST3GAL4, ST6GAL1, ST6GALNAC4, TMEM16A, TMEM45A, TMEM71, TRPM6, VAT1, ZDHHC16 ABCB6, B3GALT2, CDH12, CRTAM, DHCR24, ERMAP, KIAA0090, SFXN2, SLC2A9, TMEM45B
BP	proteolysis	13	806	1.44E-02	ADAM9, BACE2, CD55, CPA3, CTSC, DPP4, NRIP3, PCSK6, SEC11C, SERPINB4, SMURF2, TIMP1 C2
BP	immune response	13	938	4.06E-02	CCL26, CD274, CD55CTSC, DPP4, IL1R1, IL1RL1, SERPINB4, ST6GAL1, TGFB2, XBP1 C2, CRTAM
BP	monocarboxylic acid metabolic process	6	244	3.31E-02	ALOX15, ATP8B1, ELOVL5, LTB4DH, PTGS1 MTR

Supplementary Table E4. Literature analysis of former microarray studies.

Probe Set ID	mRNA Accession	Symbol	log ratio asthma <i>versus</i> control	Asthma				Rhinitis
				bronchial sample				nasal sample
				Kicic et al., 2010	Woodruff et al., 2007	Lilly et al., 2005	Laprise et al., 2004	Benson et al., 2005
8011680	NM_0011140	ALOX15	0.7				X	X
8147132	NM_000067	CA2	1.26	X				
8133876	NM_001001547	CD36	0.86					X
7939341	NM_000610	CD44	0.92	X				
8083260	NM_001870	CPA3	2.51		X		X	
8065412	NM_001898	CST1	7.15					X
8065416	NM_001322	CST2	1.87					X
7984001	NM_004751	GCNT3	1.39	X				
7938777	NM_005566	LDHA	0.68	X		X		
7971077	NM_006475	POSTN	3.56	X	X		X	
8021635	NM_002575	SERPINB2	1.64		X	X	X	
8023688	NM_002974	SERPINB4	1.15			X	X	
7970810	NM_003045	SLC7A1	0.61			X		
8109001	NM_006846	SPINK5	1.09					X
8070567	NM_003226	TFF3	1.26	X				
7998434	NM_003294	TPSAB1	0.96		X		X	X

Supplementary Table E5. 46 genes differentially induced *in vitro* by IL-4 and IL-13 in primary HNECs.

Probe Set ID	mRNA Accession	UniGene ID	GeneSymbol	Gene Name	Cytoband	log2(signal)	log2(IL-4/C)	log2(IL-13/C)
7971077	NM_006475	Hs.136348	POSTN	periostin, osteoblast specific factor	13q13.3	8.21	6.78	4.32
8065412	NM_001898	Hs.123114	CST1	cystatin SN	20p11.21	9.71	5.02	4.67
8140358	NM_006072	Hs.131342	CCL26	chemokine (C-C motif) ligand 26	7q11.23	8.85	4.43	3.44
8135436	NM_000441	Hs.571246	SLC26A4	solute carrier family 26, member 4	7q31	6.84	3.86	2.19
8063761	NM_177980	Hs.54973	CDH26	cadherin-like 26	20q13.2-q13.33	9.81	3.70	3.50
8084657	NM_014375	Hs.81073	FETUB	fetuin B	3q27	7.44	3.62	3.17
8013536	NM_000625	Hs.434386	NOS2A	nitric oxide synthase 2A (inducible, hepatocytes)	17q11.2-q12	8.63	3.39	3.10
8021645	NM_005024	Hs.158339	SERPINB10	serpin peptidase inhibitor, clade B (ovalbumin), member 10	18q21.3	7.99	3.29	2.43
8056222	NM_001935	Hs.368912	DPP4	dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)	2q24.3	7.12	2.76	1.96
8154233	NM_014143	Hs.521989	CD274	CD274 molecule	9p24	6.61	2.46	1.76
8152617	NM_005328	Hs.571528	HAS2	hyaluronan synthase 2	8q24.12	8.49	2.45	1.66
7942135	NM_018043	Hs.503074	TMEM16A	transmembrane protein 16A	11q13.3	8.43	2.35	1.33
7921900	NM_053282	Hs.350581	SH2D1B	SH2 domain containing 1B	1q21	6.13	2.24	1.30
7954330	NM_019844	Hs.504966	SLCO1B3	solute carrier organic anion transporter family, member 1B3	12p12	7.84	2.18	1.53
7918694	NM_001010922	Hs.123106	C1orf178	chromosome 1 open reading frame 178	1p13.2	9.22	2.14	1.68
8021635	NM_002575	Hs.594481	SERPINB2	serpin peptidase inhibitor, clade B (ovalbumin), member 2	18q21.3	10.5	2.07	1.34
7935553	NM_032211	Hs.306814	LOXL4	lysyl oxidase-like 4	10q24	9.42	2.06	1.23
8157524	NM_138554	Hs.174312	TLR4	toll-like receptor 4	9q32-q33	8.58	2.04	2.06
8152976	ENST00000356838	---	TMEM71	transmembrane protein 71 (TMEM71)	8q24.22	7.92	1.96	1.40
8169504	NM_007231	Hs.522109	SLC6A14	solute carrier family 6 (amino acid transporter), member 14	Xq23-q24	11.6	1.90	1.49
7933043	---	---	---	---	---	4.84	1.75	1.21
8040458	NM_002252	Hs.414489	KCNS3	potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3	2p24	9.06	1.74	1.76
8011678	---	---	---	---	---	5.49	1.71	1.23
7934916	NM_003956	Hs.47357	CH25H	cholesterol 25-hydroxylase	10q23	7.27	1.62	1.12
8112668	NM_016591	Hs.272404	GCNT4	glucosaminyl (N-acetyl) transferase 4, core 2 (beta-1,6-N-acetylglucosaminyltransferase)	5q12	7.1	1.59	1.47
8092769	NM_198505	Hs.658032	ATP13A5	ATPase type 13A5	3q29	8.68	1.58	1.05
7922200	NM_003005	Hs.73800	SELP	selectin P (granule membrane protein 140kDa, antigen CD62)	1q22-q25	6.57	1.55	1.14
7939341	NM_000610	Hs.502328	CD44	CD44 molecule (Indian blood group)	11p13	11.5	1.53	1.18
8109001	NM_006846	Hs.331555	SPINK5	serine peptidase inhibitor, Kazal type 5	5q32	9.29	1.49	1.22
7988563	NM_203349	Hs.642615	SHC4	SHC (Src homology 2 domain containing) family, member 4	15q21.1-q21.2	6.8	1.36	1.01
7965357	NM_003774	Hs.25130	GALNT4	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylglucosaminyltransferase 4 (GalNAc-T4)	12q21.3-q22	9.84	1.35	1.21
8023688	NM_002974	Hs.123035	SERPINB4	serpin peptidase inhibitor, clade B (ovalbumin), member 4	18q21.3	10.8	1.35	1.13
8047161	NM_001031716	Hs.591610	OBFC2A	oligonucleotide/oligosaccharide-binding fold containing 2A	2q32.3	8.4	1.35	1.13
8162502	NM_000507	Hs.494496	FBP1	fructose-1,6-bisphosphatase 1	9q22.3	9.5	1.34	1.22
8161774	NM_017662	Hs.272225	TRPM6	transient receptor potential cation channel, subfamily M, member 6	9q21.13	6.87	1.26	1.18
7934411	NM_152586	Hs.657355	USP54	ubiquitin specific peptidase 54	10q22.2	9.06	1.23	1.04
8133876	NM_001001547	Hs.120949	CD36	CD36 molecule (thrombospondin receptor)	7q11.2	9.33	1.18	1.01
7991602	NM_002570	Hs.498494	PCSK6	proprotein convertase subtilisin/kexin type 6	15q26.3	7.96	1.16	1.14
7935142	---	---	---	---	---	4.2	1.14	1.55
8045088	---	---	---	---	---	5.26	1.13	1.24
8087739	NM_145071	Hs.655334	CISH	cytokine inducible SH2-containing protein	3p21.3	7.83	1.00	1.06
8127362	---	---	---	---	---	3.23	-1.03	-1.10
7988350	NM_014080	Hs.71377	DUOX2	dual oxidase 2	15q15.3	7.94	-1.05	-1.01
7959482	---	---	---	---	---	3.98	-1.29	-1.24
8163616	---	---	---	---	---	3.1	-1.89	-1.40
7939056	NM_003986	Hs.591996	BBOX1	butyrobetaine (gamma), 2-oxoglutarate dioxygenase (gamma-butyrobetaine hydroxylase) 1	11p14.2	6.84	-1.89	-1.45

Supplementary Table E6. 160 genes differentially expressed in nasal epithelial brushings (patients with uncontrolled asthma *versus* patients with controlled asthma).

Probe Set ID	mRNA Accession	UniGene	Gene Symbol	Gene Name	Cytoband	log2(signal)	log2(uncontrolled/controlled)	p-value
7921690	NM_017625	Hs.50813	ITLN1	intelectin 1 (galactofuranose binding)	1q22-q23.5	5.82	2.73	3.16E-05
7902721	NM_001285	Hs.194659	CLCA1	chloride channel, calcium activated, family member 1	1p31-p22	4.30	2.65	4.11E-05
7971077	NM_006475	Hs.136348	POSTN	periostin, osteoblast specific factor	13q13.3	6.80	2.39	1.89E-02
8084657	NM_014375	Hs.81073	FETUB	fetuin B	3q27	5.68	1.75	4.54E-04
8056222	NM_001935	Hs.368912	DPP4	dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)	2q24.3	6.38	1.72	4.28E-03
8083260	NM_001870	Hs.646	CPA3	carboxypeptidase A3 (mast cell)	3q21-q25	6.09	1.29	1.21E-02
8147132	NM_000067	Hs.155097	CA2	carbonic anhydrase II	8q22	6.28	1.26	1.99E-02
8102050	NM_178833	Hs.546482	NHEDC2	Na ⁺ /H ⁺ exchanger domain containing 2	4q24	5.99	1.16	5.82E-04
7933933	NM_021800	Hs.260720	DNAJC12	DnaJ (Hsp40) homolog, subfamily C, member 12	10q22.1	5.16	1.15	2.52E-05
8169263	NM_182607	Hs.177164	VSIG1	V-set and immunoglobulin domain containing 1	Xq22.3	4.74	1.12	3.31E-03
7930482	NM_000681	Hs.249159	ADRA2A	adrenergic, alpha-2A-, receptor	10q24-q26	7.37	1.10	3.81E-04
8063761	NM_177980	Hs.54973	CDH26	cadherin-like 26	20q13.2-21	8.57	1.10	2.13E-02
7902527	NM_001039585	Hs.654365	PTGFR	prostaglandin F receptor (FP)	1p31.1	6.12	1.05	1.01E-02
8081288	NM_018004	Hs.658956	TMEM45A	transmembrane protein 45A	3q12.2	5.62	1.04	3.88E-02
8075049	ENST00000382744	---	BA9F11.1	hypothetical LOC255349 (bA9F11.1), mRNA	22q11.23	5.61	0.93	2.63E-02
8062576	NM_021931	Hs.444520	DHX35	DEAH (Asp-Glu-Ala-His) box polypeptide 35	20q11.22-q12	7.07	0.90	2.97E-03
8081826	NM_006952	Hs.271580	UPK1B	uroplakin 1B	3q13.3-q21	10.19	0.89	4.53E-02
8106448	NM_003719	Hs.584830	PDE8B	phosphodiesterase 8B	5q14.1	7.20	0.86	2.17E-02
8003667	NM_002615	Hs.532768	SERPINF1	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	17p13.1	8.94	0.85	3.56E-02
8029754	NM_004497	Hs.36137	FOXA3	forkhead box A3	19q13.2-q13.4	6.82	0.80	1.54E-02
7963313	NM_007210	Hs.505575	GALNT6	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 6 (GalNAc-T6)	12q13	6.65	0.78	4.11E-02
8042917	NM_001381	Hs.103854	DOK1	docking protein 1, 62kDa (downstream of tyrosine kinase 1)	2p13	6.60	0.77	1.12E-03
8040430	NM_003385	Hs.444212	VSNL1	visinin-like 1	2p24.3	4.98	0.76	1.74E-03
8164496	NM_004059	Hs.495250	CCBL1	cysteine conjugate-beta lyase; cytoplasmic (glutamine transaminase K, kynurenine aminotransferase)	9q34.11	6.21	0.76	6.67E-04
7933312	NM_001040084	Hs.535306	ANXA8	annexin A8	10q11.22	6.99	0.75	4.67E-02
8122038	ENST00000296078	---	KIAA1913	KIAA1913 (KIAA1913), mRNA	6q23.1	5.70	0.75	1.12E-03
8171248	NM_000216	Hs.521869	KAL1	Kallmann syndrome 1 sequence	Xp22.32	5.88	0.73	2.00E-02
8105302	NM_006350	Hs.9914	FST	Follistatin	5q11.2	5.46	0.73	1.97E-02
8126853	NM_001013732	Hs.659409	C6ORF138	chromosome 6 open reading frame 138	6p12.3	4.22	0.73	1.21E-02
7950140	---	---	-	---	-	6.63	0.71	2.61E-04
8053200	NM_133637	Hs.191705	DQX1	DEAQ box polypeptide 1 (RNA-dependent ATPase)	2p13.1	5.83	0.70	3.07E-02
8167690	---	---	-	---	-	5.04	0.69	1.96E-02
8172719	---	---	-	---	-	5.04	0.69	1.96E-02
8101992	NM_022154	Hs.288034	SLC39A8	solute carrier family 39 (zinc transporter), member 8	4q22-q24	8.66	0.67	2.60E-02
7961306	NM_005039	Hs.631726	PRB1	proline-rich protein BstNI subfamily 1	12p13.2	6.29	0.67	3.30E-03
8088560	NM_182920	Hs.656071	ADAMTS9	ADAM metalloproteinase with thrombospondin type 1 motif, 9	3p14.3-p14.2	5.11	0.65	2.24E-02
8105348	---	---	-	---	-	6.25	0.64	4.75E-02
8167673	NM_030801	---	MAGED4B	melanoma antigen family D, 4B	-	6.06	0.63	3.97E-02
8172722	NM_030801	---	MAGED4B	melanoma antigen family D, 4B	-	6.06	0.63	3.97E-02
7908397	NM_002927	Hs.497220	RGS13	regulator of G-protein signaling 13	1q31.2	4.21	0.62	7.17E-03
7961320	NM_006248	Hs.654486	PRB2	proline-rich protein BstNI subfamily 2	12p13.2	6.21	0.60	1.72E-02
8041206	NM_030915	Hs.567598 // Hs.593113	LBH	limb bud and heart development homolog (mouse)	2p23.1	7.50	0.59	1.26E-02
8125843	NM_012391	Hs.485158	SPDEF	SAM pointed domain containing ets transcription factor	6p21.3	9.50	0.59	1.27E-02

8069269	NM_001848	Hs.474053	COL6A1	collagen, type VI, alpha 1	21q22.3	7.51	0.59	6.55E-03
8107769	NM_001046	Hs.162585 // Hs.643978	SLC12A2	solute carrier family 12 (sodium/potassium/chloride transporters), member 2	5q23.3	9.82	0.55	2.00E-03
7946292	NM_016229	Hs.414362	CYB5R2	cytochrome b5 reductase 2	11p15.4	7.42	0.53	3.82E-02
8123446	NM_022138	Hs.487200	SMOC2	SPARC related modular calcium binding 2	6q27	5.03	0.53	3.67E-02
8113800	NM_001999	Hs.519294	FBN2	fibrillin 2 (congenital contractural arachnodactyly)	5q23-q31	4.21	0.51	3.14E-02
7905677	NM_130898	Hs.372924	CREB3L4	cAMP responsive element binding protein 3- like 4	1q21.3	6.98	0.50	2.25E-02
7967874	---	---	-	---	-	4.23	0.49	4.52E-03
7912887	NM_002403	Hs.389137	MFAP2	microfibrillar-associated protein 2	1p36.1-p35	6.64	0.46	3.48E-02
7898431	NM_016233	Hs.149195	PADI3	peptidyl arginine deiminase, type III	1p36.13	6.29	0.46	4.17E-02
8088192	NM_015576	Hs.476389	ERC2	ELKS/RAB6-interacting/CAST family member 2	3p14.3	4.84	0.45	2.07E-02
7923578	NM_002023	Hs.519168	FMOD	Fibromodulin	1q32	4.18	0.45	4.39E-02
8104107	ENST00000326 754	---	FLJ25801	hypothetical protein FLJ25801 (FLJ25801), mRNA	4q35.2	4.03	0.44	3.32E-02
8032751	---	---	-	---	-	5.94	0.44	3.86E-02
8086391	NM_000729	Hs.458426	CCK	Cholecystokinin	3p22-p21.3	5.45	0.42	2.07E-02
8115730	---	---	-	---	-	4.93	0.41	4.69E-02
8031843	---	---	-	---	-	4.97	0.41	3.62E-04
8046360	NM_199227	Hs.298250	MAP1D	methionine aminopeptidase 1D	2q31.1	4.40	0.40	1.18E-02
8060940	NM_012261	Hs.22920	C20ORF10 ?	chromosome 20 open reading frame 103 ?	20p12	4.30	0.40	3.20E-02
7995350	---	---	-	---	-	8.68	0.40	4.37E-02
8121112	---	---	-	---	-	5.45	0.40	2.32E-02
8174092	NM_177949	Hs.48924	ARMCX2	armadillo repeat containing, X-linked 2	Xq21.33-q22.2	5.61	0.39	2.99E-02
8037835	NM_005628	Hs.631582	SLC1A5	solute carrier family 1 (neutral amino acid transporter), member 5	19q13.3	8.96	0.38	4.17E-02
8174389	NM_033641	Hs.145586	COL4A6	collagen, type IV, alpha 6	Xq22	5.62	0.37	4.49E-02
8175860	NM_032512	Hs.92732	PDZD4	PDZ domain containing 4	Xq28	5.39	0.37	1.33E-02
7923442	NM_177402	Hs.585015	SYT2	synaptotagmin II	1q32.1	5.13	0.36	4.92E-02
7954243	---	---	-	---	-	6.03	0.36	4.77E-02
8169441	---	---	-	---	-	4.97	0.36	4.49E-02
7994981	ENST00000318 663	---	ORAI3	transmembrane protein 142C (TMEM142C), mRNA	16p11.2	7.54	0.35	4.39E-02
8077970	NM_001004019	Hs.198862	FBLN2	fibulin 2	3p25.1	5.71	0.34	3.94E-02
7970542	---	---	-	---	-	4.59	0.34	1.70E-02
7914516	---	---	-	---	-	6.42	0.32	2.15E-02
8026712	NM_152363	Hs.379097	ANKRD41	ankyrin repeat domain 41	19p13.11	4.97	0.32	4.03E-02
8079124	NM_207404	Hs.656856	ZNF662	zinc finger protein 662	3p22.1	5.97	0.32	3.14E-02
7961259	NM_006250	Hs.654485	PRH1	proline-rich protein HaeIII subfamily 1	12p13.2	4.90	0.32	3.08E-02
8132557	NM_001129	Hs.439463	AEBP1	AE binding protein 1	7p13	6.47	0.31	2.23E-02
8053165	NM_053050	Hs.534527	MRPL53	mitochondrial ribosomal protein L53	2p13.1	8.56	0.31	4.40E-02
8097443	---	---	-	---	-	5.47	0.30	1.95E-02
7960283	NM_172364	Hs.13768	CACNA2D4	calcium channel, voltage-dependent, alpha 2/delta subunit 4	12p13.33	5.48	0.29	2.14E-02
7961300	NM_006249	Hs.73031	PRB3	proline-rich protein BstNI subfamily 3	12p13.2	6.83	0.29	4.53E-02
7933498	---	---	-	---	-	6.02	0.28	4.42E-02
7897154	NM_014448	Hs.87435	ARHGEF16	Rho guanine exchange factor (GEF) 16	1p36.3	7.23	0.28	1.99E-02
8084887	---	---	-	---	-	4.85	0.27	2.30E-02
7996331	NM_005182	Hs.37014	CA7	carbonic anhydrase VII	16q22.1	4.19	0.27	3.35E-02
8076815	---	---	-	---	-	4.63	0.24	3.77E-02
8009693	ENST00000335	---	TMEM104	transmembrane protein 104 (TMEM104), mRNA	17q25.1	6.53	-0.25	3.72E-02
8034286	NM_016581	Hs.515146	ECSIT	ECSIT homolog (Drosophila)	19p13.2	7.59	-0.25	2.25E-02
7999044	NM_004380	Hs.459759	CREBBP	CREB binding protein (Rubinstein-Taybi syndrome)	16p13.3	8.53	-0.26	4.81E-02
8155707	NM_004817	Hs.50382	TJP2	tight junction protein 2 (zona occludens 2)	9q13-q21	10.21	-0.27	3.28E-02
7897520	---	---	-	---	-	4.89	-0.27	2.37E-02
8142415	NM_152556	Hs.489734	FLJ31818	hypothetical protein FLJ31818	7q31.1	7.46	-0.27	4.07E-02
8013307	NM_001455	Hs.220950	FOXO3	forkhead box O3	6q21	9.52	-0.28	2.57E-02

7932023	NM_001080491	Hs.498661	USP6NL	USP6 N-terminal like	10p13	7.57	-0.28	2.64E-02
7978595	NM_013448	Hs.696045	BAZ1A	bromodomain adjacent to zinc finger domain, ^{1A}	14q12-q13	8.35	-0.30	3.41E-02
8063211	NM_006534	Hs.592142	NCOA3	nuclear receptor coactivator 3	20q12	9.05	-0.31	3.30E-03
8005407	NM_004140	Hs.513983	LLGL1	lethal giant larvae homolog 1 (Drosophila)	17p11.2	7.37	-0.32	4.63E-02
8119926	NM_018426	Hs.414473	TMEM63B	transmembrane protein 63B	6p21.1	8.59	-0.32	2.57E-02
7952290	NM_012101	Hs.504115	TRIM29	tripartite motif-containing 29	11q22-q23	10.40	-0.33	3.65E-02
8064766	NM_007219	Hs.589884	RNF24	ring finger protein 24	20p13-p12.1	7.85	-0.34	3.56E-02
8166705	NM_000950	Hs.190341	PRRG1	proline rich Gla (G-carboxyglutamic acid) 1	Xp21.1	5.57	-0.34	3.07E-02
8079140	NM_017719	Hs.476052	SNRK	SNF related kinase	3p22.1	8.03	-0.35	7.91E-04
7939546	NM_002231	Hs.527778	CD82	CD82 molecule	11p11.2	10.43	-0.36	3.32E-02
8117243	NM_017640	Hs.649550	LRRC16	leucine rich repeat containing 16	6p22.2	8.49	-0.36	2.19E-02
7925062	NM_020808	Hs.268774	SIPA1L2	signal-induced proliferation-associated 1 like 2	1q42.2	7.13	-0.36	1.54E-02
7982102	NM_000810	Hs.612087	GABRA5	gamma-aminobutyric acid (GABA) A receptor, alpha 5	15q11.2-q12	4.62	-0.38	2.63E-02
7976976	NM_014844	Hs.195667	KIAA0329	KIAA0329	14q32.31	6.64	-0.38	3.95E-02
8060627	NM_139321	Hs.276252	ATRN	Attractin	20p13	8.80	-0.38	6.06E-03
7964701	NM_002076	Hs.334534	GNS	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	12q14	9.73	-0.38	3.36E-02
8038029	NM_014959	Hs.446146	CARD8	caspase recruitment domain family, member 8	19q13.32	6.49	-0.38	2.13E-02
7947462	NM_145804	Hs.23361	ABTB2	ankyrin repeat and BTB (POZ) domain containing 2	11p13	5.41	-0.38	2.85E-02
8133326	NM_001099415	---	LOC100101	POM121 membrane glycoprotein (rat)-like	7q11.2	7.30	-0.38	3.04E-02
7922689	NM_002065	Hs.518525	²⁶⁷ GLUL	glutamate-ammonia ligase (glutamine synthetase)	1q31	10.54	-0.38	4.84E-03
8082012	NM_021082	Hs.518089	SLC15A2	solute carrier family 15 (H+/peptide transporter), member 2	3q13.33	10.59	-0.39	3.77E-02
8063386	NM_005194	Hs.517106	CEBPB	CCAAT/enhancer binding protein (C/EBP), ^{hata}	20q13.1	8.19	-0.40	3.47E-02
7910111	NM_000120	Hs.89649	EPHX1	epoxide hydrolase 1, microsomal (xenobiotic)	1q42.1	9.79	-0.42	4.02E-02
8071069	NM_014339	Hs.129751	IL17RA	interleukin 17 receptor A	22q11.1	7.57	-0.43	1.11E-02
8145954	NM_006283	Hs.279245	TACC1	transforming, acidic coiled-coil containing protein 1	8p11	7.80	-0.45	1.95E-02
8105436	---	---	-	---	-	8.66	-0.45	1.97E-02
7961546	NM_004447	Hs.591160	EPS8	epidermal growth factor receptor pathway substrate 8	12q13	9.19	-0.45	3.07E-02
8091352	NM_020131	Hs.283739	UBQLN4	ubiquilin 4	1q21	6.20	-0.45	3.82E-02
8126382	---	---	-	---	-	8.04	-0.47	1.89E-02
8096116	NM_032717	Hs.99196	MAG1	lung cancer metastasis-associated protein	4q21.23	7.33	-0.47	4.71E-02
7908003	NM_030769	Hs.496969	NPL	N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)	1q25	5.38	-0.48	6.67E-03
7904572	---	---	-	---	-	4.60	-0.48	3.08E-02
8160297	NM_001122	Hs.3416	ADFP	adipose differentiation-related protein	9p22.1	6.60	-0.49	3.45E-03
7905154	---	---	-	---	-	6.78	-0.50	3.67E-03
7948397	---	---	-	---	-	5.22	-0.50	9.79E-03
8053484	NM_003896	Hs.415117	ST3GAL5	ST3 beta-galactoside alpha-2,3- sialyltransferase 5	2p11.2	6.67	-0.52	2.07E-02
7927658	NM_003338	Hs.129683	UBE2D1	ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast)	10q11.2-q21	8.02	-0.55	8.12E-03
7984779	NM_033240	Hs.526464	PML	promyelocytic leukemia	15q22	7.30	-0.55	4.19E-02
7936856	NM_015892	Hs.287537	GALNAC4S	B cell RAG associated protein	10q26	7.23	-0.56	1.14E-03
7961891	NM_030762	Hs.177841	^{6ST} BHLHB3	basic helix-loop-helix domain containing, class B, 3	12p11.23- p12.1	6.90	-0.57	4.40E-02
8009653	NM_007261	Hs.9688	CD300A	CD300a molecule	17q25.1	6.19	-0.58	3.82E-02
7960794	NM_004244	Hs.504641	CD163	CD163 molecule	12p13.3	4.88	-0.58	2.11E-02
8124798	NM_133471	Hs.696054	KIAA1949	KIAA1949	6p21.3	6.08	-0.58	2.19E-02
7943413	NM_001165	Hs.127799	BIRC3	baculoviral IAP repeat-containing 3	11q22	7.73	-0.60	2.24E-02
7964834	NM_001005502	Hs.654387	CPM	carboxypeptidase M	12q14.3	6.66	-0.61	3.61E-02
8121861	NM_181782	Hs.171426	NCOA7	nuclear receptor coactivator 7	6q22.31- ^{22 22} 22q13	6.23	-0.61	3.87E-02
8076734	NM_058238	Hs.512714	WNT7B	wingless-type MMTV integration site family, member 7B	22q13	7.56	-0.64	9.51E-03
7931832	NM_205845	Hs.567256	AKR1C2	aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III)	10p15-p14	9.73	-0.66	5.84E-05
8153959	NM_203447	Hs.132599	DOCK8	dedicator of cytokinesis 8	9p24.3	6.98	-0.66	4.84E-02

8075306	---	---	-	---	-	4.66	-0.67	1.97E-02
8115623	NM_025153	Hs.109358	ATP10B	ATPase, Class V, type 10B	5q34	8.40	-0.71	4.26E-03
7978644	NM_020529	Hs.81328	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	14q13	9.46	-0.71	1.04E-02
8090823	NM_005630	Hs.518270	SLCO2A1	solute carrier organic anion transporter family, member 2A1	3q21	7.72	-0.72	3.97E-02
8026456	NM_000896	Hs.106242	CYP4F3	cytochrome P450, family 4, subfamily F, polypeptide 3	19p13.2	7.50	-0.73	2.93E-02
8068697	NM_002463	Hs.926	MX2	myxovirus (influenza virus) resistance 2	21q22.3	7.91	-0.75	2.71E-02
8103601	NM_001012967	Hs.535011	FLJ31033	hypothetical protein FLJ31033	4q32.3	7.07	-0.79	1.21E-02
7997152	NM_005769	Hs.251383	CHST4	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 4	16q22.3	6.14	-0.79	7.64E-04
8125919	NM_004117	Hs.407190	FKBP5	FK506 binding protein 5	6p21.3-p21.2	7.28	-0.80	1.01E-02
7957962	NM_152323	Hs.577097	SPIC	Spi-C transcription factor (Spi-1/PU.1 related)	12q23.2	4.50	-0.85	5.82E-04
8142120	NM_005746	Hs.489615	PBEF1	pre-B-cell colony enhancing factor 1	7q22.2	10.43	-0.86	6.27E-03
8014903	NM_001042471	Hs.306777	GSDML	gasdermin-like	17q12	8.29	-0.86	1.10E-03
8100798	NM_014465	Hs.129742	SULT1B1	sulfotransferase family, cytosolic, 1B, member 1	4q13.3	4.20	-0.90	2.03E-02
7933084	ENST00000222553	---	PBEF1	pre-B-cell colony enhancing factor 1 (PBEF1), mRNA	7q22.2	11.11	-0.91	8.53E-03
7929047	NM_001547	Hs.437609	IFIT2	interferon-induced protein with tetratricopeptide repeats 2	10q23-q25	7.01	-0.93	4.73E-02
8086600	NM_001295	Hs.301921	CCR1	chemokine (C-C motif) receptor 1	3p21	4.94	-0.94	2.51E-02
7921031	---	---	-	---	-	4.59	-0.94	2.93E-03
8086330	NM_033027	Hs.370950	AXUD1	AXIN1 up-regulated 1	3p22	7.30	-1.22	4.49E-06
7917530	---	---	-	---	-	6.58	-1.29	1.80E-02

Supplementary Table E7. Functional annotation of the 160 genes regulated in patients with uncontrolled, compared with controlled, asthma.

Category	Term	Count	Pop Hits	p-value	Gene symbol
CC	proteinaceous extracellular matrix	12	344	4.06E-05	ADAMTS9, CLCA1, COL4A6, COL6A1, FBLN2, FBN2, FMOD, KAL1, MFAP2, POSTN, SMOC2 WNT7B
MF	exopeptidase activity	6	102	7.00E-04	AEBP1, CPA3, DPP4, MAP1D ATRN, CPM
BP	response to stress	14	1081	2.65E-02	8105348, ADRA2A, ANXA8, CREB3L4, FOXA3 8105436, ATRN, CCR1, CD163, CEBPB, CHST4, CREBBP, GABRA5, PML
CC	integral to plasma membrane	16	1246	3.56E-02	ADRA2A, CLCA1, KCND2, PTGFR, SLC12A2, SLC1A5 ATRN, CCR1, CD163, CD82, GABRA5, IL17RA, PRRG1, SLCO2A1, ST3GAL5, TJP2

Supplementary Table E8. 316 genes differentially expressed in primary HNECs treated with IFN- α , IFN- β or IFN- γ .

Probe Set ID	mRNA Accession	UniGene ID	Gene Symbol	Gene Name	Cytoband	log2(signal)	log2 (IFN α /C)	log2(IFN β /C)	log2(IFN γ /C)
8101126	NM_001565	Hs.632586	CXCL10	chemokine (C-X-C motif) ligand 10	4q21	8.3	6.97	1.63	8.02
7929065	NM_001548	Hs.20315	IFIT1	interferon-induced protein with tetratricopeptide repeats 1	10q25-q26	10.5	6.33	5.37	2.12
8040080	NM_080657	Hs.17518	RSAD2	radical S-adenosyl methionine domain containing 2	2p25.2	9.7	6.22	4.65	3.2
8068697	NM_002463	Hs.926	MX2	myxovirus (influenza virus) resistance 2 (mouse)	21q22.3	9.95	5.9	5.23	2.24
7902541	NM_006820	Hs.389724	IFI44L	interferon-induced protein 44-like	1p31.1	9.39	5.86	5.38	3.63
7929047	NM_001547	Hs.437609	IFIT2	interferon-induced protein with tetratricopeptide repeats 2	10q23-q25	10.2	5.66	3.88	4
8096361	NM_016323	Hs.26663	HERC5	hect domain and RLD 5	4q22.1	7.27	5.37	3.58	1.1
7967117	NM_003733	Hs.118633	OASL	2'-5'-oligoadenylate synthetase-like	12q24.2	8.56	4.94	2.6	1.56
8101131	NM_005409	Hs.632592	CXCL11	chemokine (C-X-C motif) ligand 11	4q21.2	7.45	4.67	0.23	6.18
7929052	NM_001031683	Hs.47338	IFIT3	interferon-induced protein with tetratricopeptide repeats 3	10q24	9.6	4.67	3.75	3.79
7902553	NM_006417	Hs.82316	IFI44	interferon-induced protein 44	1p31.1	9.06	4.67	4.16	2.65
7971296	NM_001002264	Hs.546467	EPSTI1	epithelial stromal interaction 1 (breast)	13q13.3	7.95	4.47	3.03	3.66
8074606	ENST00000292729	---	---	---	---	9.54	4.41	3.28	1.51
8160559	NM_014314	Hs.190622	DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	9p12	9.66	4.33	3.2	2.42
7917561	NM_052941	Hs.409925	GBP4	guanylate binding protein 4	1p22.2	8.7	4.29	1.26	5.79
8068713	NM_002462	Hs.517307	MX1	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	21q22.3	11.1	4.25	3.51	2.65
8140971	NM_152703	Hs.489118	SAMD9L	sterile alpha motif domain containing 9-like	7q21.2-q21.3	8.68	4.21	2.67	3.12
8103601	NM_001012967	Hs.535011	FLJ31033	hypothetical protein FLJ31033	4q32.3	9.09	4.09	2.69	1.23
8103563	ENST00000260184	---	FLJ20035	hypothetical protein FLJ20035 (FLJ20035)	4q32.3	8.62	4.07	2.99	2.6
7958913	NM_002535	Hs.414332	OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa	12q24.2	10.21	3.99	3.4	2.58
8146092	NM_002164	Hs.840	INDO	indoleamine-pyrrole dioxygenase	2,3 8p12-p11	9.69	3.93	0.37	5.71
7914127	NM_022872	Hs.523847	IFI6	interferon, alpha-inducible protein 6	1p35	10.69	3.68	2.61	1.12
8050102	NM_207315	Hs.7155	LOC129607	hypothetical protein LOC129607	2p25.2	8.63	3.66	2.16	1.63
7896817	NM_005101	Hs.458485	ISG15	ISG15 ubiquitin-like modifier	1p36.33	9.36	3.59	2.03	1.09
8071155	NM_017414	Hs.38260	USP18	ubiquitin specific peptidase 18	22q11.21	8.92	3.56	2.24	0.86
8004184	NM_017523	Hs.441975	XAF1	XIAP associated factor-1	17p13.2	9.5	3.51	2.68	2.59
8059650	NM_080424	Hs.145150	SP110	SP110 nuclear body protein	2q37.1	8.68	3.47	2.34	2.69
8056285	NM_022168	Hs.163173	IFIH1	interferon induced with helicase C domain 1	2q24	9.14	3.46	1.94	1.89
7958895	NM_006187	Hs.528634	OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa	12q24.2	9.77	3.46	2.39	2.23
7958884	NM_016816	Hs.524760	OAS1	2'-5'-oligoadenylate synthetase 1, 40/46kDa	12q24.1	11.19	3.38	2.34	1.6
8117034	NM_006877	Hs.484741	GMPR	guanosine monophosphate reductase	6p23	7.58	3.34	1.13	0.44
8096335	NM_017912	Hs.529317	HERC6	hect domain and RLD 6	4q22.1	9.48	3.32	2.37	1.21
7921434	NM_004833	Hs.281898	AIM2	absent in melanoma 2	1q22	5.84	3.23	1	2.39
8007446	NM_005533	Hs.632258	IFI35	interferon-induced protein 35	17q21	9.86	3.18	1.73	2.52
8086125	NM_014831	Hs.170999	LBA1	lupus brain antigen 1	3p22.2	8.42	3.04	1.54	1.41
7988350	NM_014080	Hs.71377	DUOX2	dual oxidase 2	15q15.3	9.43	3.01	1.26	2.58
7983405	NM_207581	Hs.497987	DUOX2	dual oxidase maturation factor 2	15q15.1	8.27	2.81	1.08	2.72
8082100	NM_017554	Hs.696194	PARP14	poly(ADP-ribose) polymerase family member 14	3q21.1	10.66	2.75	1.85	2.72
8066117	NM_015474	Hs.580681	SAMHD1	SAM domain and HD domain 1	20pter-q12	10.3	2.7	1.78	2.37
8041422	NM_170672	Hs.143674	RASGRP3	RAS guanyl releasing protein 3 (calcium and DAG-regulated)	2p25.1-p24.1	5.63	2.68	0.97	1.02
7917516	NM_002053	Hs.62661	GBP1	guanylate binding protein 1, interferon-inducible, 67kDa	1p22.2	11	2.64	0.99	3.83
8073088	NM_021822	Hs.660143	APOBEC3G	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G	22q13.1-q13.2	6.13	2.59	0.12	1.52
8140967	NM_017654	Hs.65641	SAMD9	sterile alpha motif domain containing 9	7q21.2	10.14	2.52	1.27	0.66
8084732	NM_022147	Hs.43388	RTP4	receptor (chemosensory) transporter protein 4	3q27.3	8.65	2.51	1.73	1.72
8057744	NM_007315	Hs.642990	STAT1	signal transducer and activator of transcription 1, 91kDa	2q32.2	11.75	2.5	1.89	2.48
7948982	NM_017878	Hs.272805	HRASLS2	HRAS-like suppressor 2	11q12.3	7.55	2.47	0.12	0.09
8122334	AF110640	Hs.310512	CCRL1	chemokine (C-C motif) receptor-like 1	3q22	7.43	2.47	0.81	3.45
8010426	ENST00000319902	---	---	---	---	10.37	2.46	1.2	2.23
8010454	NM_020914	Hs.195642	RNF213	ring finger protein 213	17q25.3	11	2.4	1.17	2.19
8090018	NM_031458	Hs.518200	PARP9	poly (ADP-ribose) polymerase family, member 9	3q13-q21	10.4	2.38	1.94	2.17

8136388	NM_018295	Hs.658637	TMEM140	transmembrane protein 140	7q33	8.29	2.37	1.08	2.4
7948493	NM_016582	Hs.237856	SLC15A3	solute carrier family 15, member 3	11q12.2	8.52	2.31	0.69	1.67
8026533	ENST00000253680	---	---	---	---	7.99	2.31	1.36	0.68
8052331	NM_033109	Hs.388733	PNPT1	polyribonucleotide nucleotidyltransferase 1	2p15	9.75	2.29	1.37	0.01
8015511	NM_024119	Hs.55918	DHX58	DEXH (Asp-Glu-X-His) box polypeptide 58	17q21.2	8.28	2.28	1.26	1.04
8101701	NM_152542	Hs.291000	PPM1K	protein phosphatase 1K (PP2C domain containing)	4q22.1	6.94	2.28	1.18	0.12
7899394	NM_004848	Hs.10649	C1ORF38	chromosome 1 open reading frame 38	1p35.3	7.47	2.26	0.34	0.23
8055702	NM_004688	Hs.54483	NMI	N-myc (and STAT) interactor	2q23	8.93	2.25	0.85	2.02
7964119	NM_005419	Hs.530595	STAT2	signal transducer and activator of transcription 2, 113kDa	12q13.2	10.14	2.24	1.25	1.81
8156688	NM_014290	Hs.193842	TDRD7	tudor domain containing 7	9q22.33	8.3	2.23	1.23	0.89
8092348	NM_014398	Hs.518448	LAMP3	lysosomal-associated membrane protein 3	3q26.3-q27	9.62	2.22	0.81	0.97
8051501	NM_002759	Hs.131431	EIF2AK2	eukaryotic translation initiation factor 2-alpha kinase 2	2p22-p21	10.42	2.22	1.63	0.92
7946089	NM_033034	Hs.370515	TRIM5	tripartite motif-containing 5	11p15	9.25	2.16	1.34	0.77
8067680	NM_001037335	Hs.517180	PRIC285	peroxisomal proliferator-activated receptor A interacting complex 285	20q13.33	8.73	2.15	1.66	1.02
8143327	NM_022750	Hs.12646	PARP12	poly(ADP-ribose) polymerase family member 12	7q34	9.59	2.13	1.2	1.35
7945962	NM_003141	Hs.532357	TRIM21	tripartite motif-containing 21	11p15.5	9.02	2.12	1.17	2.12
7969986	NM_006573	Hs.525157	TNFSF13B	tumor necrosis factor (ligand) superfamily, member 13b	13q32-q34	5.96	2.11	0.08	0.66
7917576	ENST00000370459	---	---	---	---	7.69	2.09	0.49	5.96
7990839	NM_181900	Hs.513075	STARD5	StAR-related lipid transfer (START) domain containing 5	15q26	8.85	2.09	0.92	1.22
8028744	ENST00000354596	---	---	---	---	6.26	2.07	0.3	5.06
8092169	NM_003810	Hs.478275	TNFSF10	tumor necrosis factor (ligand) superfamily, member 10	3q26	11.47	2.06	0.92	1.31
8179495	NM_002800	Hs.654585	PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	6p21.3	9.97	2.06	0.61	2.46
8178211	NM_148954	Hs.654585	PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	6p21.3	9.97	2.06	0.61	2.46
8118571	NM_148954	Hs.654585	PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	6p21.3	9.97	2.06	0.61	2.46
8125993	NM_016135	Hs.272398	ETV7	ets variant gene 7 (TEL2 oncogene)	6p21	7.75	2.04	0.85	2.58
7968637	NM_003914	Hs.417050	CCNA1	cyclin A1	13q12.3-q13	8.51	2.04	0.5	0.07
8154233	NM_014143	Hs.521989	CD274	CD274 molecule	9p24	7.67	2.02	0.36	3.66
7937335	NM_003641	---	IFITM1	interferon induced transmembrane protein 1 (9-27)	11p15.5	11.37	2.02	1.32	1.28
8098611	NM_003265	Hs.657724	TLR3	toll-like receptor 3	4q35	8.72	2.01	0.45	0.95
7917530	ENST00000294663	---	---	---	---	7.17	1.98	0.29	5.09
7938035	NM_006074	Hs.501778	TRIM22	tripartite motif-containing 22	11p15	10.41	1.97	1.29	1.36
8002778	NM_152649	Hs.119878	MLKL	mixed lineage kinase domain-like	16q22.3	8.78	1.96	0.57	1.69
8161964	NM_174938	Hs.127535	FRMD3	FERM domain containing 3	9q21.32	6.8	1.95	0.01	1.47
8067869	NM_144770	Hs.283828	RBM11	RNA binding motif protein 11	21q11	4.32	1.9	0.02	0.48
7984779	NM_033240	Hs.526464	PML	promyelocytic leukemia	15q22	9	1.87	0.97	1.53
7995926	NM_032206	Hs.528836	NLR5	NLR family, CARD domain containing 5	16q13	8.33	1.86	0.83	2.01
8007188	NM_033133	Hs.273621	CNP	2',3'-cyclic nucleotide phosphodiesterase	17q21	9.68	1.85	0.75	0.51
8016847	NM_005082	Hs.528952	TRIM25	tripartite motif-containing 25	17q23.2	10.35	1.84	1.07	1.28
8180061	NM_000593	Hs.352018	TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	6p21.3	11.43	1.83	0.7	2.63
8125512	NM_000593	Hs.352018	TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	6p21.3	11.43	1.83	0.7	2.63
8178867	NM_000593	Hs.352018	TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	6p21.3	11.43	1.83	0.7	2.63
7976443	NM_005532	Hs.532634	IFI27	interferon, alpha-inducible protein 27	14q32	10.35	1.83	1.25	0.51
7949340	NM_138456	Hs.124840	BATF2	basic leucine zipper transcription factor, ATF-like 2	11q13.1	8.34	1.82	0.87	2.31
8066905	NM_021035	Hs.371794	ZNFX1	zinc finger, NFX1-type containing 1	20q13.13	9.92	1.81	1.02	1.28
8025551	ENST00000253110	---	---	---	---	8.72	1.8	0.86	0.73
8082075	NM_138287	Hs.518201	DTX3L	deltex 3-like (Drosophila)	3q21.1	10.21	1.8	1.23	1.71
8019486	NM_003004	Hs.558009	SECTM1	secreted and transmembrane 1	17q25	10.01	1.79	0.28	2.75
8048940	NM_001080391	Hs.369056	SP100	SP100 nuclear antigen	2q37.1	9.08	1.76	1	1.21
8091327	NM_021105	Hs.130759	PLSCR1	phospholipid scramblase 1	3q23	9.62	1.74	1.08	1.05
7951397	NM_033292	Hs.2490	CASP1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	11q23	8.56	1.72	0.32	1.47
8081710	NM_017699	Hs.591291	SIDT1	SID1 transmembrane family, member 1	3q13.2	7.91	1.66	0.65	0.48
8158560	NM_014506	Hs.654672	TOR1B	torsin family 1, member B (torsin B)	9q34	9.54	1.66	0.94	0.24
8137414	NM_016118	Hs.647082	NUB1	negative regulator of ubiquitin-like	7q36	10.22	1.64	0.34	1.4

8094259	NM_015907	Hs.570791	LAP3	proteins 1					
7973618	NM_006084	Hs.1706	ISGF3G	leucine aminopeptidase 3	4p15.32	9.91	1.62	0.68	1.66
				interferon-stimulated transcription factor 3, gamma 48kDa	14q11.2	9.44	1.62	1.46	1.39
8075709	NM_030643	Hs.115099	APOL4	apolipoprotein L, 4	22q11.2-q13.2	8.16	1.61	0.51	3.76
7948274	NM_004223	Hs.425777	UBE2L6	ubiquitin-conjugating enzyme E2L 6	11q12	9.86	1.6	0.73	1.5
8073081	NM_145298	Hs.659991	APOBEC3F	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3F	22q13.1	7.25	1.54	0.25	0.89
7951413	NM_001007232	Hs.44102	INCA	inhibitory caspase recruitment domain (CARD) protein	11q22.3	6.65	1.54	0.14	1.27
8075695	NM_014349	Hs.474737	APOL3	apolipoprotein L, 3	22q13.1	6.87	1.54	0.46	3.12
7979721	ENST00000357461	---	---	---	---	7.74	1.52	0.62	1.26
8119198	ENST00000373451	---	KIAA0082	KIAA0082 (KIAA0082), mRNA	6p21.2	9.47	1.52	0.91	0.72
8046003	NM_012198	Hs.377894	GCA	grancalcin, EF-hand calcium binding protein	2q24.2	7.07	1.52	0.26	0.34
8154245	NM_025239	Hs.532279	PDCD1LG2	programmed cell death 1 ligand 2	9p24.2	6.61	1.51	0.47	1.77
7945462	NM_004031	Hs.166120	IRF7	interferon regulatory factor 7	11p15.5	8.61	1.51	0.8	0.4
8072710	NM_030641	Hs.257352	APOL6	apolipoprotein L, 6	22q12.3	9.59	1.5	0.49	1.92
8090180	NM_033049	Hs.5940	MUC13	mucin 13, cell surface associated	---	6.1	1.5	0.2	1.7
8138941	NM_001002009	Hs.487933	NT5C3	5'-nucleotidase, cytosolic III	7p14.3	7.85	1.48	0.32	0.41
7917532	NM_004120	Hs.386567	GBP2	guanylate binding protein 2, interferon-inducible	1p22.2	7.95	1.48	0.15	4.15
8005458	NM_001042685	Hs.656100	LOC284194	galectin-9 like	17p11.2	9.57	1.46	0.74	0.47
7956269	---	---	---	---	---	4.58	1.44	1.09	0.47
7969129	NM_001040443	Hs.369039	PHF11	PHD finger protein 11	13q14.3	8.69	1.44	0.76	0.85
8005809	NM_009587	Hs.81337	LGALS9	lectin,galactoside-binding,soluble,9 (galectin 9)	17q11.1	7.68	1.44	0.8	0.38
8170704	NM_000033	Hs.159546	ABCD1	ATP-binding cassette, sub-family D (ALD), member 1	Xq28	7.63	1.43	0.51	0.45
8091141	NM_019001	Hs.435103	XRN1	5'-3' exoribonuclease 1	3q23	9.11	1.43	0.22	1.19
7953993	NM_030766	Hs.210343	BCL2L14	BCL2-like 14 (apoptosis facilitator)	12p13-p12	7.49	1.42	0.47	1.27
7935639	NM_031212	Hs.403790	SLC25A28	solute carrier family 25, member 28	10q23-q24	8.96	1.42	0.92	0.95
8162729	NM_014788	Hs.575631	TRIM14	tripartite motif-containing 14	9q22.33	8.83	1.41	0.98	0.58
8013450	NM_001042685	Hs.656100	LOC284194	galectin-9 like	17p11.2	10.35	1.41	0.69	0.47
8154178	NM_004972	Hs.656213	JAK2	Janus kinase 2 (a protein tyrosine kinase)	9p24	8.31	1.4	0.1	1.85
8078729	NM_002468	Hs.82116	MYD88	myeloid differentiation primary resp. gene (88)	3p22	9.82	1.39	0.67	0.51
8067221	NM_030776	Hs.302123	ZBP1	Z-DNA binding protein 1	20q13.31	6.76	1.38	0.35	0.34
8096808	NM_017918	Hs.234149	CCDC109B	coiled-coil domain containing 109B	4q25	8.81	1.37	0	2.37
8157362	NM_133374	Hs.349208	ZNF618	zinc finger protein 618	9q32	7.1	1.37	0.42	0.52
8092251	NM_021629	Hs.173030	GNB4	guanine nucleotide binding protein (G protein), beta polypeptide 4	3q26.32-q26.33	7.38	1.36	0.23	0.58
8072735	NM_145343	Hs.114309	APOL1	apolipoprotein L, 1	22q13.1	11.16	1.33	0.43	1.78
7947156	NM_145650	Hs.407152	MUC15	mucin 15, cell surface associated	11p14.3	8.09	1.33	0.22	-0.49
7926821	NM_032844	Hs.276905	MASTL	microtubule associated	10p12.1	7.94	1.33	0.33	-0.23
8055697	NM_198557	Hs.302442	RBM43	serine/threonine kinase-like					
8107044	NM_022350	Hs.591249	LRAP	RNA binding motif protein 43	2q23.3	6.47	1.3	0.42	0.22
				leukocyte-derived arginine aminopeptidase	5q15	7.64	1.29	0.55	1.5
8075720	NM_030882	Hs.474740	APOL2	apolipoprotein L, 2	22q12	9.03	1.29	0.24	1.97
8037794	NM_016457	Hs.466987	PRKD2	protein kinase D2	19q13.3	8.46	1.29	0.45	0.48
7929072	NM_012420	Hs.252839	IFIT5	interferon-induced protein with tetratricopeptide repeats 5	10q23.31	9.18	1.28	1	1.55
8157381	NM_133374	Hs.349208	ZNF618	zinc finger protein 618	9q32	6.1	1.28	0.44	0.47
7985777	NM_002201	Hs.459265	ISG20	interferon stimulated exonuclease gene 20kDa	15q26	7.94	1.27	0.63	0.85
7987772	NM_139265	Hs.143703	EHD4	EH-domain containing 4	15q11.1	9.95	1.27	0.28	0.04
8004510	NM_001251	Hs.647419	CD68	CD68 molecule	17p13	9.52	1.27	0.28	-0.13
8113358	NM_005668	Hs.308628	ST8SIA4	ST8 alpha-N-acetyl-neuraminidase	5q21	7.62	1.25	-0.1	2.88
8171449	NM_021804	Hs.178098	ACE2	alpha-2,8-sialyltransferase 4					
				angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	Xp22	7.77	1.25	0.24	0.26
8114010	NM_002198	Hs.436061	IRF1	interferon regulatory factor 1	5q31.1	10	1.25	0.18	3.45
7961120	NM_197947	Hs.143929	CLEC7A	C-type lectin domain family 7, member A	12p13.2	5.83	1.25	0.19	-0.31
8117321	NM_006355	Hs.584851	TRIM38	tripartite motif-containing 38	6p21.3	8.84	1.25	0.49	0.52
8101699	AK092450	---	---	---	---	4.65	1.23	0.58	-0.46
8117458	NM_007048	Hs.191510	BTN3A1	butyrophilin, subfamily 3, member A1	6p22.1	7.26	1.23	0.01	1.41
8015060	NM_019016	Hs.87383	KRT24	keratin 24	17q11.2	6.85	1.23	0.1	-0.39
7940028	NM_000062	Hs.384598	SERPING1	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)	11q12-q13.1	9.13	1.23	0.16	2.74
8014903	NM_001042471	Hs.306777	GSDML	gasdermin-like	17q12	7.78	1.22	0.09	0.91
8125483	NM_000544	Hs.502	TAP2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	6p21.3	9.29	1.22	0.31	1.7
7920531	NM_001111	Hs.12341	ADAR	adenosine deaminase, RNA-specific	1q21.1-2	10.36	1.22	0.77	0.85
8117476	NM_006994	Hs.167741	BTN3A3	butyrophilin, subfamily 3, member A3	6p21.3	7.48	1.22	0.18	1.35

8180034	NM_000544	Hs.502	TAP2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	6p21.3	9.32	1.21	0.32	1.69
8077103	NM_001953	Hs.592212	ECGF1	endothelial cell growth factor 1 (platelet-derived)	22q13	9.76	1.21	0.62	0.97
7914603	NM_153341	Hs.591504	RNF19B	ring finger protein 19B	1p35.1	10.08	1.2	0.35	1.94
8137250	NM_015660	Hs.647071	GIMAP2	GTPase, IMAP family member 2	7q36.1	5.08	1.2	0.22	1.19
8153609	NM_032789	Hs.348609	PARP10	poly(ADP-ribose) polymerase fam. member 10	8q24.3	8.2	1.2	0.64	0.68
7910674	---	---	---	---	---	5.08	1.2	1.38	1.33
8143279	NM_020119	Hs.133512	ZC3HAV1	zinc finger CCCH-type, antiviral 1	7q34	10.34	1.19	1.23	1.35
8178841	NM_018833	Hs.502	TAP2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	6p21.3	9.34	1.19	0.31	1.68
8038225	NM_020904	Hs.9469	PLEKHA4	pleckstrin homology domain contain. Family A (phosphoinositide binding specific) member 4	19q13.33	7.82	1.18	0.49	0.26
8035304	NM_004335	Hs.118110	BST2	bone marrow stromal cell antigen 2	19p13.2	8.88	1.17	0.67	0.62
7917503	NM_018284	Hs.656774	GBP3	guanylate binding protein 3	1p22.2	9.67	1.15	0.23	1.5
8048976	---	---	---	---	---	7.05	1.15	0.56	0.75
7907492	NM_014857	Hs.585378	RABGAP1L	RAB GTPase activating protein 1-like	1q24	7.54	1.14	0.62	0.24
8101429	NM_016619	Hs.546392	PLAC8	placenta-specific 8	4q21.22	8.25	1.14	-0.59	0.44
7975238	NM_020715	Hs.594236	PLEKHH1	pleckstrin homology domain containing, family H (with MyTH4 domain) member 1	14q24.1	9.09	1.13	0.63	0.28
7986789	NM_024490	Hs.659258	ATP10A	ATPase, Class V, type 10A	15q11.2	7.71	1.13	0.41	1.17
7937940	ENST00000380435	---	---	---	---	6.51	1.13	0.29	0.43
8093230	BC033615	Hs.478868	KIAA0226	KIAA0226	3q29	9.19	1.13	0.39	0.26
8087485	NM_003335	Hs.16695	UBE1L	ubiquitin-activating enzyme E1-like	3p21	8.87	1.12	0.37	0.39
8006531	NM_144975	Hs.14691	SLFN5	schlafen family member 5	17q12	11.47	1.12	0.5	0.67
8048926	ENST00000243810	---	---	---	---	8.09	1.12	0.29	0.91
8173848	ENST00000297977	---	HDX	chromosome X open reading frame 43 (CXorf43), mRNA	Xq21.1	6.07	1.12	0.6	0.23
8012841	---	---	---	---	---	4.36	1.11	1.99	1.84
8094240	NM_001775	Hs.479214	CD38	CD38 molecule	4p15	6.04	1.11	-0.02	0.1
8063369	NM_018683	Hs.144949	ZNF313	zinc finger protein 313	20q13.13	10.47	1.11	0.33	0.91
8127778	NM_017633	Hs.10784	FAM46A	family with sequence similarity 46, member A	6q14	9.03	1.1	0.43	0.25
8057959	NM_024989	Hs.229988	PGAP1	GPI deacylase	2q33.1	6.84	1.1	0.04	0.05
7983157	NM_024956	Hs.511175	TMEM62	transmembrane protein 62	15q15.2	8.93	1.09	0.3	0.22
8017262	NM_032043	Hs.532799	BRIP1	BRCA1 interacting protein C-term. helicase 1	17q22-q24	5.69	1.09	0.05	-0.17
8048749	NM_080671	Hs.591620	KCNE4	potassium voltage-gated channel, Isk-related family, member 4	2q36.3	6.15	1.09	0.42	0.82
7979757	NM_015346	Hs.98041	ZFYVE26	zinc finger, FYVE domain containing 26	14q24.1	8.27	1.08	0.2	0.29
8113491	NM_139164	Hs.93842	STARD4	StAR-related lipid transfer (START) domain containing 4	5q22.1	9.08	1.08	0.28	-0.04
8097903	NM_003264	Hs.519033	TLR2	toll-like receptor 2	4q32	7.58	1.07	0.03	0.49
8111739	NM_001465	Hs.370503	FYB	FYN binding protein (FYB-120/130)	5p13.1	7.14	1.07	0.1	0.89
8120967	NM_002526	Hs.153952	NT5E	5'-nucleotidase, ecto (CD73)	6q14-q21	7.56	1.07	0.52	0.32
8061073	---	---	---	---	---	7.16	1.07	0.27	0.2
8097080	NM_133477	Hs.655519	SYNPO2	synaptopodin 2	4q26	6.13	1.06	0.48	0.65
7996027	NM_002996	Hs.531668	CX3CL1	chemokine (C-X3-C motif) ligand 1	16q13	9.35	1.06	0.04	1.85
7904050	NM_020963	Hs.514941	MOV10	Mov10, Moloney leukemia virus 10, homolog	1p13.2	9.52	1.05	0.37	0.64
8063955	NM_007346	Hs.67896	OGFR	opioid growth factor receptor	20q13.3	10.11	1.05	0.49	0.89
7950641	NM_023930	---	KCTD14	potassium channel tetramerisation domain containing 14	11q14.1	8.05	1.05	-0.01	1.32
7907171	NM_003666	Hs.130746	BLZF1	basic leucine zipper nuclear factor 1 (JEM-1)	1q24	9.51	1.04	0.18	-0.06
8127107	---	---	---	---	---	4.71	1.04	0.86	1.05
8041542	NM_138801	Hs.435012	GALM	galactose mutarotase (aldose 1-epimerase)	2p22.1	6.89	1.04	0.04	-0.29
8014257	ENST00000361112	---	---	---	---	6.78	1.03	0.37	0.62
8000574	NM_001042483	Hs.513463	NUPR1	nuclear protein 1	16p11.2	11.97	1.02	-0.12	0.56
8011599	NM_016376	Hs.696087	ANKFY1	ankyrin repeat and FYVE domain containing 1	17p13.3	9.25	1.02	0.59	0.54
8091306	NM_020353	Hs.477869	PLSCR4	phospholipid scramblase 4	3q24	6.21	1.02	0.25	1.61
8094378	NM_018323	Hs.638037	PI4K2B	phosphatidylinositol 4-kinase type 2 beta	4p15.2	8.75	1.01	0.17	0.16
7957510	NM_001037671	Hs.326303	LOC338809	hypothetical protein LOC338809	12q22	6.38	1.01	0.15	0.06
7999423	NM_003745	Hs.50640	SOCS1	suppressor of cytokine signaling 1	16p13.13	7.85	1	0.33	1.88
7981290	NM_004184	Hs.497599	WARS	tryptophanyl-tRNA synthetase	14q32.31	11.57	0.99	-0.18	2.21
8129666	NM_145176	Hs.486508	SLC2A12	solute carrier family 2 (facilitated glucose transporter), member 12	6q23.2	6.02	0.96	0.43	1.33
8125500	NM_004159	Hs.180062	PSMB8	proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)	6p21.3	9.73	0.91	0.11	1.02
7897728	NM_018438	Hs.464419	FBXO6	F-box protein 6	1p36.23-p36.11	8.74	0.87	0.32	1.09
8066214	NM_004613	Hs.517033	TGM2	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	20q12	9.87	0.86	-0.09	1.56

7917528	NM_001085489	Hs.567809	HCG_2028557	hCG2028557	1p22.2	5.22	0.82	0.27	4.41
7917514	---	---	---	---	---	4.84	0.81	0.16	1.9
7958828	NM_006700	Hs.5148	TRAFD1	TRAF-type zinc finger domain containing 1	12q	9.57	0.8	0.03	1.03
7930577	NM_033339	Hs.9216	CASP7	caspase 7, apoptosis-related cysteine peptidase	10q25	10.18	0.79	0.16	1.07
8047403	NM_032977	Hs.5353	CASP10	caspase 10, apoptosis-related cysteine peptidase	2q33-q34	7.23	0.79	0.18	1.24
8123606	ENST00000380726	---	---	---	---	7.66	0.78	0.04	1.03
8018731	NM_024599	Hs.464157	RHBDF2	rhomboid 5 homolog 2 (Drosophila)	17q25.1	7.93	0.76	0.26	1.3
7979269	NM_000161	Hs.86724	GCH1	GTP cyclohydrolase 1 (dopa-responsive dystonia)	14q22.1-q22.2	8.75	0.75	-0.14	1.39
7902850	NM_198460	Hs.254338	GBP6	guanylate binding protein family, member 6	1p22.2	9.94	0.74	0.01	2.46
8117435	NM_007047	Hs.376046	BTN3A2	butyrophilin, subfamily 3, member A2	6p22.1	7.7	0.7	-0.01	1.23
8002133	NM_002801	Hs.9661	PSMB10	proteasome (prosome, macropain) subunit, beta type, 10	16q22.1	8.71	0.7	0.2	1.12
8118455	NM_001002029	Hs.534847	C4B	complement comp. 4B (Childo blood group)	6p21.3	7.51	0.69	0.11	1.2
8179399	NM_007293	Hs.655564	C4A	complement comp. 4A (Rodgers blood group)	6p21.3	7.51	0.69	0.11	1.2
8118409	ENST00000375177	---	C4A	complement comp. 4A (Rodgers blood group)	6p21.3	7.51	0.69	0.11	1.2
8150034	---	---	---	---	---	6.54	0.67	1.12	0.23
8074106	NM_138433	Hs.592211	KLHDC7B	kelch domain containing 7B	22q13.33	8.33	0.67	0.46	1.1
8151447	NM_000880	Hs.591873	IL7	interleukin 7	8q12-q13	5.88	0.66	0.21	1.03
7917548	NM_207398	Hs.591450	GBP7	guanylate binding protein 7	1p22.2	6.36	0.63	0.37	1.01
8090193	BC067235	---	---	---	---	7.95	0.62	0.18	1.01
7931899	NM_172200	Hs.524117	IL15RA	interleukin 15 receptor, alpha	10p15-p14	8.77	0.61	0.18	1.04
8040430	NM_003385	Hs.444212	VSNL1	visinin-like 1	2p24.3	8.31	0.6	-0.1	1.44
8175683	hsa-mir-224	---	---	---	---	6.45	0.6	-0.37	-1.14
8030002	NM_153608	Hs.511883	ZNF114	zinc finger protein 114	19q13.32	5.4	0.56	0.36	1.07
8013536	NM_000625	Hs.434386	NOS2A	nitric oxide synthase 2A (inducible)	17q11.2-q12	7.08	0.53	0.42	3.36
7919971	NM_000449	Hs.695975	RFX5	regulatory factor X, 5 (influences HLA class II expression)	1q21	8.84	0.51	-0.11	1.02
8014233	NM_152270	Hs.462829	SLFN11	schlafen family member 11	17q12	7.53	0.48	0.15	1.15
7967322	NM_006018	Hs.458425	GPR109B	G protein-coupled receptor 109B	12q24.31	7.73	0.48	-0.23	1.07
8047419	NM_001228	Hs.655983	CASP8	caspase 8, apoptosis-related cysteine peptidase	2q33-q34	7.85	0.47	-0.05	1.19
8091600	NM_014996	Hs.567423	PLCH1	phospholipase C, eta 1	3q25.31	6.36	0.47	-0.01	1.4
7903786	NM_000757	Hs.591402	CSF1	colony stimulating factor 1 (macrophage)	1p21-p13	8.21	0.44	0.08	1.01
8072108	NM_020437	Hs.567547	ASPHD2	aspartate β -hydroxylase domain containing 2	22q12.1	7.57	0.41	0.15	1.03
7981011	---	---	---	---	---	5.51	0.41	1.13	1.14
8040365	NM_021643	Hs.467751	TRIB2	tribbles homolog 2 (Drosophila)	2p25.1-p24.3	8.48	0.38	0.01	1.24
8037422	NM_001031749	Hs.44289	LYPD5	LY6/PLAUR domain containing 5	19q13.31	7.14	0.36	0.17	1.05
8026971	NM_006332	Hs.14623	IFI30	interferon, gamma-inducible protein 30	19p13.1	10.63	0.36	-0.18	1.28
8021470	NM_021127	Hs.96	PMAIP1	phorbol-12-myristate-13-acetate-induced prot. 1	18q21.32	8.54	0.29	-0.13	1.46
8121257	NM_001198	Hs.436023	PRDM1	PR domain containing 1, with ZNF domain	6q21-q22.1	8.11	0.28	-0.06	1.38
7991224	NM_178232	Hs.447530	HAPLN3	hyaluronan and proteoglycan link protein 3	15q26.1	7.82	0.28	0.01	2.26
7997712	NM_002163	Hs.137427	IRF8	interferon regulatory factor 8	16q24.1	6.18	0.27	0.06	2.26
7960744	NM_001733	Hs.524224	C1R	complement component 1, r subcomponent	12p13	8.76	0.22	-0.02	1.24
8101118	NM_002416	Hs.77367	CXCL9	chemokine (C-X-C motif) ligand 9	4q21	4.84	0.2	0.14	5.5
8105411	NM_139017	Hs.55378	IL31RA	interleukin 31 receptor A	5q11.2	5.82	0.19	0.05	1.69
8147206	NM_003821	Hs.103755	RIPK2	receptor-interacting serine-threonine kinase 2	8q21	8.27	0.19	-0.15	2.08
8006433	NM_002982	Hs.303649	CCL2	chemokine (C-C motif) ligand 2	17q11.2-12	5.38	0.18	-0.17	2.09
7993195	NM_000246	Hs.126714	CIITA	class II, major histocompatibility complex, transactivator	16p13	7.58	0.18	0.13	1.09
8060997	NM_018327	Hs.425023	SPTLC3	serine palmitoyltransferase, long chain base subunit 3	20p12.1	6.84	0.18	0.14	1.01
7931951	NM_001029880	Hs.407983	SFMBT2	Scm-like with four mbt domains 2	10p14	6.48	0.17	0.05	1.04
8082965	NM_012219	Hs.527021	MRAS	muscle RAS oncogene homolog	3q22.3	7.26	0.17	0.01	1.16
7998637	NM_016332	Hs.655346	SEPX1	selenoprotein X, 1	16p13.3	9.87	0.17	-0.13	1.88
8146863	NM_015170	Hs.409602	SULF1	sulfatase 1	8q13.2-3	6.46	0.16	-0.07	1.69
8018864	NM_003955	Hs.527973	SOCS3	suppressor of cytokine signaling 3	17q25.3	7.61	0.14	0.04	1.05
8099240	---	---	---	---	---	5.67	0.13	-1.24	0.25
8070584	NM_024022	Hs.208600	TMPRSS3	transmembrane protease, serine 3	21q22.3	6.61	0.13	0.07	1.46
8057578	NM_005795	Hs.470882	CALCR	calcitonin receptor-like	2q32.1	5.14	0.12	-0.1	1.01
8100393	NM_002253	Hs.479756	KDR	kinase insert domain receptor (a type III receptor tyrosine kinase)	4q11-q12	5.39	0.06	-0.15	1.12
8125530	NM_002118	Hs.654428	HLA-DMB	major histocompatibility complex, class II, DMB	6p21.3	8.62	0.05	-0.16	1.04
7996908	NM_006750	Hs.461117	SNTB2	syntrophin, beta 2 (dystrophin-	16q22-q23	8.84	0.04	0.02	1.08

7981273	AK123840	Hs.653294	C14ORF65	associated protein A1, 59kDa, basic component 2)					
8132406	NM_005402	Hs.6906	RALA	chromosome 14 open reading frame 65	14q32.31	8.23	0.03	-0.16	1.12
8023401	NM_025214	Hs.120790	CCDC68	v-ral simian leukemia viral oncogene homolog A (ras related)	7p15-p13	9.05	0.02	-0.2	1.02
7992828	NM_001012631	Hs.943	IL32	coiled-coil domain containing 68	18q21	6.29	0.01	-0.28	1.25
8146967	NM_031461	Hs.436542	CRISPLD1	interleukin 32	16p13.3	8.05	-0.04	-0.06	1.32
8102362	NM_052864	Hs.310640	TIFA	cysteine-rich secretory protein LCCL domain containing 1	8q21.11	5.94	-0.06	0.04	1.18
8158059	NM_003165	Hs.288229	STXBP1	TRAF-interacting protein with a forkhead-associated domain	4q25	7.33	-0.11	-0.08	1.1
7964834	NM_001005502	Hs.654387	CPM	syntaxin binding protein 1	9q34.1	8.46	-0.12	0.05	1
8056491	NM_002977	Hs.439145	SCN9A	carboxypeptidase M	12q14.3	8.75	-0.13	0.15	1.01
8025601	NM_000201	Hs.643447	ICAM1	sodium channel, voltage-gated, type IX, α sub.	2q24	6.19	-0.15	-0.09	1
8097692	NM_001957	Hs.183713	EDNRA	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	19p13.3-p13.2	9.36	-0.15	-0.37	3.02
8017867	NM_017565	Hs.268874	FAM20A	endothelin receptor type A	4q31.23	7.57	-0.16	-0.2	2.4
8083469	---	---	---	family with sequence similarity 20, member A	17q24.2	6.77	-0.16	0.11	1.6
8161444	---	---	---	---	---	5.52	-0.18	-1.03	-0.42
7934997	NM_005398	Hs.303090	PPP1R3C	protein phosphatase 1, regulatory (inhibitor) subunit 3C	10q23-q24	8.49	-0.27	-0.09	-1.03
8174226	---	---	---	---	---	4.74	-0.23	-1.09	-0.91
8019643	NM_003585	Hs.648240	DOC2B	double C2-like domains, beta	17p13.3	8.09	-0.31	-0.04	1.35
8107673	NM_023927	Hs.363558	GRAMD3	GRAM domain containing 3	5q23.2	8.89	-0.36	-0.34	1.24
8178295	NM_006398	Hs.44532	UBD	ubiquitin D	6p21.3	7.31	-0.38	-0.48	2.86
8124650	NM_006398	Hs.44532	UBD	ubiquitin D	6p21.3	7.28	-0.38	-0.44	2.87
8043697	NM_025190	Hs.532921	KIAA1641	KIAA1641	2q11.2	7.24	-0.44	-0.33	-1.02
8053801	AK123626	Hs.646318	LOC400986	protein immuno-reactive with anti-PTH polyclonal antibodies	2q11.2	8.24	-0.46	-0.25	-1.13
7950810	NM_206927	Hs.369520	SYTL2	synaptotagmin-like 2	11q14	6.76	-0.47	-0.34	-1.04
7981960	NR_003321	---	SNORD116-6	small nucleolar RNA, C/D box 116-6	15q11.2	5.61	-0.5	-1.06	-0.4
8103769	NM_000860	Hs.655491	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)	4q34-q35	7.93	-0.51	-0.1	-1.16
8112980	NM_005711	Hs.482730	EDIL3	EGF-like repeats and discoidin I-like domains 3	5q14	6.3	-0.59	-1.04	-0.36
8101952	NM_145244	Hs.480378	DDIT4L	DNA-damage-inducible transcript 4-like	4q23	7.94	-0.62	-0.07	-1.16
8089568	NM_138806	Hs.309158	CD200R1	CD200 receptor 1	3q13.2	6.42	-0.62	-0.45	-1.03
8103094	NM_000901	Hs.163924	NR3C2	nuclear receptor subfamily 3 group C member 2	4q31.1	8.52	-0.7	-0.4	-1.03
8084878	---	---	---	---	---	5.52	-0.71	-0.59	-1.26
8064098	---	---	---	---	---	5.09	-0.74	-0.41	-1.02
7981943	NR_001294	Hs.564847	SNORD64	small nuclear ribonucleoprotein polypeptide N // Prader-Willi/Angelman syndrome-5 // small nucleolar RNA, C/D box 64 // paternally expressed transcript PAR-SN	15q11-q13	4.16	-0.87	-0.39	-1.08
7909789	NM_003238	Hs.133379	TGFB2	transforming growth factor, beta 2	1q41	8.68	-0.89	-0.31	-1.39
7972239	NM_032229	Hs.525105	SLITRK6	SLIT and NTRK-like family, member 6	13q31.1	6.76	-0.95	-0.76	-1.59
7907271	NM_001460	Hs.144912	FMO2	flavin containing monooxygenase 2 (non-functional)	1q23-q25	6.54	-0.95	-0.44	-1.2
8106516	NM_152405	Hs.482605	JMY	junction-mediating and regulatory protein	5q14.1	7.49	-1	-0.53	-0.15
8065071	NM_198391	Hs.41296	FLRT3	fibronectin leucine rich transmembrane prot. 3	20p11	6.92	-1.01	-0.53	-1.47
8165667	NC_001807	---	---	---	---	9.46	-1.02	-0.45	0.07
7896752	NC_001807	---	---	---	---	9.46	-1.02	-0.45	0.07
8121749	NM_000165	Hs.74471	GJA1	gap junction protein, alpha 1, 43kDa	6q21-q23.2	8.8	-1.06	-0.51	-0.52
7911329	BC131690	---	---	---	---	6.92	-1.06	-0.42	-0.1
8113356	ENST00000388663	---	---	---	---	4.34	-1.07	-1.44	-0.6
8102800	NM_014331	Hs.390594	SLC7A11	solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	4q28-q32	8.92	-1.08	-0.9	-0.42
7967386	NM_022782	Hs.577404	MPHOSPH9	M-phase phosphoprotein 9	12q24.31	6.65	-1.09	-0.34	-0.56
7965359	NM_001001323	Hs.506276	ATP2B1	ATPase Ca++ transporting plasma membr. 1	12q21.3	8.66	-1.1	-0.38	-0.65
8176276	NM_138270	Hs.533526	ATRX	alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae)	Xq13.1-q21.1	6.98	-1.11	-0.53	-0.86
7970392	GENSCAN 00000064825	---	---	---	---	6.79	-1.14	-1.05	-0.37
8099612	NM_145290	Hs.99195	GPR125	G protein-coupled receptor 125	4p15.31	7.64	-1.16	-0.25	-0.64
7896750	NC_001807	---	---	---	---	4.81	-1.26	-1.26	-0.79
8100310	GENSCAN 00000033673	---	---	---	---	6.23	-1.37	-0.95	-0.07

7973900	NR_002937	Hs.639271	C14ORF19	chromosome frame 19	14	open	reading	14q13	4.65	-1.43	-0.3	-0.26
8165663	NC_001807	---	---	---				---	5.12	-1.52	-1.35	-0.8
8165672	NC_001807	---	---	---				---	6.94	-1.73	-1.05	-0.02

Supplementary file 1

Materials and Methods

Subjects and samples

The study included 45 children aged 6 to 17 years old. Thirty-three patients were followed for an allergic rhinitis to dust mite. Fourteen of them were also asthmatic, with controlled asthma (group “a”, n = 7) or uncontrolled asthma (group "A", n = 7). Nineteen had rhinitis without asthma (group “R”, n=19). The control group (group "C") comprised 12 children. This project received the approval of the Nice University Hospital Ethics Committee and all volunteers' parents gave written, informed consent.

The diagnosis of dust-mite-allergic rhinitis was based on clinical criteria (ARIA: rhinorrhea, nasal obstruction, nasal itching and sneezing) and positive prick-tests with *Dermatophagoïdes pteronyssinus* and *Dermatophagoïdes farinae*. Prick-tests used 9% phosphate codeine and 10 mg/mL histamine as positive controls and 50% glycerol solution as a negative control. The test was positive when the diameter of the papule was greater than 3 mm or greater than 50% of the largest papule observed with the 2 positive controls. All allergic children presented a perennial rhinitis with mild or moderate-severe form. Standard spirometry was performed on all patients. None were being treated with nasal local glucocorticosteroids for at least 1 month at time of recruitment.

Asthma was defined by the following clinical criteria (NAEPP/Expert Panel Report 3 (EPR3) 2007): recurring episodes of wheezing, dyspnea, cough, and obstructive syndrome reversible under beta2-mimetic. This last criterion was detected by spirometry.

Asthma control classification

Asthma severity and control were assessed following NAEPP/EPR3 criteria. We only considered two statuses: “well-controlled class” (controlled) and “not-well-controlled or poorly-controlled class” (uncontrolled). Long-term inhaled treatments (glucocorticosteroids +/- long-acting β 2-agonists) of asthmatic subjects were not stopped.

Subjects, aged 6-11 years of age, were defined with (1) controlled asthma if all the following points were present during the preceding three months: no more than two days/wk with trouble breathing, no more than one night-time awakening, no interference with normal activity, use of short-acting beta agonist ≤ 2 days/wk, FEV1 $>80\%$ predicted, FEV1/FVC $>80\%$ and no more than one exacerbation requiring oral systemic corticosteroids during the last year, (2) uncontrolled asthma if one or more of these features was absent.

Subjects, aged ≥ 12 years of age, were defined with (1) controlled asthma if all the following points were present during the preceding three months: no more than two days/wk with trouble breathing, no more than two night-time awakenings, no interference with normal activity, use of short-acting beta agonist ≤ 2 days/wk, FEV1 $>80\%$ predicted, ACT ≥ 20 , and no more than one exacerbation requiring oral systemic corticosteroids during the last year, (2) uncontrolled asthma if one or more of these features was absent.

Control subject selection was based on the absence of any personal or family history of atopy, rhinitis or asthma. We relied on the clinical definition of asthma and no spirometry measurements were performed in the healthy donor group.

Before inclusion, all subjects underwent a clinical examination. Subjects with symptoms of infectious rhinitis or bronchitis in the previous 15 days were excluded.

Nasal respiratory epithelium sampling was performed by brushing the inferior turbinates and the adjacent nasal wall using cytological brushes (Ventimed, France). Brushings were recovered in 5 ml culture medium Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Carlsbad, CA 92008, United States). For anatomo-pathological analysis, 200 μ l of the sample

were centrifuged on cytospin in order to perform cell counting and identification according to morphological criteria, after staining by the Papanicolaou stain method. The cell pellet was immediately lysed with 100 µl Absolutely RNA® Microprep Kit lysis buffer (Stratagene, La Jolla, CA 92037, United States), mixed with 0.7 µl β-mercaptoethanol 14.2 M and stored at -70°C.

Viral respiratory tract infection detection by Real-time quantitative PCR (qPCR)

The RealAccurate™ Respiratory Kit (PathoFinder, 6229 EV Maastricht, The Netherlands) was used according to the manufacturer's instructions, for each subject, to detect the possibility of viral respiratory tract infection. The kit contained optimized sets of primers and probes for the detection of 13 respiratory viruses (influenza types A and B, respiratory syncytial virus (RSV) types A and B, parainfluenza (PIV) types 1,2,3,4, coronavirus types 229E and OC43, rhinovirus, human metapneumovirus, adenovirus). All probes were 5'-labeled with 6-carboxyfluorescein (FAM) and 3'-labeled with tetramethylrhodamine (TAMRA), used as quencher. The amplicons were detected by measuring the FAM fluorescence. The RT-PCR reaction was performed with 10 ng total subject RNA isolated as described above. A positive RNA control, an internal control and a negative control (dilution buffer) were included in each test performed. Using the LightCycler 480 Real-Time PCR System (Roche, Indianapolis, IN 46250, United States), the following real-time cycler conditions were used: 30 min at 50°C for reverse transcription, 15 min at 97°C for Taq DNA polymerase activation and inactivation of the reverse transcriptase, then 42 cycles ((a) denaturation at 94°C for 15 s, (b) annealing/extension at 55°C for 60 s). A strong positive sample was characterized by a Ct (cycle threshold) value of < 30 (between 25 - 30), a positive sample with a value of 30 < Ct < 35, whereas a value of 35 < Ct < 40 indicated a sample showing low levels of expression.

Seven children (1 asthmatic, 5 rhinitis without asthma, 1 control) were positive for respiratory viruses. Five were positive for rhinovirus and 2 for parainfluenza virus type 1 (PIV1). These children were not included in subsequent analyses. Characteristics of the patients and samples (sex, age, ARIA score, asthma control, treatment and respiratory parameters, percentage of epithelial cells) are summarized in Table E1. These values do not significantly differ among experimental groups except for respiratory parameters of patients with uncontrolled asthma compared with the other groups (Table 1).

Isolation and culture of primary human nasal epithelial cells (HNECs)

Inferior turbinates were collected after surgical turbinectomy. Cystic fibrosis, asthmatic or allergic patients were excluded from the study. All procedures were approved by the Nice University Hospital Ethics Committee. The primary cultures of human epithelial cells derived from nasal mucosa of inferior turbinates were performed as described elsewhere (1). Briefly, after excision, the tissue was immediately immersed in HBSS (Ca²⁺/Mg²⁺-free; Invitrogen)-HEPES (25 mM, Gibco product, Invitrogen) culture medium containing 100 U/ml penicillin (Gibco, Invitrogen), 100 mg/ml streptomycin (Gibco, Invitrogen), 100 mg/ml gentamicin sulfate (Gibco, Invitrogen), and 2.5 mg/ml amphotericin B (Gibco, Invitrogen). Tissue was then digested with 0.1% Pronase (Sigma-Aldrich, St. Louis, MO 63103, USA) at 4°C overnight. Cells were gently removed from the digestion medium and HNECs were detached from the stroma by gentle agitation with a medium containing 10% fetal calf serum (FCS). The cell suspension was centrifuged (150 g, 10 min, 4°C) and the pellet was re-suspended in 10% FCS-HBSS-HEPES medium. After a second centrifugation, the second cell pellet was then suspended in the 10% FCS-Dulbecco's modified Eagle's (DMEM) medium (Invitrogen). Cells were gently dissociated mechanically by several passages through a 0.8 mm diameter needle using a 10 ml syringe. The cells were counted and then plated at a density of 3.10^4

cells/cm² on type IV collagen (Sigma-Aldrich)-coated Transwell permeable supports (6.5 or 12 mm diameter; 0.4 mm pore size; Sigma-Aldrich). They were incubated overnight in a humidified atmosphere of 5% CO₂ at 37°C. The next day, the culture medium was replaced by a proliferating Bronchial Epithelial Growth Medium (BEGM), reconstituted from Bronchial Epithelial Basal Medium (BEBM, Lonza, Basel, Switzerland), and supplemented with insulin, apo-transferrin, epidermal growth factor, epinephrine, hydroxycortisone, 3,30,5-triiodothyronine, endothelial cell growth supplement, retinoic acid (low concentration ~10 nM), amphotericin B (2.5 mg/ml), streptomycin (100 mg/ml), penicillin (100 U/ml), gentamicin sulfate (50 mg/ml) and L-glutamine (2 mM), according to the manufacturer's recommendation. Confluent cell monolayers were obtained after 7 days. Apical medium was then removed so that cells were cultured at an air-liquid interface (ALI). Basolateral medium was replaced by a differentiating medium, corresponding to an equal mix of BEBM and DMEM high glucose (Invitrogen). The differentiating medium was supplemented with the same growth factors as above except for retinoic acid which was added at a final concentration of 300 nM, in order to induce differentiation. Differentiation was assessed during 21 days of culture at an air-liquid interface (ALI). This protocol allows the recapitulation of the different steps of the regeneration, including proliferation (ALI D0), polarization (ALI D7) and differentiation (early=ALI D14, full=ALI D21). Cells were cultured until ALI D 25-28 before treatment with cytokines. All cultures used in this study had a transepithelial resistance greater than 500 Ω/cm². Each experiment was replicated using samples from three different donors. Tested agents: IL-4 (BD Pharmingen, San Diego, CA 92121, United States), IL-13 (PeproTech, Rocky Hill, NJ 08553, United States), IFN-αA/D (Sigma-Aldrich), IFN-β1a (Sigma-Aldrich), were added on the basolateral side at each time and concentration indicated in the text.

Microarray analysis

RNA extraction

Total RNA was isolated from nasal brushings using the Absolutely RNA® Microprep Kit (including the optional RNase-free DNase I digestion of contaminating DNA), and from primary HNECs using Trizol (Invitrogen), RNEasy kit (Qiagen, France) and RQ1 RNase-free DNase (Promega), Madison, WI 53711, United States), according to the manufacturers' instructions. The amount of RNA was estimated by spectrophotometry with a Nanodrop ND-1000 UV spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). RNA quality was verified by electrophoresis on a Bioanalyser 2100 (Agilent Technologies, Santa Clara, CA 95051, USA) with RNA Pico LabChip® (Agilent Technologies, Santa Clara, CA 95051, USA).

GeneChip Human Gene 1.0 ST Array

cDNA was generated from 300 ng total RNA using the GeneChip WT cDNA Synthesis and Amplification Kit (Affymetrix, Santa Clara, CA 95051, USA), according to the manufacturer's instructions. cDNA was fragmented and end-labeled using the GeneChip WT Terminal Labeling Kit (Affymetrix). Labeled DNA targets were hybridized to the Affymetrix GeneChip Human Gene 1.0 ST Array at 45°C for 17 h, according to the manufacturer's recommendation. At the end of the hybridization, microarrays were washed and stained on a GeneChip Fluidics Station 450 and scanned on a GeneChip Scanner 3000 7G (Affymetrix). Gene-expression levels were summarized using the Gene Level-RMA sketch method available in the Expression Console software (Affymetrix). Normalized data were analyzed using R with packages from Bioconductor (Biobase, limma, randomForest, bioDist).

Data storage

Microarray data are archived in GEO under reference GSE19190 and GSE22147. Experimental design, Tiff images and files resulting from the quantification stage were

initially stored in the MEDIANTE database developed by the laboratory and are available at: <http://www.microarray.fr:8080/merge/index> (2, 3).

Bioanalysis programs and statistical analyses

Most calculations were performed using limma and Random Forest, two packages of the R statistical program (4). The sizepower package was used to evaluate *a priori* the minimal size of our groups. With a $\log_2$ fold change superior to 1 and a false discovery rate of 5%, groups of 6-7 patients appeared sufficient to reach a power of 90%. Differentially-expressed genes in each comparison group were identified using linear modeling associated with Benjamini-Hochberg correction of p-value for multiple tests. For each gene, we define an A-value, which is the average $\log_2$ expression level for each gene across all microarrays, and M-values, which are the $\log_2$ fold-change for the comparisons that are considered. Prediction analysis was performed using the Random Forest (RF) approach developed by Breiman (5), according to the specifications made by Diaz-Uriarte (5, 6). This classification algorithm has comparable performance to other robust classification methods such as SVM in multi-class problems with group sizes similar to the one in the present study. Moreover, the gene selection procedure yields very small sets of genes while preserving predictive accuracy (6). Independent cross-validations were performed in order to control the robustness of the classifiers. In order to limit over-fitting, a partition of the datasets in two independent sets (i.e. training and test) was carried out. Predictions were based on the data obtained for the test groups only, and did not take into account the results obtained with the training groups. A 10-fold partition was performed in the Allergic versus Healthy comparison, including 38 patients. The training set varied from 34 to 35 subjects and the test set from 3 to 4 subjects. A 5-fold cross partition was performed in the uncontrolled versus controlled asthmatics comparison, including 13 patients. The training set varied from 10 to 11 subjects and the test set from 2 to 3 subjects in this latter case. The number of feature sets was reduced in the training group with limma before running

the Random Forest in order to perform the random forest on the most relevant probes. We performed 20 independent cross-validations. Every cross-validation was associated with a set of genes, which corresponded to the most influential variables contributing to determine the class of the sample. A gene set was defined as the group of genes that belonged to all twenty cross-validations. Prediction accuracy was based on the averaged results obtained at the end of the 20 cross validations. Random Forest classified each patient into one of the following categories: false positive (FP), true positive (TP), false negative (FN) and true negative (TN). The prediction accuracy (ACC) for each cross validation is given by:

$$ACC = \frac{TP + TN}{TP + TN + FP + FN} \times 100\%$$

We presented in the text the prediction accuracy based on the averaged results obtained at the end of 20 independent cross validations for the patients belonging to the “test” groups.

So the numerator was replaced by: $\sum(\%TP + \%TN)$ for each patient among the 20 cross validations.

Ontologies attached to each gene were then used to classify relevant genes according to main biological themes. We used for this the Functional Annotation Clustering report available from DAVID on <http://david.abcc.ncifcrf.gov/home.jsp> (7). The underlying grouping algorithm is based on the hypothesis that similar annotations should have similar gene members. It uses Kappa statistics to measure the relationships. Hierarchical clusterings were performed using the TIGR MultiExperiment Viewer (MeV) program, version 4.3 available at <http://www.tm4.org/mev.html> (Manhattan distance metric and average linkage) (8) or using R statistical environment.

Biological networks were built using Ingenuity Pathway Analysis software (<http://www.ingenuity.com/>). We presented the networks with the highest “network score” in the comparison. The network score was calculated with a right-tailed Fisher’s exact test. Gene Set Enrichment Analysis (GSEA) was used to determine whether an *a-priori* defined set of

genes can characterize differences between two biological states (e.g. phenotypes) <http://www.broad.mit.edu/gsea/index.jsp> (9, 10). Four key values were generated at the end of the gene set enrichment analysis: (1) the enrichment score (ES) reflected the extent of the over-representation of a gene set at the top or the bottom of a ranked list of genes; (2) the normalized enrichment score (NES) took into account differences in gene set size and in correlations between gene sets and the expression data set; (3) the false discovery rate (FDR) was the estimated probability that a gene set with a given NES represented a false-positive finding; (4) the nominal p-value estimated the statistical significance of the enrichment score for a single gene set.

ENCODE functional genomics data were used to determine potential binding sites for interferon regulatory factors. Genomic regions and Encyclopedia of DNA Elements (ENCODE) functional genomics data were retrieved and displayed using the UCSC Genome Browser on Human March 2006 (NCBI36/hg18) Assembly (<http://genome.ucsc.edu/>) (11, 12). H3K4Me1 histone marks (monomethylated histone H3 at lysine 4) are associated with enhancer and promoter regions. ENCODE information about histones was derived from 8 cell lines. Histone H3K4Me3 (trimethylated histone H3 at lysine 4) binding was associated with promoter regions. ENCODE Histone Mark H3K4Me3 was derived from 9 cell lines. Hypersensitivity to DNase I treatment is associated with accessible chromatin regions. DNase I hypersensitivity clusters are represented as gray boxes where the darker values represent the highest sensitivity. ENCODE Transcription Factor ChIP-seq (chromatin immunoprecipitation followed by deep-sequencing) data include IRF4 binding site experimental analysis on the GM12878 cell line. IRF4 binding sites are represented as gray boxes where the darker values represent the highest binding signal. Predicted IRF sites were retrieved from HMR conserved transcription factor binding sites. Mammalian conservation within 28 species is shown in grayscale where darker values indicate the highest conservation

according to phastCons score (PHYlogenetic Analysis with Space/Time Conservation), a phylo-HMM-based method for detecting conserved regions in multiple sequence alignments.

Real-time quantitative PCR (qPCR)

First-strand cDNA was synthesized from 500 ng total RNA using 1 μ L ImProm-II™ Reverse Transcriptase (Promega) in a final volume of 20 μ l with 500 ng oligo-dT (Proligo, Sigma-Aldrich). Quantitative PCR was carried out in 96-well plates using the LightCycler 480 Real-Time PCR System (Roche Applied Science, Indianapolis, IN46250, USA) with the qPCR Master Mix Plus for SYBRs Green I (Roche). All reactions were performed in a total volume of 20 μ l and contained 5 ng reverse-transcribed RNA (based on the initial RNA concentration) and 500 nM of each primer set. The primer sets were designed using the Primer Express software from Applied Biosystems (Carlsbad, California 92008, United States) for the following human transcripts: CST1 (NM_001898), POSTN (NM_006475), NTS (NM_006183), CD44 (NM_000610), GSN (NM_000177), ALOX15 (NM_001140), GSDML (NM_001042471), MX2 (NM_002463), IFIT2 (NM_001547), DUOX2 (NM_014080), CXCL10 (NM_001565), GSDML (NM_001042471), ST8SIA4 (NM_005668), MX1 (NM_002462) and housekeeping genes GAPDH (NM_002046), ACTB (NM_001101), RPL13A (NM_012423), TTC1 (NM_003314) (Supplementary Table 1). Primer sets were usually designed to span an intron in order to avoid amplification from potential traces of genomic DNA in the total RNA preparations, except for NTS, MX2, IFIT2, DUOX2 and GAPDH. Additional negative controls corresponded to the same samples reverse transcribed in the absence of reverse transcriptase. The efficiency and specificity of each primer set were validated using serial dilutions. Thermal cycling was performed at 95°C for 5 min, followed by 40 cycles comprising each a denaturation step at 95°C for 10 s and an annealing/extension step at 60°C for 10 s, then, finally, 72°C for 10 s. Amplification of the

appropriate product was verified by analyzing the dissociation curve that was obtained after PCR with the following steps: 5 s at 95°C, 1 min at 65°C, and then a slow ramp from 65°C to 97°C. The purity of the amplified product was determined as a single peak of dissociation curve. The SYBR Green dye was measured at 530 nm during the extension phase. The threshold cycle (Ct) value was determined as the cycle number at which the fluorescence generated within a reaction crosses a given threshold. The relative mRNA amount in each sample was calculated from the Ct compared with the Ct of the two most stable housekeeping genes. These latter were chosen by the software geNorm from a set of four tested candidate reference genes (GAPDH, ACTB, RPL13A, TTC1) (13). A gene expression normalization factor was calculated for each sample based on the geometric mean of the selected reference genes. Each sample was measured in duplicate and the mean value was calculated. Results were calculated with the delta-Ct method, relating the Ct value of our genes (either target or reference) to a control/calibrator (i.e. the sample with the highest expression, characterized by the lowest Ct value):

$$\frac{Efficiency^{(lowest\ Ct\ value - gene\ Ct\ value)}}{Normalization\ factor}$$

For detecting IFN- α , IFN- β , IFN- λ 1 and IFN- λ 2/3 transcripts, we used Taqman® probes, primers (Integrated DNA Technologies, Leuven, Belgium) previously described elsewhere (14). Briefly, quantitative PCR was carried out in 96-well plates using the LightCycler 480 Real-Time PCR System (Roche Applied Science) with the Quantitect Probe One-step RT-PCR kit (Qiagen). All reactions were performed in a total volume of 20 μ l and contained 100 ng total RNA, 200 nM Taqman® probe and 1 μ M of each primer set. The primer and probe sets corresponded to those used by Khaitov and col.: IFN- α 1, IFN- α 2, IFN- β , IFN- λ 1 and IFN- λ 2/3 (14). We used as a housekeeping gene, GAPDH (NM_002046), designed using Integrated DNA Technologies online-available software. Real-time cycler conditions consisted of an initial reverse transcription step of 30

min at 50°C, then a two-step cycling was performed at 95°C for 15 min, followed by 40 cycles comprising each a denaturation step at 94°C for 15 s, and an annealing/extension step at 60°C for 60 s. Results were normalized with respect to GAPDH:

$$2^{-(\text{GAPDH Ct value} - \text{gene Ct value})}$$

For MUC5AC and FOXJ1 transcripts, real-time PCR was performed using Taqman gene expression Assay (Applied Biosystems) following manufacturer's instructions.

Immunohistochemistry

Four-micrometer sections from formalin-fixed and paraffin-embedded normal human bronchial, nasal and sinus mucosa were mounted on glass slides (SuperFrost Plus; Menzel, Braunschweig, Germany), then air-dried overnight at 57°C. After rehydration, antigens were retrieved with the CC1 protocol for the anti-GSDML antibody, and with the CC2 protocol for anti-Duox1/ThOx1, as developed by Ventana (Ventana Medical Systems, Roche Group, Inc., Tucson, AR). Intrinsic peroxidase activity was blocked by 3% hydrogen peroxide for 20 min. Non-specific antibody binding was blocked with normal goat serum (5%; Sigma). The anti-GSDML rabbit polyclonal antibody (Sigma-Aldrich, St. Louis, MO) was used at a 1:200 dilution. Anti-Duox1/Duox2 rabbit polyclonal antibody (kindly provided by Professor Françoise Miot, Université Libre de Bruxelles, Belgium) was used at a 1:600 dilution (15). Staining procedures were performed on a Benchmark immunohistochemistry staining system (Benchmark XT, Ventana Medical Systems). The primary antibodies were incubated for 32 min. Slides were washed in TBST (50 mM Tris pH 7.6, 300 mM NaCl, 0.1% Tween 20), before incubation with labeled polymer-HRP anti-rabbit or anti-mouse secondary antibody for 30 min at room temperature. For visualization, the EnVision FLEX kit (Dako) was used according to the manufacturer's instructions.

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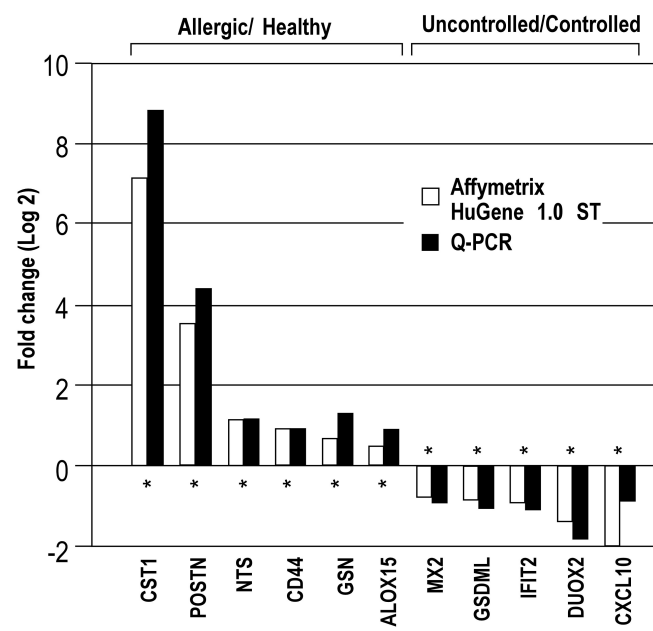


FIGURE E1

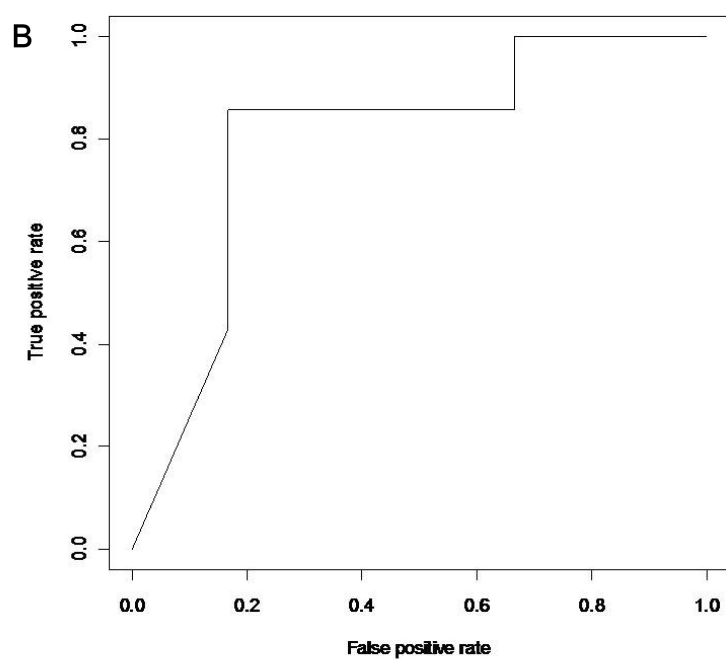
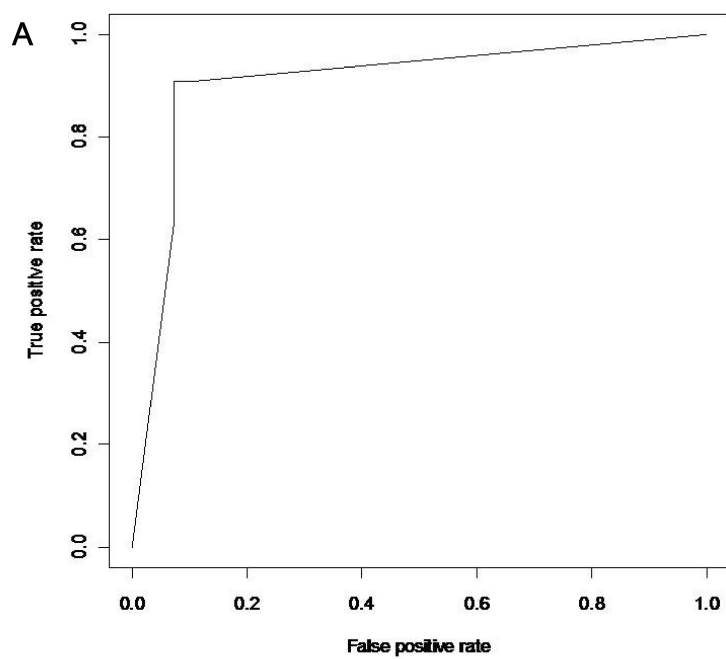


FIGURE E2

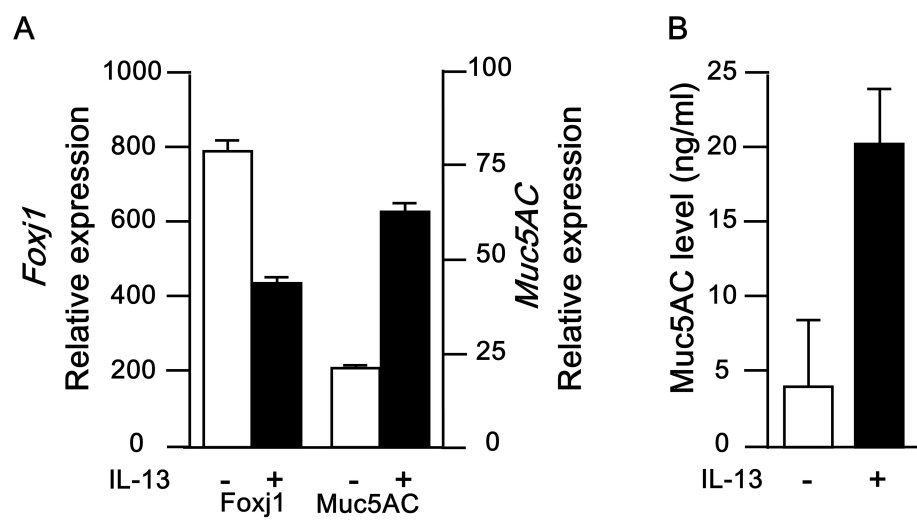


FIGURE E3

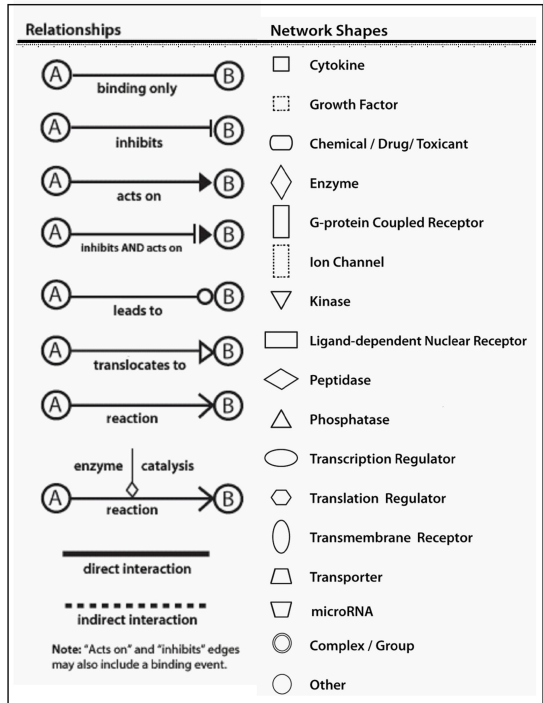


FIGURE E4

GSDML

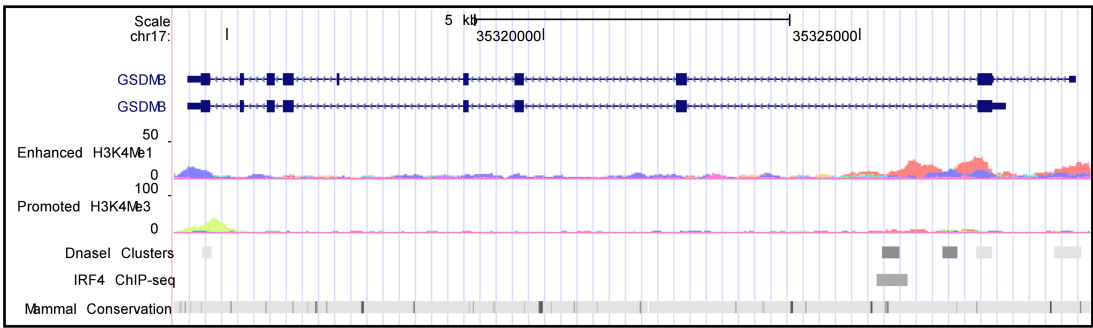


FIGURE E5