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Early View

Original article

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The "one airway, one disease" concept in light of Th2 inflammation

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Asthma, allergic rhinitis, nasal epithelium, bronchial epithelium, Th2 inflammation, transcriptome, RNAseq

Take home message: Nasal epithelial cells are not good surrogates of bronchial cells to evaluate Th2 inflammation

Abstract

In line with the pathophysiological continuum described between nose and bronchus in allergic respiratory diseases, we assessed whether nasal epithelium could mirror the Th2 status of bronchial epithelium.

Nasal and bronchial cells were collected by brushings from patients with allergic rhinitis and asthma (AR, n=12), isolated allergic rhinitis (R, n=14) and healthy controls (C, n=13). Cellular composition was assessed by flow cytometry. Gene expression was analyzed by RNA sequencing. Th2, Th17 and interferon signatures were derived from the literature.

Infiltration by polymorphonuclear neutrophils in nose excluded 30% of the initial cohort. All bronchial samples from AR group were Th2-high. Nasal samples gene expression profile from the AR group correctly predicted the paired bronchial sample Th2 status in 71% of cases. Nevertheless, nasal cells did not appear as a reliable surrogate of the Th2 response, in particular due to a more robust influence of the interferon response in 14/26 nasal samples. Th2 scores correlated with mast cells counts ($p<0.001$) and numbers of sensitizations ($p=0.006$ and 0.002), while Th17 scores correlated with PMN counts ($p<0.014$).

The large variability in nasal cell composition and type of inflammation restricts its use as a surrogate for assessing bronchial Th2 inflammation in AR patients.

Introduction

A pathophysiological continuum has been described between the nose and bronchus in allergic respiratory diseases[1, 2]. Most patients with allergic asthma also have allergic rhinitis, while twenty to fifty percent of patients with allergic rhinitis have asthma[3]. Rhinitis has been demonstrated to be an independent risk factor for the development of asthma in atopic (odds ratio, 8.1) and non-atopic (odds ratio, 11.6) patients[4, 5]. Consequently, nasal cells have been suggested as potential surrogates for bronchial cells in the study of allergic respiratory diseases[6]. Indeed, nasal cells can be collected easily and repeatedly, including in children, through a noninvasive sampling[7].

Th2 inflammation is a major component of the epithelial response in allergic respiratory disease in adults[8] and children[9] and has been used to predict response to anti-Th2 biotherapies in asthmatic patients[10, 11]. These biotherapies are emerging as relevant opportunities for severe asthma, with promising results from phase IIb[12] and III[13, 14] trials. Th17[15] and interferon (IFN)-driven[16] epithelial responses represent more recently elucidated asthma pathways which seem distinct and mutually exclusive of Th2 response in cross-sectional studies. Studies on biotherapies targeting these pathways are, on the other hand, still not convincing.

The definition of Th2-high and Th2-low status in asthma, as derived from initial bronchial transcriptomics studies[17] remains a challenge in clinical studies. Being able to predict Th2 bronchial status in asthma by gentle and innocuous nasal brushings could represent an interesting opportunity at the era of Th2 biotherapies development.

No study has yet compared the extent of Th2 inflammation in the same individual at the levels of nasal and bronchial epithelia. This led us to analyze whether Th2 inflammation was of similar amplitude in nose and bronchi in a context of allergic diseases and whether nasal Th2 inflammation was a good surrogate for bronchial Th2 inflammation in asthma. As Th17 and

IFN signatures have also been described in asthma, we investigated whether these pathways were activated in our patients' samples and also their interplay with Th2 responses.

Material and Methods

Subjects and samples

We collected nasal and bronchial brushings from 39 subjects: 12 had both allergic rhinitis and asthma (Group AR), 14 had allergic rhinitis only (Group R), and 13 were healthy controls (Group C).

Allergic rhinitis was defined according to 2010 ARIA (*Allergic Rhinitis and its Impact on Asthma*) recommendations[18]. Asthma was defined according to 2012 GINA (Global Initiative for Asthma)[19] and 2007 EPR3 (Expert Panel Report)[20] recommendations.

The project received the approval of the CPP Sud Méditerranée V Ethics Committee (ref 13.032) on July 23, 2013 and all volunteers gave their written informed consent.

Flow cytometry analysis

Seventy-eight samples were analyzed by flow cytometry. Brushed cells were first incubated in a blocking solution containing 10% normal mouse serum and 50 µg/ml human IgG (Sigma) and then stained. The following monoclonal antibodies were purchased from BD Biosciences (Le Pont de Claix, France): anti-EPCAM (FITC), anti-CD45 (V510), anti-CD20 (PERCPCY55), anti-CD3 (APCH7), anti-CD16 (A700), anti-CD117 (PECY7); and from Biolegend (San Diego, California): anti-SIGLEC-8 (APC).

Cells were fixed with Cytofix/Cytoperm reagent from BD Biosciences then analyzed on a BD LSRII Fortessa. Data were acquired with BD FACSDiva 6.1 software and processed using Kaluza software (Beckman Coulter). Doublet cells were excluded from the analysis.

Gene expression analysis

Gene expression analyses were conducted on 52 paired samples obtained from the 26 patients whose nasal and bronchial samples fulfilled our criteria in terms of epithelial cellularity **(Figure E1)**. Libraries were generated from 500 ng total RNA using the Truseq Stranded Total RNA ribozero gold kit (Illumina). Libraries were quantified with the KAPA library quantification kit (Kapa biosystems) and sequenced on a NextSeq 500 platform (Illumina) with 2×75 bp paired-end chemistry. RNA sequencing data were initially archived in Mediante, a database developed by our laboratory[21], then uploaded to the Gene Expression Omnibus (GEO) under reference GSE101720.

Th2 signature construction

A specific Th2 signature was compiled from four independent datasets analyzing the response to IL-13 in human airway epithelia: (1) and (2), human nasal epithelial cells incubated with 10 ng/mL and 100 ng/mL IL-13 for 1 and 3 day(s), respectively (Giovannini-Chami et al., GSE19190[9]; and GSE110799); (3) human tracheal epithelial cells incubated with 50 ng/ml IL-13 for 2 days (submerged conditions), then for 21 days (air-liquid interface conditions) (Alevy et al, 2012 ; GSE37693[22]); (4) human bronchial epithelial cells incubated with 25 ng/ml IL-13 for 2 days (Zhen et al, 2007; GSE4804[23]). Differentially expressed genes were defined by a positive B statistic in all analyses, combined with a log2 fold change between IL-13 and control at least equal to 2 for GSE19190 and GSE4804, and 4 for the other two datasets[24]. Datasets were analyzed with the GEO2R function and custom R scripts.

Th17 and IFN signature

Th17+TNF alpha and IFN alpha signature were defined by Choy[15] and Bhakta et al.[16] in air-liquid interface (ALI) cultures of normal human bronchial epithelium (NHBE). Bronchial

IFN alpha signature was entirely overlapping with our previous IFN alpha, beta, gamma signature defined in primary cultures of nasal cells (NHNE) at ALI and suitable for analysis in nasal epithelium[9].

Metagenomics analysis of viral transcriptome

De novo transcriptome assembly was independently performed for each sample with 100,000 R1/R2 paired-end reads not mapping the human genome, using Trinity software with default parameters. We used the Trinity script *align_and_estimate_abundance.pl* to estimate the abundance of each reconstructed contigs and extract the first 20 most expressed for annotation using Blast alignment tool against a blast database gathering 9,532 sequences belonging to one of the 7,474 viral complete genomes available at NCBI (https://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?taxid=10239).

Biostatistics

All statistical analyses outside of bioinformatics programs were performed using the R statistical package. Statistical significance was assessed using nonparametric tests (Wilcoxon rank sum test or Kruskal-Wallis rank sum test). Associations between continuous variables were measured using Spearman Rho. These tests were two sided and a nominal p-value of 0.05 was considered as statistically significant.

Results

Subjects characteristics

Characteristics of the 39 subjects are detailed in **Table E1**. Flow cytometry analyses showed that 13 nasal samples (5 AR, 4 R, and 4 C) were composed of less than 80% epithelial cells, preventing comparison with bronchial samples **(Table E2, Figure 1A)**. These 13 samples and

their paired bronchial samples were removed from further analysis, so that roughly equivalent levels of epithelial cells were observed in the remaining nasal and bronchial samples in each group **(Figure 1B).** These thirteen patients had similar clinical and functional characteristics compared to the 26 remaining subjects, excepted nasal samples cellularity **(Table E3)**. **Table 1** shows the detailed characteristics of the 26 remaining subjects. Three patients were taking medications. One was taking L-thyroxine for hypothyroidism. Two required either antihistamines and inhaled salbutamol (1 patient) or only inhaled salbutamol (1 patient) as reliever treatment. There were more inflammatory cells (mast cells, eosinophils and polymorphonuclear neutrophils (PMN)) as well as more T-cells in nasal samples from patients belonging to groups AR and R than from controls and more mast cells in bronchial samples from patients belonging to the groups AR and R than from controls.

Table 2 shows the composition of nasal and bronchial cells in the three clinical groups (AR, R, C). There was a higher percentage of mast cells in the bronchi than in the nose in the AR and R groups. There was also a higher percentage of eosinophils and PMN in the nose than in the bronchi in the AR and R groups.

Comparison of nasal and bronchial samples within groups

A high correlation was noticed between the gene expression levels in nose and bronchi (**Figure 2A**). However, we also noticed some robust variations in gene expression between the two tissues. Nasal and bronchial cells were easily distinguished, as evidenced by a list of 63 most differentially expressed genes, which showed an absolute log2 fold change above 4 and an adjusted p-value below 0.05 in all three independent clinical groups. **(Figure 2B,C)**. This nasal/bronchial signature is presented in **Figure 2D** and in **Table E4**. A loosest cut-off with an absolute log2 fold change above 2 and an adjusted p-value below 0.05 in the three clinical groups selected a list of 323 genes. **(Figure E2).**

A functional annotation of the genes overexpressed in the nasal epithelium revealed many transcription factors for which expression has to date been considered to be restricted to neurons, such as PAX6 or OTX2 **(Figure E3)**. The increased expression of markers such as MARCO, FABP4, VSIG4, and RBP4 in bronchi probably illustrates the presence of resident macrophages.

Definition of Th2 signature

In order to assess Th2 inflammation, we defined a Th2 signature that could be applied to nasal as well as bronchial epithelia. This was done after analyzing four independent microarray datasets defined in the Methods section. 28 genes were found to be differentially expressed in at least three datasets. Strong differences in gene expression levels between nose and bronchi characterized some markers (**Figure E4**), and this effect was stronger than the impact of the pathological status itself **(Figure E5**). We decided to restrict our signature to transcripts that displayed equivalent levels of expression between nose and bronchi. The resulting signature contained POSTN, previously included in Woodruff's signature[17], CST1, FETUB and ALOX15 previously included in Choy's signature[25]. These genes were moreover the most correlated (rho> 0.55) in our data set among the initial 28 IL-13 inducible genes **(Figure E6).** Of note, CST1 and FETUB were specifically expressed in allergic patients **(Figure E4**).

Hierarchical clustering according to Th2 signature

A hierarchical clustering of the 52 samples from our three groups was performed using the 4-genes Th2 signature **(Figure 3A and E7A**). Subjects were stratified according to their Th2 response. Th2 high response was defined by at least three out of four transcripts (i.e. 75%) from the signature displaying an expression above the average. For the AR group, all nasal samples except two (N.AR30 and N.AR33) and all bronchial samples were characterized by a Th2-high status. For the C group, all nasal and bronchial samples except for one patient

(N.C28 and B.C28) were characterized by a Th2-low status. The R group was more heterogeneous; four R patients had a nasal and bronchial Th2-low status and two R patients had a nasal and bronchial Th2-high status. The nasal and bronchial Th2 statuses of the remaining four R patients were discordant (two with nasal Th2-low and bronchial Th2-high, and two with nasal Th2-high and bronchial Th2-low). Degree of concordance of Th2 status (high or low) within groups was 71% in AR group, 60% in R group and 100% in C group **(Figure 3C)**.

Hierarchical clustering according Th17 and IFN signature

Hierarchical clustering was also performed using Th17 and IFN signatures **(Figure E7 B,C)**. A Th17 high or IFN high responses were defined as more than 75% of transcripts above the average. 19 samples displayed a Th17-high signature (10 bronchial and 9 nasal samples) and 14 samples an IFN-high signature (14 nasal samples). Of note a strong tissue effect was observed concerning IFN signature, nasal samples showing a global higher level of IFN response transcripts than bronchial ones. This tissue effect was not observed with the Th17 response.

Relationships between Th2, Th17 and IFN signatures

Compiling Th2 hierarchical clustering with gene expression level of Th17 and IFN showed that outliers, such as N. AR33, had a low Th2 status but a high Th17 and IFN status and that some samples like N. R14 had a triple (Th2, Th17 and IFN) high status **(Figure 3A,C)**. Th2 response was distinct from IFN and Th17 responses in the whole dataset **(Figure 3B)**. Th17 response was moreover correlated globally to the IFN response, as illustrated by the fact that one of the transcript (IFI6) belonging to the IFN-response signature clearly clusterized with

genes from the Th17 signature. **Figure 3C** summarizes the Th2, Th17 and IFN status in a same individual for the two tissues.

Th2, Th17 and IFN scores: correlations with clinical and biological parameters, relationships with group of patients and tissue

We then calculated a Th2 score in order to assess the relationships between clinical and biological quantitative traits. First, normalized gene expression data for the genes included in the final Th2 signature were transformed into z-scores by gene, then for each sample the Th2 score was calculated as the median of these z-scores. Th2 score was not related to any of the clinical severity parameters or spirometry scores (not shown). Th2 score was only correlated with the number of sensitizations and the number of mast cells in nasal and bronchial epithelium **(Figure 4A)**. Th2 score in bronchial epithelium in the R group was correlated with asthma history. Four out of the five subjects with a history of childhood asthma, with complete remission for more than two years, displayed the highest scores **(Figure E8).**

Th17 and IFN scores were calculated as described previously for Th2 scores. Th17 score correlated positively with the number of PMN in nasal and bronchial epithelium and negatively with the percentage of epithelial cells in nasal epithelium **(Figure 4B)**. IFN score in bronchial epithelium was inversely correlated to FEV1 and FVC **(Figure 4C)**.

As expected Th2 scores were significantly higher in AR+R group in nasal and bronchial samples versus C and not different between nose and bronchi. Th17 scores were not related either to the group of patients or the tissue. IFN score was significantly higher in nasal versus bronchial samples. We further extended the 9-gene IFN signature[16] to a 33-gene signature common to Bhakta's 50-top list of transcripts induced in bronchi[16] and our 79-top list of transcripts induced in nose[9] **(Figure E9)**. The IFN score was still higher in nasal samples

using this broader list, even after removing the four nasal samples with the highest scores **(Figure E10)**.

Metagenomic analysis of viral transcriptome

We looked for the presence of viruses among the unmapped reads (i.e. RNA sequences that did not map to the human genome) of the different samples. This analysis identified the presence of rhinovirus in N.R3 and N.R14 samples, which both displayed a very strong interferon signature (**Figure E7B).** We did not identify viruses in the other samples with a high IFN signature, probably because our sampling was performed long after infection.

Discussion

A link between upper and lower airways in allergic respiratory diseases has been documented for many years, but the concept of a "united airway disease" (UAD), quoted as "one airway, one disease", is more recent. This relies on epidemiological, clinical, functional, immunological and histological relationships which have led to a global management approach for allergic respiratory diseases[26]. At the same time, upper and lower epithelia were shown to display striking differences regarding defenses against viral infections[27], remodeling[28], inflammation[29] and epithelial shedding. This led us to analyze whether Th2 inflammation was of similar amplitude in nose and bronchi in a context of allergic diseases and whether nasal Th2 inflammation was a good surrogate for bronchial one in asthma. Others non-invasive Th2 biomarkers usable in clinical studies have been developed in order to reflect Th2 bronchial status such as serum periostin, blood eosinophils, FeNO, and total IgE. They have moreover been used using a machine learning inference scheme in order to predict the subtypes of gene expression within bronchial biopsies and epithelial cells [30].

They have shown their own merits, but they may also display some limits in the context of viral infection, cigarette smoking, parasitic infections, or bone disease[31]. Moreover, use of serum periostin in large clinical trials has not yet shown clear benefits [32]. Induced sputum has also been analyzed and could be a good proxy, nevertheless it has not yet been directly compared to bronchial Th2 status[33, 34]. Though not invasive, this technique, which needs cooperation, is hardly feasible in young children, and is unsafe in severe asthmatics. Based on our data set, it is unlikely that nasal samples can be sufficiently reliable to be used as a surrogate for bronchial epithelium to assess Th2 status in asthmatic patients with rhinitis. Even if some discrepancies could be explained by expositions to some specific pathogens, we demonstrate in this cross-sectional study that a same individual can display different inflammation profiles in the two sites. We moreover show that Th2, Th17 and IFN inflammation are not mutually exclusive in a same sample.

As previously reported by Poole et al., it remains that a large overlap exists between gene expression profiles in nasal and bronchial brushings (rho=0.943)[35]. Some differences can be explained by the presence of specific populations of immune cells. This is the case for MARCO, a marker of macrophages, which is more highly expressed in bronchi. But differences can also be intrinsic to the two epithelia, and a same tissue-specific signature was easily identified and consistently observed in the three independent experimental groups. A striking observation was the robust expression in nasal epithelium of well-established neuronal markers. The expression of neuronal markers such as OTX2, PAX6, SIX3 and FOXG1 has been confirmed in primary cultures of nasal epithelia[9, 36], thus suggesting an intrinsic expression in nasal epithelial cells rather than contamination by olfactory or sensory neurons, which are absent in bronchi. Additional evidence comes from immunolabelling experiments, in which some of these proteins have been detected in the nuclei of nasal but not

bronchial epithelial cells (e.g., http://www.proteinatlas.org/ENSG00000007372-PAX6/antibody).

We noticed the extreme variability of the cellular composition in nasal brushings. Compared with bronchial samplings, nasal samplings displayed more frequently a lower percentage of epithelial cells and an excessive number of inflammatory cells, mostly PMN. As this situation occurred in more than 30% of nasal samples, we suspect that such a sampling can introduce a strong bias, due to the abundance of non-epithelial cells. On the opposite, we noticed that the quality of the bronchial samples remained quite stable over the full study, even with a lower quality of the corresponding nasal-brushing samples and in the context of mild-to-moderate asthma. We emphasize the importance of a careful checking of the cellular composition of brushings before undergoing any gene expression study[37].

We initially considered the Th2 signature defined by Woodruff et al., which comprises the three transcripts POSTN, CLCA1 and SERPINB2[8]. CLCA1 and SERPINB2 were strongly differentially expressed between nose and bronchi (adjusted $p < 0.001$ and adjusted $p = 0.0013$, respectively) **(Figure E3)**. These results led us to test a larger list of IL-13-responsive transcripts, differentially expressed in nasal as well as in bronchial cells, in order to define a Th2 signature that could be interoperable between these two tissues. We derived this new 28-transcripts signature from four independent studies using nasal, tracheal and bronchial cells. After some optimizations, we restricted this new set to a four-gene signature including POSTN previously used by Woodruff and coworkers, ALOX15 which has been linked to asthma in functional studies and CST1 and FETUB. CST1 and FETUB were the most differentially expressed between AR and C and between R and C in nasal cells as well as in bronchial cells. The functions of these two cystatins in the context of asthma are unknown.

This 4-transcript signature defines a strong Th2 response that is common to nasal and bronchial epithelial cells, with a clear clinical relevance to asthma and allergic rhinitis. Th17 and IFN signature were derived from recently published studies.

All patients of our AR group had a Th2-high level in bronchi, probably explained by the selection of our asthmatic cohort (young, atopic mild asthma). Degree of correlation between nose and bronchi can be first considered as acceptable in AR group, with an average level of 71%. At the same time, this value is probably insufficiently high, and its use would probably lead to misdiagnose an excessive number of Th2 subjects. Indeed, two nasal samples were Th2 low (bronchi being high) but were at the same time Th17 and/or IFN high. Different signatures can consequently co-exist at different levels of a same airway epithelium. Th17 and IFN signatures are probably driven by a more important exposure of nose to viruses, bacteria, irritants and pollutants and by a more robust nasal antiviral response[27].

The R group appeared to be much more heterogeneous, with some patients even displaying a Th2-low score in the nose. This situation may indicate that Th2 signaling has a lower impact on allergic rhinitis than on asthma in adults. We also noticed a very high degree of IFN response in R nasal epithelial cells, 7/10 R patients being IFN-high, including 5 of them with also a Th17 high signature. Of note, patient R14 displayed a triple high (Th2, Th17 and IFN) status in nasal epithelium. In the subset of R patients who displayed a Th2-high score in the bronchi, all appeared to have a history of childhood asthma or asthma with clinical remission. This suggests that a high bronchial Th2 status may persist for a long time after clinical remission of disease.

We also defined quantitative Th2 scores by the median of z-scores for the 4 genes that defined our Th2 signature. This approach was applied in nasal and bronchial brushings, thus defining for each patient a Th2 nasal and a Th2 bronchial score[38]. It was used to investigate possible

relationships with clinical parameters. Th2 scores in nasal and bronchial samples correlated well with mast cells infiltration of the two epithelia together with the number of sensitizations (**Figure 4**. These observations are in line with the description of allergic inflammation, including an increased membrane basement thickness and an epithelial desquamation in the bronchi of atopic subjects even before the onset of clinical symptoms[39]. Th17 scores, correlated well with PMN infiltration and lower epithelial cell percentage. IFN scores were significantly higher in nose compared to bronchi. This could correspond to a different level of innate immunity in each tissue as suggested by the more robust anti-rhinovirus response in nose[27]. The association of IFN, Th2 and Th17 inflammation in this study in the same nasal samples and of Th2 and Th17 inflammation in the same bronchial samples indicate moreover that the new inflammatory pathways of asthma are not strictly mutually exclusive as previously evoked[15]. This phenomenon occurs despite a reciprocal regulation that has long been described between IFN and Th2 inflammation in bronchi[40] leading to distinct phenotypes[16]. IFN scores in bronchial samples were correlated to FEV1 and CVF in asthmatic patients as described previously by Bhakta et al[16].

Our study has some restrictions. First, the AR group comprised a majority of Th2-high subjects, even though we anticipated an inclusion of 50% Th2-low subjects in this group, based on the report by Woodruff et al.[17]. Th2-low subjects can represent up to 70% of patients in cohorts like U-BIOPRED containing a high percentage of severe asthma [30]. Our high percentage of Th2-high AR patients may be related to the younger age of our allergic groups (14 years younger on average than those of Woodruff et al. and 27 years younger than U-BIOPRED) combined with our choice to select only allergic subjects. Our complete asthmatic group had a median number of positive skin prick-tests similar to the Woodruff Th2-high group. Another potential difference with U-BIOPRED and Woodruff's studies is the

lower severity of our asthmatic cohort. We included patients with mild-to-moderate persistent asthma, and only two of these patients had asthma that was not fully controlled. Overall, our patients had a better FEV1 (91.4% vs 87%) compared with Woodruff's group. Some of our results may thus not apply well to patients with severe asthma.

A second limitation of our study is the initial exclusion of 30% of patients of our cohort due to an insufficient percentage of epithelial cells in the nasal samples. This value is similar to the percentage measured in a previous study[9], where 21% (8/38 patients) of the nasal samples contained less than 80% epithelial cells, even after exclusion of patients positive for respiratory viruses. Because our bronchial samples had higher cell counts, we decided to exclude patients with an insufficient percentage of nasal epithelial cells, so that a meaningful comparison between the paired nasal/bronchial samples could be made. Our work was consequently restricted to samples with a high percentage of epithelial cells. This does not preclude a future use of more heterogeneous samples to assess Th2 status, after having defined highly specific markers of the epithelial inflammation.

The last limitation is the cross-sectional nature of our study enabling to drive conclusions on the origin and stability of non-Th2 inflammatory profiles over time[41], which was however partially compensated by the detection of viral sequences in RNA sequencing data.

Conclusion

Nasal epithelium displays a gene expression profile that slightly, but consistently, differs from bronchial epithelium. Infiltration by PMN is more frequent in the nose, leading to unsuitable samples in up to 30% of cases. The remaining nasal samples displayed consistently a higher IFN response, probably linked with intercurrent environmental exposures. Collectively, our results suggest that nasal samples cannot be reliably used as surrogates for bronchial epithelium to assess Th2 status in asthma. In addition, in isolated allergic rhinitis, asthma

remission is not associated with a switch from high to low Th2 status, showing the long-lasting nature of high Th2 status and inflammation, which persist long after the clinical remission of asthma.

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Tables

Table 1. Subject characteristics (n=26)

Data are presented as median (first quartile-third quartile). Statistically significant p-values appear in bold.

Footnotes: AR: allergic rhinitis and asthma, R: rhinitis, C: healthy controls.

Table 2. Comparison between nasal and bronchial cells within groups (n=26)

Data are presented as median (first quartile-third quartile). Statistically significant p-values appear in bold.

Footnotes: AR: allergic rhinitis and asthma, R: rhinitis, C: healthy controls.

Table E1. Subjects characteristics (n=39)

Data are presented as median (first quartile-third quartile). Statistically significant p-values appear in bold.

Footnotes: AR: allergic rhinitis and asthma, R: rhinitis, C: healthy controls.

Table E2. Comparison of nasal and bronchial cells within groups (n=39)

Data are presented as median (first quartile-third quartile). Statistically significant p-values appear in bold.

Footnotes: AR: allergic rhinitis and asthma, R: rhinitis, C: healthy controls.

Table E3. Comparison between included (n=26) and excluded (n=13) subject characteristics.

Data are presented as median (first quartile-third quartile). Statistically significant p-values appear in bold.

Footnotes: AR: allergic rhinitis and asthma, R: rhinitis, C: healthy controls, y: years.

Table E4. 63 Most-regulated genes between nasal and bronchial cells in healthy subjects (C).

Table 1. Subject characteristics (n=26)

	AR	R	C	p-value
Sample size	7	10	9	
Age, years	22(21;24)	26(22.5;28.75)	29(25;36)	**0.045**
Sex ratio (M/F)	5/2	7/3	2/7	0.069
PAREO score	8(6.5;8.5)	7(6.25;8)	na	0.586
SNOT-22 score	32(22;47)	26(22;35.75)	na	0.561
Number of sensitizations	5(4.5;6.5)	3(2;5.25)	0	**0.001**
Dust mite senstitization (n)	5	8	0	**0.001**
Intermittent/ persistent rhinitis	1/6	2/8	na	0
Mild/moderate-severe Rhinitis	1/6	3/7	na	0
Rhinitis duration (years)	6.4(3.98;18.64)	14.47(6.43; 15.46)	na	0.66
Partly controlled/ controlled asthma	2/5	na	na	na
FEV1, % predicted	91.4(86.8;97.1)	100.1(96.6;103.2)	96.8(93.4;98.7)	0.134
FEF_{25-75}, % predicted	74(69.4;89.4)	105.3(89;116.6)	92.1(81.4;96.2)	0.11
Asthma duration (years)	2.98(1.98;11.84)	na	na	na
Nasal cell count ($x10^5$ cells)	7.5(6.85;9.5)	8.6(6.8;12)	7.2(5;8)	0.771
Nasal epithelial cells (%)	91.5(80.1;93.5)	86.9(85.5;91.7)	98(95.6;98.8)	**0.002**
Nasal mast cells (%)	0.14(0.11;0.33)	0.15(0.05;0.24)	0.01(0.01;0.04)	**0.02**
Nasal eosinophils (%)	0.04(0.03;0.10)	0.21(0.07;0.77)	0.01(0;0.04)	**0.003**
Nasal T-cells (%)	0.93(0.67;2.18)	1.73(0.88;2.5)	0.35(0.29;0.68)	**0.03**
Nasal B-cells (%)	0.05(0.01;0.38)	0.03(0.01;0.06)	0.01(0;0.03)	0.13
Nasal PMN (%)	2.66(1.09;3.56)	4.14(1.48;6.53)	0.22(0.09;0.58)	**0.03**
Bronchial cell count ($x10^5$ cells)	9.5(8.25;10.15)	15(9;21)	13(8;17)	0.441
Bronchial epithelial cells (%)	94.3(90.3;94.9)	92.5(88.3;94.3)	94.2(92.7;95.9)	0.25
Bronchial mast cells (%)	0.86(0.52;1.13)	0.39(0.29;0.46)	0.07(0.04;0.08)	**0.002**
Bronchial eosinophils (%)	0.02(0.01;0.03)	0.07(0.03;0.09)	0.02(0.01;0.03)	0.09
Bronchial T-cells (%)	1.8(1.12;2.59)	1.93(1.53;2.91)	1.59(0.82;1.65)	0.48
Bronchial B-cells (%)	0.03(0.01;0.04)	0.04(0.01;0.15)	0.05(0.02;0.07)	0.53
Bronchial PMN (%)	0.43(0.19;0.64)	1.25(0.3;2.39)	1.98(0.68;2.03)	0.06

Table 2. Comparison between nasal and bronchial cells within groups (n=26).

	AR			R			C		
	Nasal	**Bronchial**	**p-value**	**Nasal**	**Bronchial**	**p-value**	**Nasal**	**Bronchial**	**p-value**
Cells count (x10^5 cells)	7.5(6.85;9.5)	9.5(8.25;10.15)	0.47	8.6(6.8;12)	15(9;21)	0.077	7.2(5;8)	13(8;17)	0.121
Epithelial cells (%)	91.5(80.1;93.5)	94.3(90.3;94.9)	0.209	86.9(85.5;91.7)	92.5(88.3;94.3)	0.123	98(95.6;98.8)	94.2(92.7;95.9)	0.063
Mast cells (%)	0.14(0.11;0.33)	0.86(0.52;1.13)	**0.026**	0.15(0.05;0.24)	0.39(0.29;0.46)	**0.023**	0.01(0.01;0.04)	0.07(0.04;0.08)	0.077
Eosinophils (%)	0.04(0.03;0.10)	0.02(0.01;0.03)	0.097	0.21(0.07;0.77)	0.07(0.03;0.09)	**0.043**	0.01(0;0.04)	0.02(0.01;0.03)	0.185
T-cells (%)	0.93(0.67;2.18)	1.8(1.12;2.59)	0.71	1.73(0.88;2.5)	1.93(1.53;2.91)	0.481	0.35(0.29;0.68)	1.59(0.82;1.65)	**0.001**
B-cells (%)	0.05(0.01;0.38)	0.03(0.01;0.04)	0.456	0.03(0.01;0.06)	0.04(0.01;0.15)	0.796	0.01(0;0.03)	0.05(0.02;0.07)	**0.031**
PMN (%)	2.66(1.09;3.56)	0.43(0.19;0.64)	**0.026**	8.6(6.8;12)	1.25(0.3;2.39)	0.075	0.22(0.09;0.58)	1.98(0.68;2.03)	**0.031**

Table E1. Subject characteristics (n=39)

	AR	R	C	p-value
Sample size	12	14	13	
Age, years	22 (21;23)	24.5(21.25;28.75)	30(25;36)	**0.001**
Sex ratio (M/F)	9/3	9/5	4/9	0.07
PAREO score	7(5;8.25)	7(6;8)	na	0.896
SNOT-22 score	29(19.5;35.5)	26(22;37.25)	na	0.926
Number of sensitizations	5(3.75;6.25)	3(2;6)	0	**<0.001**
Dust mite senstitization (n)	9	12	0	**<0.001**
Intermittent/ persistent rhinitis	4/8	2/12	na	
Mild/ moderate-severe rhinitis	3/9	4/10	na	
Rhinitis duration (years)	14.48(6.1; 16.04)	12.55(6.4; 15.19)	na	0.724
Partly controlled/ controlled asthma	2/10	na	na	na
FEV1, % predicted	96.3(89.7;103.3)	106.6(102.7;114.6)	98.9(96.2;109.5)	**0.023**
FEF_{25-75}, % predicted	75.2(63.3;81.7)	104.3(90.7;116.6)	82.8(81.4;96.2)	**0.004**
Asthma duration (years)	8.79(2.87; 14.68)	na	na	na
Nasal cell count ($x10^5$ cells)	9.5(7.25;12)	8.6(6.8;13)	8(6 ;16)	0.987
Nasal epithelial cells (%)	78.8(59;92)	85.6(78.2;89.4)	95.6(72.8;98.3)	0.053
Nasal mast cells (%)	0.13(0.09;0.26)	0.15(0.05;0.19)	0.01(0.01;0.04)	**0.002**
Nasal eosinophils (%)	0.06(0.04;0.27)	0.25(0.09;0.51)	0.01(0;0.07)	**0.002**
Nasal T-cells (%)	1(0.5;1.7)	1.2(0.8;2.3)	0.4(0.3;0.8)	0.135
Nasal B-cells (%)	0(0;0.1)	0.1(0;0.1)	0(0;0)	0.224
Nasal PMN (%)	7.7(2.4;30.1)	6.4(2.9;12.9)	0.6(0.2;10.5)	0.107
Bronchial cell count ($x10^5$ cells)	9.5(7.25;13.8)	15(9.4;21)	12(8 ;17)	0.229
Bronchial epithelial cells (%)	92.8(89.3;95.8)	92.5(88.3;94.3)	93.7(90.8;95.4)	0.484
Bronchial mast cells (%)	1.06(0.6 ;1.23)	0.4(0.29;0.58)	0.08(0.05;0.31)	**0.001**
Bronchial eosinophils (%)	0.02(0.01;0.04)	0.07(0.03;0.1)	0.02(0.01;0.03)	0.064
Bronchial T-cells (%)	1.77(1.13;2.3)	1.93(1.53;2.91)	1.65(1.49;2.47)	0.824
Bronchial B-cells (%)	0.03(0.01 ;0.04)	0.03(0.0 ;0.15)	0.03(0.03;0.07)	0.347
Bronchial PMN (%)	0.47(0.2;0.73)	0.97(0.35;1.86)	1.98(0.7;2.17)	**0.025**

Table E2. Comparison of nasal and bronchial cells within groups (n=39).

	AR			R			C		
	Nasal	**Bronchial**	**p-value**	**Nasal**	**Bronchial**	**p-value**	**Nasal**	**Bronchial**	**p-value**
Cell count (x10^5 cells)	9.5(7.25;12)	9.5(7.25;13.8)	0.91	8.6(6.8;13)	15(9.4;21)	0.058	8(6 ;16)	12(8 ;17)	0.488
Epithelial cells(%)	78.8(59;92)	92.8(89.3;95.8)	**0.007**	85.6(78.2;89.4)	92.5(88.3;94.3)	**0.011**	95.6(72.8;98.3)	93.7(90.8;95.4)	0.65
Mast cells (%)	0.13(0.09;0.26)	1.06(0.6 ;1.23)	**0**	0.15(0.05;0.19)	0.4(0.29;0.58)	**0.004**	0.01(0.01;0.04)	0.08(0.05;0.31)	**0.003**
Eosinophils (%)	0.06(0.04;0.27)	0.02(0.01;0.04)	**0.024**	0.25(0.09;0.51)	0.07(0.03;0.1)	**0.004**	0.01(0;0.07)	0.02(0.01;0.03)	0.878
T-cells (%)	1(0.5;1.7)	1.77(1.13;2.3)	0.198	1.2(0.8;2.3)	1.93(1.53;2.91)	0.21	0.4(0.3;0.8)	1.65(1.49;2.47)	**0.001**
B-cells (%)	0(0;0.1)	0.03(0.01 ;0.04)	0.198	0.1(0;0.1)	0.03(0.0 ;0.15)	0.874	0(0;0)	0.03(0.03;0.07)	0.081
PMN (%)	7.7(2.4;30.1)	0.47(0.2;0.73)	**0**	6.4(2.9;12.9)	0.97(0.35;1.86)	**0.002**	0.6(0.2;10.5)	1.98(0.7;2.17)	0.614

Table E3. Comparison between included (n=26) and excluded (n=13) subject characteristics.

	AR	R	C	AR excluded	R excluded	C excluded	AR vs AR excluded p-value	R vs R excluded p-value	C vs C excluded p-value
Sample size	7	10	9	5	4	4			
Age, years	22(21;24)	26(22.5;28.75)	29(25;36)	22 (21;22)	22 (20.75;26.25)	32.5 (30;36)	0.51	0.48	0.44
Sex ratio (M/F)	5/2	7/3	2/7	4 / 1	2 / 2	2 / 2	1	0.58	0.53
PAREO score	8(6.5;8.5)	7(6.25;8)	na	5 (4;7)	6 (5.5;6.5)	na	0.22	0.32	na
SNOT-22 score	32(22;47)	26(22;35.75)	na	25 (18.25;29.75)	30 (23.75;38)	na	0.34	0.80	na
Number of sensitizations	5(4.5;6.5)	3(2;5.25)	0	4 (3;6)	4 (2;6)	0 (0;0)	0.46	0.94	0.39
Dust mite sensitization (n)	5	8	0	4	4	0	1.00	1.00	na
Intermittent/ persistent rhinitis	1/6	2/8	na	3 / 2	0 / 4	na	0.22	1.00	na
Mild/moderate-severe Rhinitis	1/6	3/7	na	2 / 3	1 / 3	na	0.53	1.00	na
Rhinitis duration (y)	6.4(3.98;18.64)	14.47(6.43; 15.46)	na	14.55 (14.4;15.55)	6.73 (5.61;9.64)	na	0.53	0.47	na
Partly controlled/ controlled asthma	2/5	na	na	0 / 5	na	na	0.47	na	na
FEV1, % predicted	91.4(86.8;97.1)	100.1(96.6;103.2)	96.8(93.4;98.7)	91.6 (84;95.95)	108.3 (105.4;112.2)	103.7 (99.1;109.8)	0.11	0.64	0.33
FEF_{25-75}, % predicted	74(69.4;89.4)	105.3(89;116.6)	92.1(81.4;96.2)	76.4 (49;80.6)	100.9(94.3;113)	82.5 (78.;88.4)	0.42	0.95	0.71
Asthma	2.98(1.98;11.84	na	na	14.47(11.6;15.2	na	na	0.39	na	na

duration (y)	)			9)					
Nasal cell count (x10^5 cells)	7.5(6.85;9.5)	8.6(6.8;12)	7.2(5;8)	12(11.25;12.05)	11.8 (6.6;17.45)	19.9(13.5;26.1)	0.17	0.49	0.10
Nasal epithelial cells (%)	91.5(80.1;93.5)	86.9(85.5;91.7)	98(95.6;98.8)	57.7 (56.9;59.4)	55.4 (46.3;64.5)	66.1 (60.4;71.1)	**0.00**	**0.00**	**0.00**
Nasal mastocytes (%)	0.14(0.11;0.33)	0.15(0.05;0.24)	0.01(0.01;0.04)	0.12 (0.07;0.25)	0.13 (0.08;0.16)	0.02 (0.01;0.03)	0.43	0.45	0.94
Nasal eosinophils (%)	0.04(0.03;0.10)	0.21(0.07;0.77)	0.01(0;0.04)	0.14 (0.07;0.59)	0.25 (0.23;0.33)	0.13 (0.04;0.23)	0.15	0.84	0.11
Nasal LT (%)	0.93(0.67;2.18)	1.73(0.88;2.5)	0.35(0.29;0.68)	1.09 (0.57;1.21)	0.91 (0.61;1.37)	0.81 (0.49;2.52)	0.64	0.45	0.15
Nasal LB (%)	0.05(0.01;0.38)	0.03(0.01;0.06)	0.01(0;0.03)	0.04 (0.03;0.09)	0.71 (0.11;2.26)	0.08 (0.01;2.06)	1.00	0.24	0.15
Nasal PMN (%)	2.66(1.09;3.56)	4.14(1.48;6.53)	0.22(0.09;0.58)	37.71 (27.61;37.74)	35.56 (29.8;42.82)	26.3 (15.83;34.34)	**0.00**	**0.00**	**0.01**
Bronchial cell count (x10^5 cells)	9.5(8.25;10.15)	15(9;21)	13(8;17)	11(62.5;15)	13.4(9.7;21)	10.1(6.65;15.75)	0.92	0.70	0.71
Bronchial epithelial cells (%)	94.3(90.3;94.9)	92.5(88.3;94.3)	94.2(92.7;95.9)	89.9 (88.8;95)	92.8 (88.4;94.6)	91.4 (89.1;92.9)	0.64	0.95	0.05
Bronchial mastocytes (%)	0.86(0.52;1.13)	0.39(0.29;0.46)	0.07(0.04;0.08)	1.13 (1.08;1.24)	0.5 (0.3;0.64)	0.3 (0.19;0.68)	0.43	0.95	0.05
Bronchial eosinophils (%)	0.02(0.01;0.03)	0.07(0.03;0.09)	0.02(0.01;0.03)	0.01 (0.01;0.06)	0.06 (0.01;0.11)	0.01 (0;0.03)	0.88	0.73	0.71
Bronchial LT (%)	1.8(1.12;2.59)	1.93(1.53;2.91)	1.59(0.82;1.65)	1.75 (1.31;1.98)	1.87 (1.41;2.65)	3.29 (2.86;4.63)	1.00	0.95	**0.01**
Bronchial LB (%)	0.03(0.01;0.04)	0.04(0.01;0.15)	0.05(0.02;0.07)	0.03 (0.02;0.04)	0.03 (0.02;2.86)	0.03 (0.03;0.14)	1.00	0.84	1.00
Bronchial PMN (%)	0.43(0.19;0.64)	1.25(0.3;2.39)	1.98(0.68;2.03)	0.51 (0.31;0.9)	0.97 (0.8;1.06)	1.49 (0.78;2.25)	0.76	0.95	0.60

Table E4. 63 Most-regulated genes between nasal and bronchial cells in healthy subjects (C)

Symbol	Name	logFC(C)	AveExpr(C)	adj.P.Val(C)
OTX2	orthodenticle homeobox 2	8.01	0.09	2.49E-19
RP11-89K21.1	/	7.6	1.13	4.88E-14
LGI1	leucine rich glioma inactivated 1	7.44	0.54	1.01E-13
PAX6	paired box 6	7.37	0.03	9.17E-18
LINC00461	long intergenic non-protein coding RNA 461	6.78	-0.99	1.14E-14
FEZF1	FEZ family zinc finger 1	6.28	-0.33	3.03E-14
PAX3	paired box 3	6.18	-0.22	2.45E-14
PAX7	paired box 7	6.07	2.9	1.08E-15
SIX3-AS1	SIX3 antisense RNA 1	6.02	-1.05	7.49E-15
RP11-966I7.1	/	5.94	-1.62	2.17E-18
IL19	interleukin 19	5.36	0.17	3.28E-09
ALOX15B	arachidonate 15-lipoxygenase, type B	5.29	-0.12	1.82E-11
GAPDHP24	glyceraldehyde 3 phosphate dehydrogenase pseudogene 24	5.28	-1.21	5.01E-10
CA12	carbonic anhydrase 12	5.26	4.38	6.01E-17
GALNT13	polypeptide N-acetylgalactosaminyltransferase 13	5.25	0.83	2.59E-10
CPA4	carboxypeptidase A4	5.13	0.99	1.75E-06
RP11-10H3.1	/	5.08	-0.72	1.94E-07
RP5-964N17.1	/	5.03	-1.86	1.43E-12
FEZF1-AS1	FEZF1 antisense RNA 1	4.62	1.81	5.93E-15
MEOX1	mesenchyme homeobox 1	4.5	-1.19	8.53E-10
LAMB4	laminin subunit beta 4	4.12	0.18	1.96E-10
EDN2	endothelin 2	4.08	-1.53	2.12E-05
KRT24	keratin 24	4.07	-1.42	5.82E-05
STC1	stanniocalcin 1	4.06	0.22	3.19E-07
RP11-1016B18.1	/	-4.32	-1.01	2.18E-08
SHH	sonic hedgehog	-4.4	0.14	3.17E-09
CACNA1H	calcium voltage-gated channel subunit alpha1 H	-4.46	0.6	7.09E-07
VSIG4	V-set and immunoglobulin domain containing 4	-4.69	0.89	3.68E-08
RBP4	retinol binding protein 4	-5.02	-2.46	1.35E-06
RP11-625L16.1	/	-5.14	0.07	1.52E-08
SCN3A	sodium voltage-gated channel alpha subunit 3	-5.22	-0.57	3.49E-08

LINC01014	long intergenic non-protein coding RNA 1014	-5.31	0.1	2.30E-10
PRDM16	PR/SET domain 16	-5.79	0.31	1.47E-16
CTD-3012A18.1	/	-6.25	-1.67	2.73E-20
RP11-368I23.2	/	-6.32	-0.64	1.52E-10
KIF1A	kinesin family member 1A	-6.42	-1.2	3.78E-13
RP11-429A20.3		-6.55	-2.08	9.84E-15
SCGB3A1	secretoglobin family 3A member 1	-6.56	6.6	1.62E-09
LMO3	LIM domain only 3	-6.58	1.94	1.01E-17
RP11-429A20.4	/	-6.59	-1.84	1.43E-15
PAX1	paired box 1	-6.72	-1.93	2.26E-20
EGFEM1P	EGF like and EMI domain containing 1, pseudogene	-6.84	2.78	2.04E-08
RP11-627G23.1	/	-7.1	0.28	3.16E-14
SLC5A7	solute carrier family 5 member 7	-7.14	-1.13	1.09E-17
NKX2-1	NK2 homeobox 1	-8.59	-0.79	3.03E-35
KCNA1	potassium voltage-gated channel subfamily A member 1	-8.88	0.91	1.80E-16
FABP4	fatty acid binding prot	-8.9	-0.66	1.08E-15

Figure legends

Figure 1. Nasal and bronchial cells composition in initial (A) and curated (B) sets of patients

Boxplots of the percentages of epithelial, mast cells, eosinophils, T-cells, B-cells and PMN in each group and each type of sample in (A) the initial set of patients, (B) the final set of patients

Footnotes: AR: allergic rhinitis and asthma, R: rhinitis, C: healthy controls, N: nasal, B: bronchi, EC: epithelial cells, T-cells: T-lymphocytes, B-cells: B-lymphocytes, PMN, polymorphonuclear neutrophils.

Figure 2. Differential gene expression between nasal and bronchial tissues (63 transcripts)

(A) Scatter plot of average log2 expression levels for genes commonly expressed between nasal and bronchial tissues (N=26 in each group). Rho: spearman rho statistic. Venn diagrams showing the number of genes differentially upregulated (B) and downregulated (C) between nose and bronchi, common to the three clinical groups. Differentially expressed genes were defined by an adjusted p-value<0.05 and an abs(log2 FC)>2. The values highlighted within parentheses correspond to an adjusted p-value<0.05 and an abs(log2 FC)>4. (D) Unsupervised hierarchical clustering of the RNAseq data based on a set of the 63 most differentially expressed genes (adjusted p-value<0.05 and an abs(log2 FC)>4). Each square represents the expression level of a given gene in a given sample relative to the average

expression level in all samples. A red-to-blue color scale indicates gene expression levels above (red) or below (blue) the average level of expression for this same gene.

Footnotes: B: bronchus; N: nose; AR: allergic rhinitis and asthma; R: isolated allergic rhinitis; C: healthy controls.

Figure 3. Hierarchical clustering of the 26 nasal and 26 bronchial samples according to their respective Th2, Th17, IFN status

(A) Unsupervised hierarchical clustering of the RNAseq data based on our 4-gene Th2 signature. Each square represents the expression level of a given gene in a given sample relative to the gene average expression level in all samples. A red-to-blue color scale indicates gene expression levels above (red) or below (blue) the average level of expression for the same transcript. Gene belonging to Th17 and IFN signature were added, but did not participate to this clustering. (B) Heatmap of correlation between the 18 genes of the Th2, Th17 and IFN signatures. (C). Representation of nasal and bronchial Th2, Th17 and IFN status of each patient, red colour indicating a high status.

Footnotes: B: bronchus; N: nose; AR: allergic rhinitis and asthma group; R: isolated allergic rhinitis group; C: healthy controls.

Figure 4. Comparison of Th2 and Th17 scores with clinical and biological parameters

(A) Top panels: scatter plot of Th2 score in nasal samples with % mast cells in nasal brushings and number of sensitizations. Bottom panels: scatterplot of Th2 score in bronchial samples with % mast cells in bronchial brushings and number of sensitizations. (B) Top panels: scatter plot of Th17 score in nasal samples with number of % PMN and epithelial cells

in nasal brushings. Bottom panels: Th17 score in bronchial samples with number of % PMN and epithelial cells in bronchial brushings. (C) Top panels: scatter plot of IFN score in nasal samples with FEV1 and FVC. Bottom panels: scatter plots of IFN score in bronchial samples with FEV1 and FVC.

Footnotes: #: number; AR: allergic rhinitis and asthma group; R: isolated allergic rhinitis group; C: healthy controls, FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity.

Figure E1. Flow chart of the study

Figure E2. Differential gene expression between nasal and bronchial tissues (323 transcripts)

Unsupervised hierarchical clustering of the RNAseq data based on a set of the 323 most differentially expressed genes (adjusted p-value<0.05 and an abs(log2 FC)>2). Each square represents the expression level of a given gene in a given sample relative to the average expression level in all samples. A red-to-blue color scale indicates gene expression levels above (red) or below (blue) the average level of expression for this same gene.

Figure E3. Biological theme analysis of nose/bronchus signature

Physiological system development and functional categories found to be significantly affected in an Ingenuity Pathway Analysis® downstream effect analysis of a selection of 323 genes differentially expressed between nasal and bronchial samples (adjusted p-value<0.05 and an abs(log2 FC)>2). Bars represent the strength of the statistical significance.

Figure E4. Boxplots showing the expression in AR, R and C samples from the nose and bronchi for the 28 transcripts defining the initial Th2 gene signature, defined from GSE19190, GSE110799, GSE37693 and GSE4804

Distribution of expression levels (log2) in each experimental group for the 28-gene set of our Th2 signature.

Footnotes: B: bronchus; N: nose; AR: allergic rhinitis and asthma group; R: isolated allergic rhinitis group; C: healthy controls.

Figure E5. Hierarchical clustering based on initial Th2 gene signature

Unsupervised hierarchical clustering of the RNAseq data based the initial 28 genes Th2 signature. Each square represents the expression level of a given gene in a given sample relative to the gene average expression level in all samples. A red-to-blue color scale indicates gene expression levels above (red) or below (blue) the average level of expression for the same transcript.

Footnotes: B: bronchus; N: nose; AR: allergic rhinitis and asthma group; R: isolated allergic rhinitis group; C: healthy controls.

Figure E6. Heatmap of Pearson correlation between Th2 markers

Unsupervised hierarchical clustering of the pairwise correlations between Th2 markers. Each square represents the Pearson correlation coefficient between 2 genes. A red-to-blue color scale indicates positive (red) or negative (blue) correlation.

Figure E7. Hierarchical clustering based Th2, Th17 and IFN gene signature

Unsupervised hierarchical clustering of the RNAseq data based on the (A) initial 4-genes Th2 signature (B) Th17 signature, (C) IFN signature. Each square represents the expression level of a given gene in a given sample relative to the gene average expression level in all samples. A red-to-blue color scale indicates gene expression levels above (red) or below (blue) the average level of expression for the same transcript.

Footnotes: B: bronchus; N: nose; AR: allergic rhinitis and asthma group; R: isolated allergic rhinitis group; C: healthy controls.

Figure E8. Th2 score of bronchial samples in the R group as a function of asthma history

Boxplots of Th2 score in the bronchial samples from the patients belonging to the R group according to clinical history. * p-value < 0.05

Footnotes: A: asthma history; EW: isolated wheeze on exertion history; N; no asthma or wheeze on exertion history

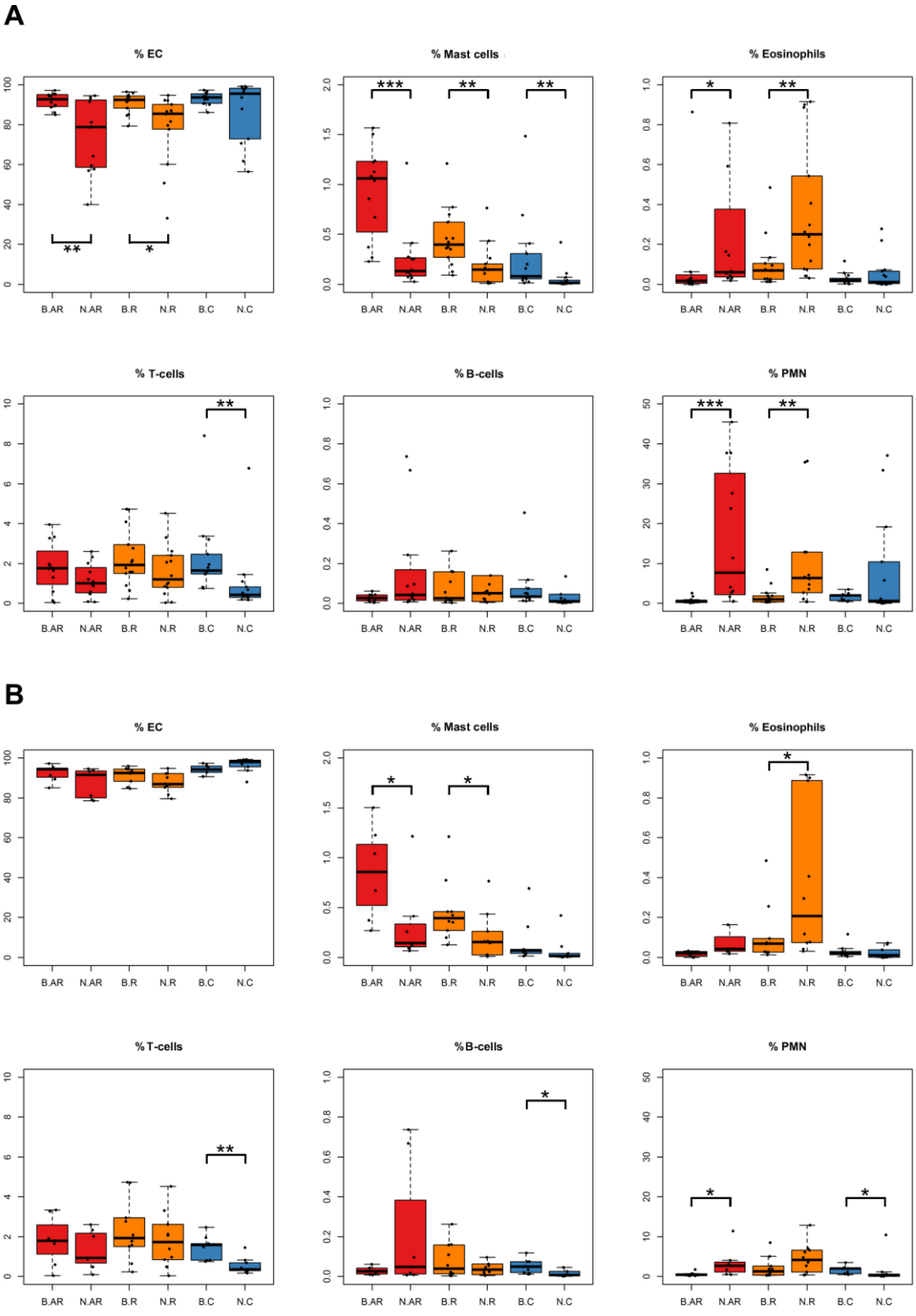
Figure E9. Hierarchical clustering based on an enlarged IFN gene signature

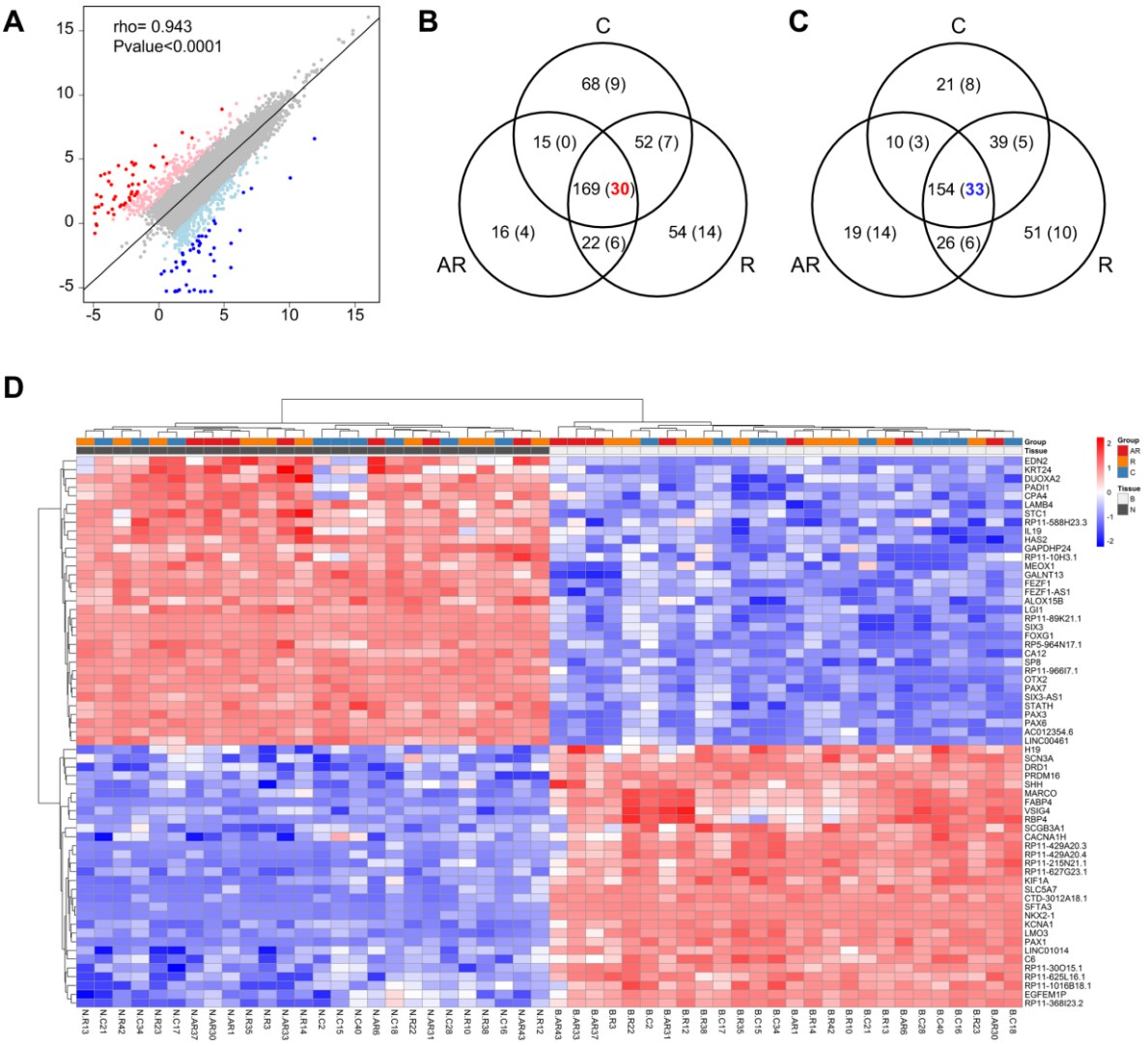
Unsupervised hierarchical clustering of the RNAseq data based on an extended 33-gene IFN signature. Each square represents the expression level of a given gene in a given sample relative to the gene average expression level in all samples. A red-to-blue color scale indicates gene expression levels above (red) or below (blue) the average level of expression for the same transcript.

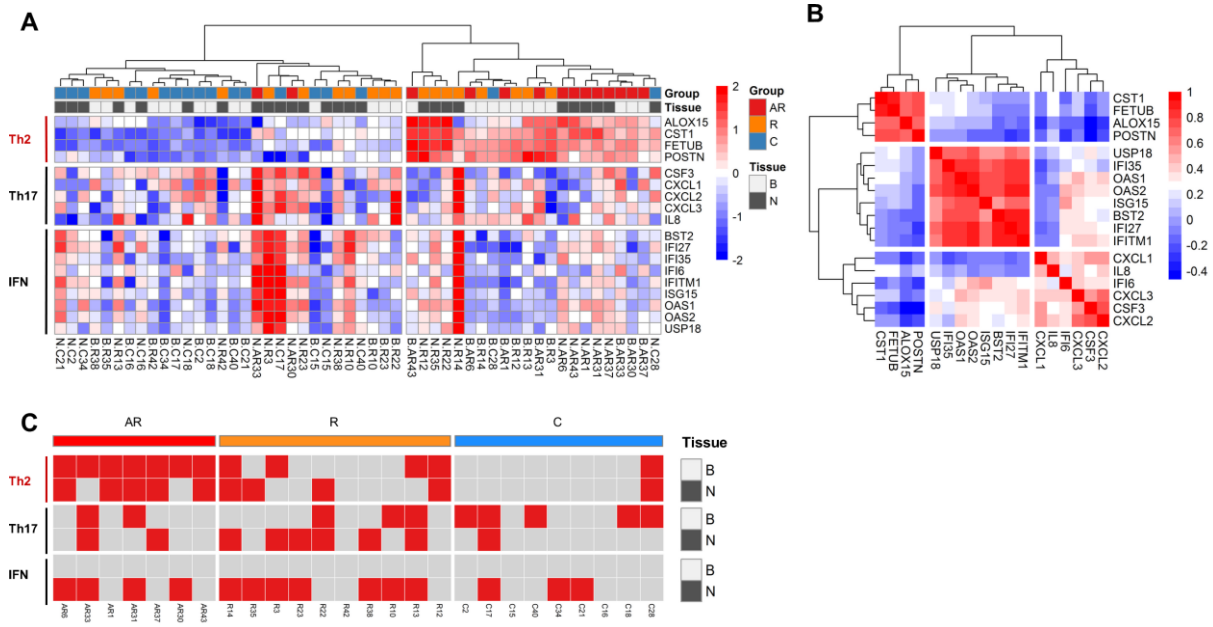
Footnotes: B: bronchus; N: nose; AR: allergic rhinitis and asthma group; R: isolated allergic rhinitis group; C: healthy controls.

Figure E10. IFN score in nasal and bronchial samples

Boxplots of IFN score in the nasal and bronchial samples (score based on the 33 genes signature), in the (A) whole data set and (B) removing the four samples with the highest scores.


A
% EC
% Mast cells
% Eosinophils
% T-cells
% B-cells
% PMN
B
% EC
% Mast cells
% Eosinophils
% T-cells
% B-cells
% PMN
B.AR
N.AR
B.R
N.R
B.C
N.C


A
rho= 0.943
Pvalue<0.0001
B
C
68 (9)
15 (0)
52 (7)
169 (30)
16 (4)
22 (6)
54 (14)
AR
R
C
C
21 (8)
10 (3)
39 (5)
154 (33)
19 (14)
26 (6)
51 (10)
AR
R
D
Group
Tissue
EDN2
KRT24
DUOXA2
PADI1
CPA4
LAMB4
STC1
RP11-588H23.3
IL19
HAS2
GAPDHP24
RP11-10H3.1
MEOX1
GALNT13
FEZF1
FEZF1-AS1
ALOX15B
LGI1
RP11-89K21.1
SIX3
FOXG1
RP5-964N17.1
CA12
SP8
RP11-966I7.1
OTX2
PAX7
SIX3-AS1
STATH
PAX3
PAX6
AC012354.6
LINC00461
H19
SCN3A
DRD1
PRDM16
SHH
MARCO
FABP4
VSIG4
RBP4
SCGB3A1
CACNA1H
RP11-429A20.3
RP11-429A20.4
RP11-215N21.1
RP11-627G23.1
KIF1A
SLC5A7
CTD-3012A18.1
SFTA3
NKX2-1
KCNA1
LMO3
PAX1
LINC01014
C6
RP11-30O15.1
RP11-625L16.1
RP11-1016B18.1
EGFEM1P
RP11-368I23.2
N.R13
N.C21
N.R42
N.C34
N.R23
N.C17
N.AR37
N.AR30
N.AR1
N.R35
N.R3
N.AR33
N.R14
N.C2
N.C15
N.C40
N.AR6
N.C18
N.R22
N.AR31
N.C28
N.R10
N.R38
N.C16
N.AR43
N.R12
B.AR43
B.AR33
B.AR37
B.R3
B.R22
B.C2
B.AR31
B.R12
B.R38
B.C17
B.R35
B.C15
B.C34
B.AR1
B.R14
B.R42
B.R10
B.C21
B.R13
B.AR6
B.C28
B.C40
B.C16
B.R23
B.AR30
B.C18
Group
AR
R
C
Tissue
B
N


A
Group
Tissue
Th2
Th17
IFN
ALOX15
CST1
FETUB
POSTN
CSF3
CXCL1
CXCL2
CXCL3
IL8
BST2
IFI27
IFI35
IFI6
IFITM1
ISG15
OAS1
OAS2
USP18
AR
R
C
B
N
B
CST1
FETUB
ALOX15
POSTN
USP18
IFI35
OAS1
OAS2
ISG15
BST2
IFI27
IFITM1
CXCL1
IL8
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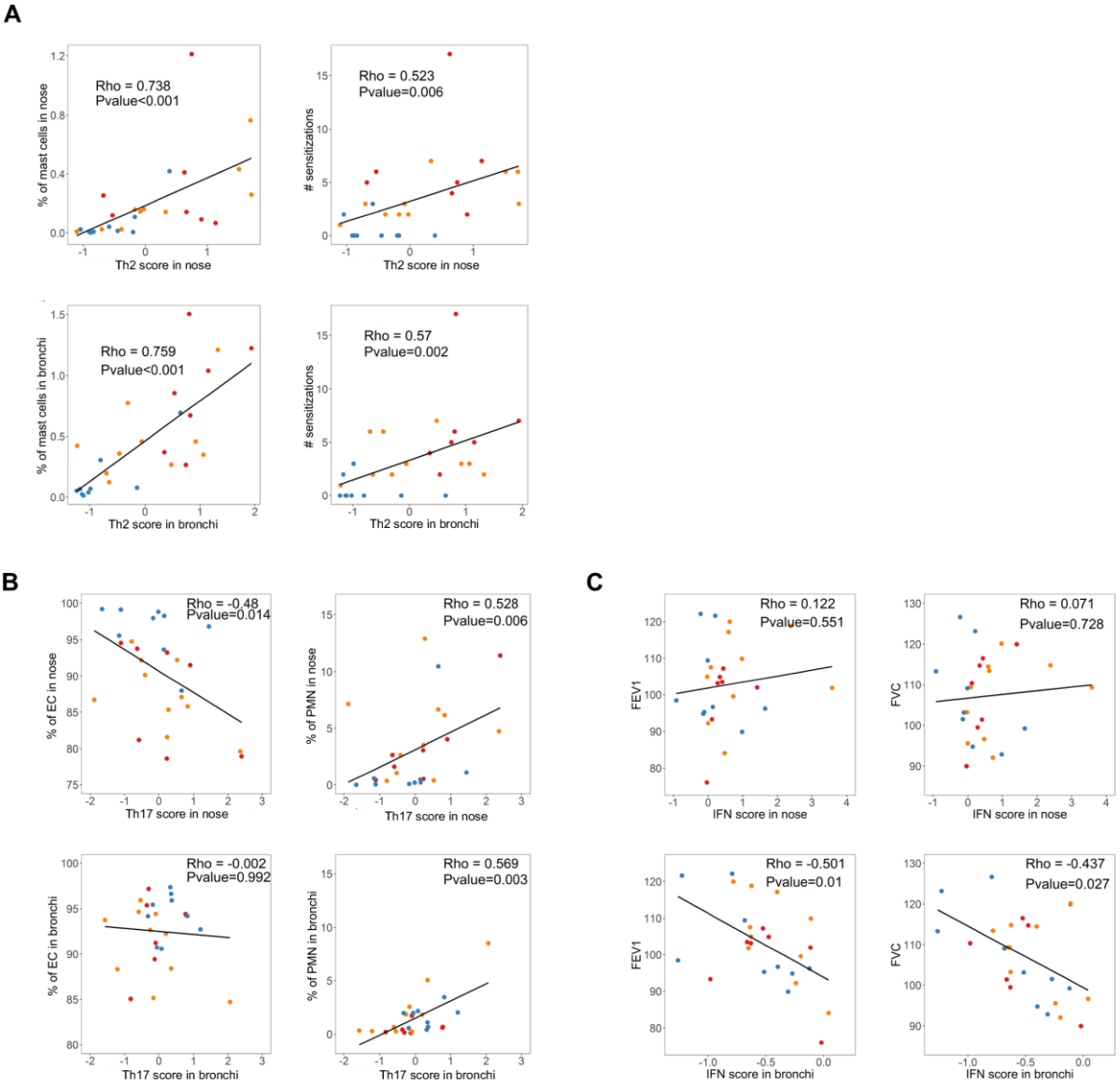
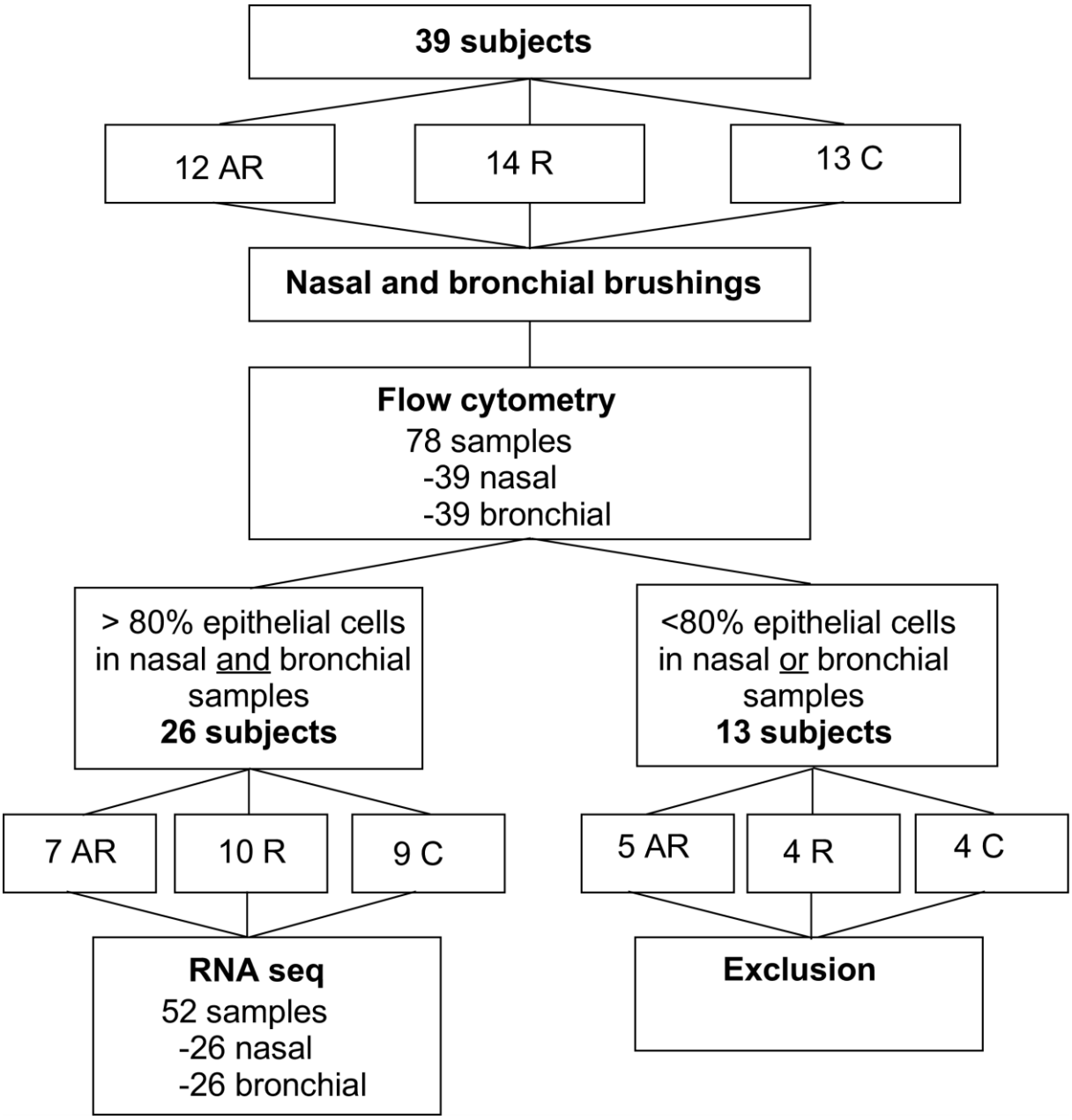
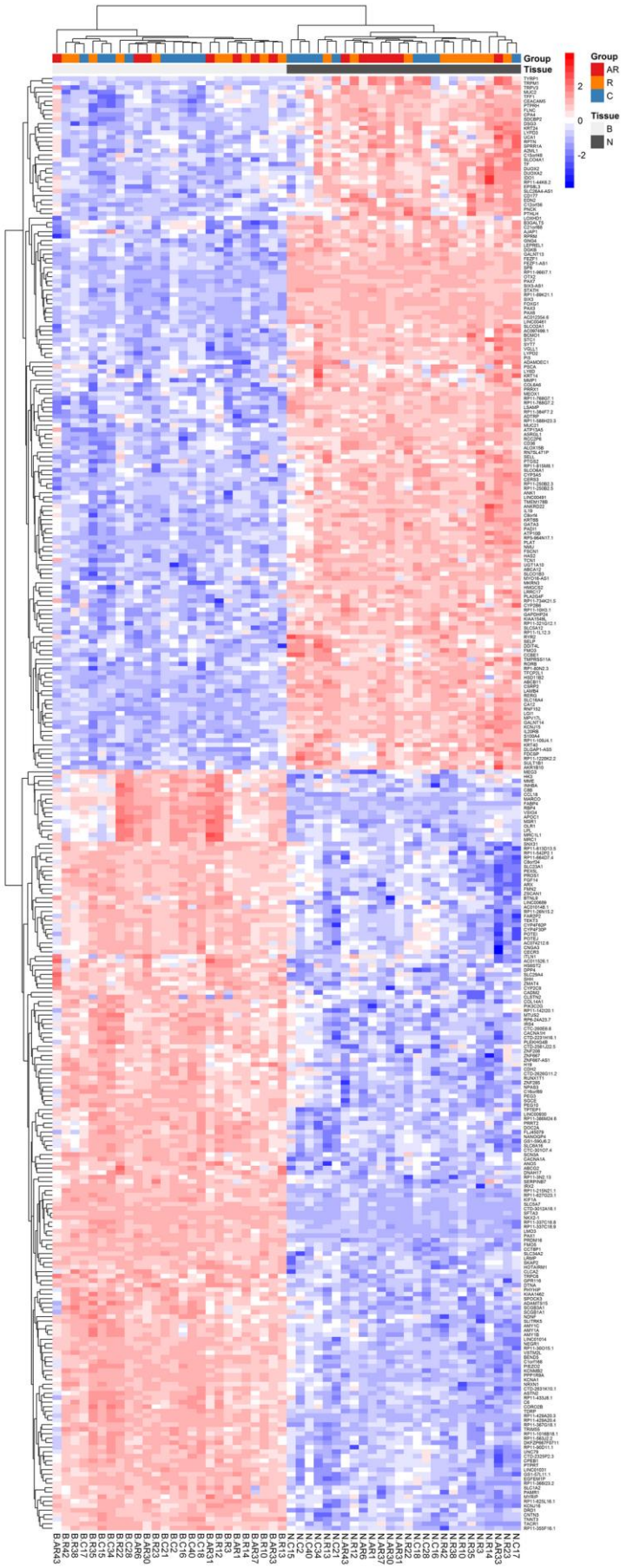
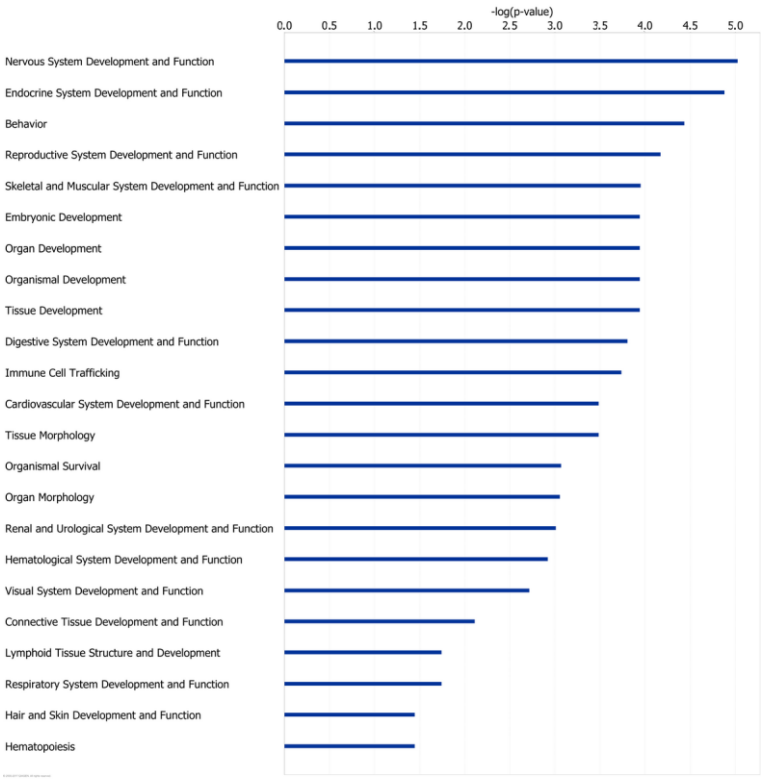
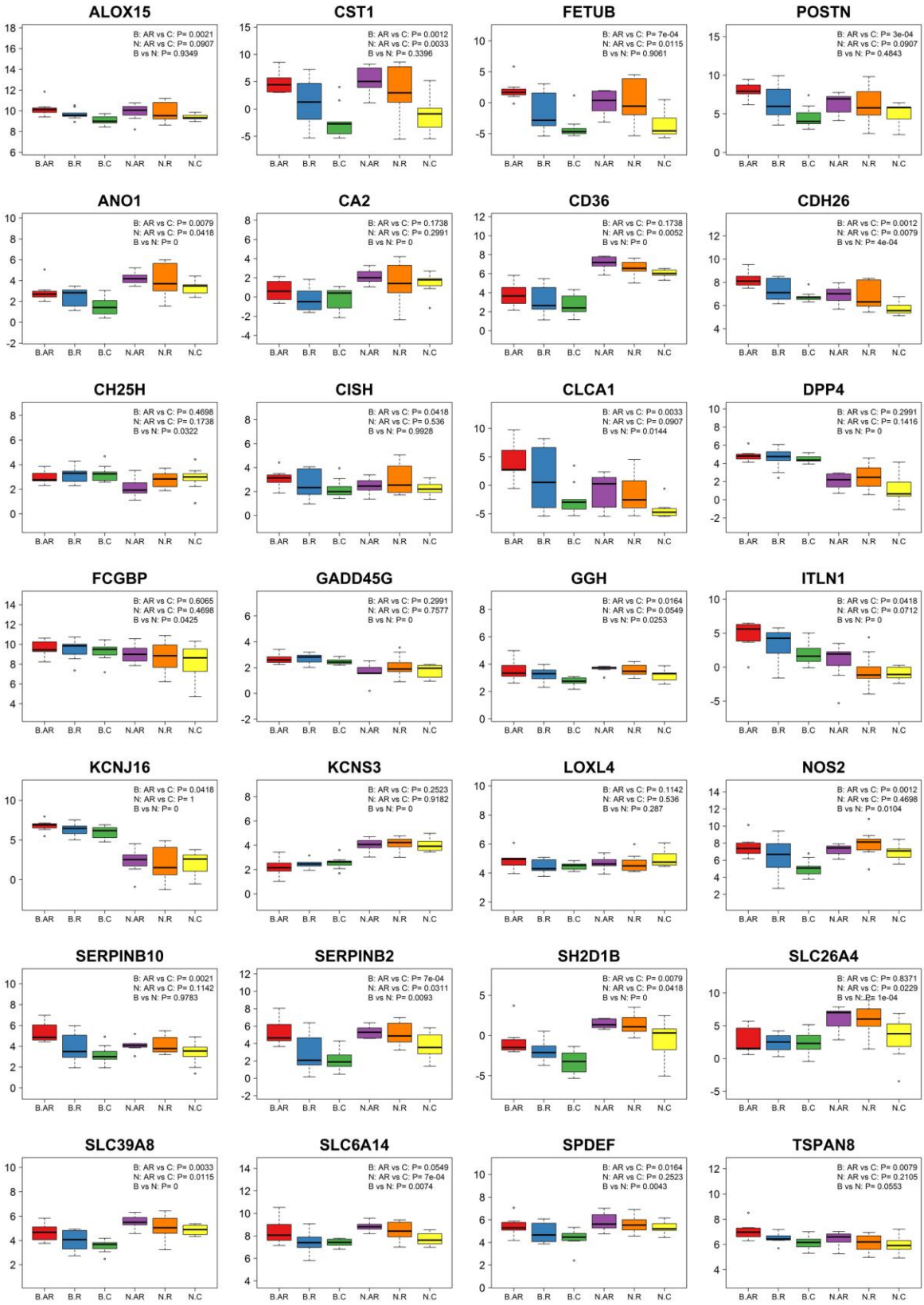
CSF3
CXCL2
C
AR
R
C
Tissue
Th2
Th17
IFN
B
N

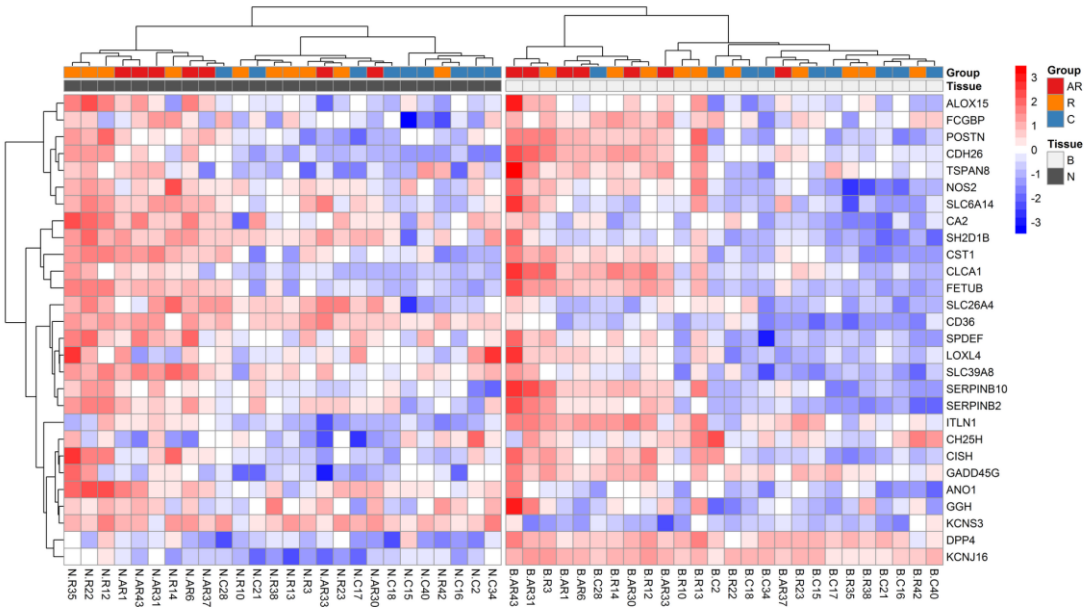

A
Rho = 0.738
Pvalue<0.001
% of mast cells in nose
Th2 score in nose
Rho = 0.523
Pvalue=0.006
# sensitizations
Th2 score in nose
Rho = 0.759
Pvalue<0.001
% of mast cells in bronchi
Th2 score in bronchi
Rho = 0.57
Pvalue=0.002
# sensitizations
Th2 score in bronchi
B
Rho = -0.48
Pvalue=0.014
% of EC in nose
Th17 score in nose
Rho = 0.528
Pvalue=0.006
% of PMN in nose
Th17 score in nose
Rho = -0.002
Pvalue=0.992
% of EC in bronchi
Th17 score in bronchi
Rho = 0.569
Pvalue=0.003
% of PMN in bronchi
Th17 score in bronchi
C
Rho = 0.122
Pvalue=0.551
FEV1
IFN score in nose
Rho = 0.071
Pvalue=0.728
FVC
IFN score in nose
Rho = -0.501
Pvalue=0.01
FEV1
IFN score in bronchi
Rho = -0.437
Pvalue=0.027
FVC
IFN score in bronchi


39 subjects
12 AR
14 R
13 C
Nasal and bronchial brushings
Flow cytometry
78 samples
-39 nasal
-39 bronchial
> 80% epithelial cells in nasal and bronchial samples
26 subjects
<80% epithelial cells in nasal or bronchial samples
13 subjects
7 AR
10 R
9 C
5 AR
4 R
4 C
RNA seq
52 samples
-26 nasal
-26 bronchial
Exclusion


Group
Tissue
Group
AR
R
C
Tissue
B
N
2
0
-2


-log(p-value)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Nervous System Development and Function
Endocrine System Development and Function
Behavior
Reproductive System Development and Function
Skeletal and Muscular System Development and Function
Embryonic Development
Organ Development
Organismal Development
Tissue Development
Digestive System Development and Function
Immune Cell Trafficking
Cardiovascular System Development and Function
Tissue Morphology
Organismal Survival
Organ Morphology
Renal and Urological System Development and Function
Hematological System Development and Function
Visual System Development and Function
Connective Tissue Development and Function
Lymphoid Tissue Structure and Development
Respiratory System Development and Function
Hair and Skin Development and Function
Hematopoiesis


ALOX15
B: AR vs C: P= 0.0021
N: AR vs C: P= 0.0907
B vs N: P= 0.9349
CST1
B: AR vs C: P= 0.0012
N: AR vs C: P= 0.0033
B vs N: P= 0.3396
FETUB
B: AR vs C: P= 7e-04
N: AR vs C: P= 0.0115
B vs N: P= 0.9061
POSTN
B: AR vs C: P= 3e-04
N: AR vs C: P= 0.0907
B vs N: P= 0.4843
ANO1
B: AR vs C: P= 0.0079
N: AR vs C: P= 0.0418
B vs N: P= 0
CA2
B: AR vs C: P= 0.1738
N: AR vs C: P= 0.2991
B vs N: P= 0
CD36
B: AR vs C: P= 0.1738
N: AR vs C: P= 0.0052
B vs N: P= 0
CDH26
B: AR vs C: P= 0.0012
N: AR vs C: P= 0.0079
B vs N: P= 4e-04
CH25H
B: AR vs C: P= 0.4698
N: AR vs C: P= 0.1738
B vs N: P= 0.0322
CISH
B: AR vs C: P= 0.0418
N: AR vs C: P= 0.536
B vs N: P= 0.9928
CLCA1
B: AR vs C: P= 0.0033
N: AR vs C: P= 0.0907
B vs N: P= 0.0144
DPP4
B: AR vs C: P= 0.2991
N: AR vs C: P= 0.1416
B vs N: P= 0
FCGBP
B: AR vs C: P= 0.6065
N: AR vs C: P= 0.4698
B vs N: P= 0.0425
GADD45G
B: AR vs C: P= 0.2991
N: AR vs C: P= 0.7577
B vs N: P= 0
GGH
B: AR vs C: P= 0.0164
N: AR vs C: P= 0.0549
B vs N: P= 0.0253
ITLN1
B: AR vs C: P= 0.0418
N: AR vs C: P= 0.0712
B vs N: P= 0
KCNJ16
B: AR vs C: P= 0.0418
N: AR vs C: P= 1
B vs N: P= 0
KCNS3
B: AR vs C: P= 0.2523
N: AR vs C: P= 0.9182
B vs N: P= 0
LOXL4
B: AR vs C: P= 0.1142
N: AR vs C: P= 0.536
B vs N: P= 0.287
NOS2
B: AR vs C: P= 0.0012
N: AR vs C: P= 0.4698
B vs N: P= 0.0104
SERPINB10
B: AR vs C: P= 0.0021
N: AR vs C: P= 0.1142
B vs N: P= 0.9783
SERPINB2
B: AR vs C: P= 7e-04
N: AR vs C: P= 0.0311
B vs N: P= 0.0093
SH2D1B
B: AR vs C: P= 0.0079
N: AR vs C: P= 0.0418
B vs N: P= 0
SLC26A4
B: AR vs C: P= 0.8371
N: AR vs C: P= 0.0229
B vs N: P= 1e-04
SLC39A8
B: AR vs C: P= 0.0033
N: AR vs C: P= 0.0115
B vs N: P= 0
SLC6A14
B: AR vs C: P= 0.0549
N: AR vs C: P= 7e-04
B vs N: P= 0.0074
SPDEF
B: AR vs C: P= 0.0164
N: AR vs C: P= 0.2523
B vs N: P= 0.0043
TSPAN8
B: AR vs C: P= 0.0079
N: AR vs C: P= 0.2105
B vs N: P= 0.0553
B.AR
B.R
B.C
N.AR
N.R
N.C


Group
Tissue
ALOX15
FCGBP
POSTN
CDH26
TSPAN8
NOS2
SLC6A14
CA2
SH2D1B
CST1
CLCA1
FETUB
SLC26A4
CD36
SPDEF
LOXL4
SLC39A8
SERPINB10
SERPINB2
ITLN1
CH25H
CISH
GADD45G
ANO1
GGH
KCNS3
DPP4
KCNJ16
3
2
1
0
-1
-2
-3
Group
AR
R
C
Tissue
B
N
N.R35
N.R22
N.R12
N.AR1
N.AR43
N.AR31
N.R14
N.AR6
N.AR37
N.C28
N.R10
N.C21
N.R38
N.R13
N.R3
N.AR33
N.R23
N.C17
N.AR30
N.C18
N.C15
N.C40
N.R42
N.C16
N.C2
N.C34
BAR43
BAR31
BR3
BAR1
BAR6
BC28
BR14
BAR30
BR12
BAR33
BR10
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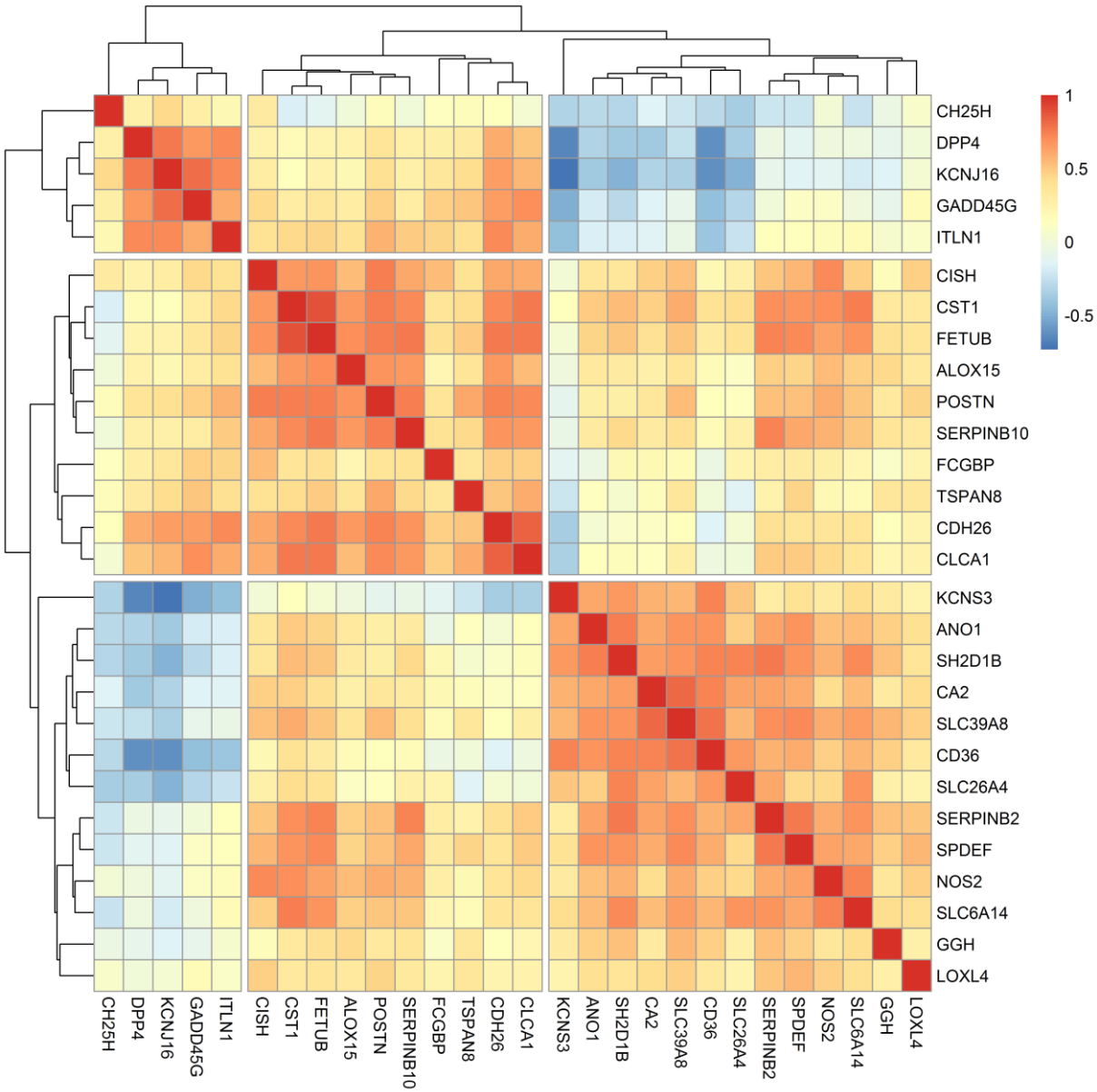
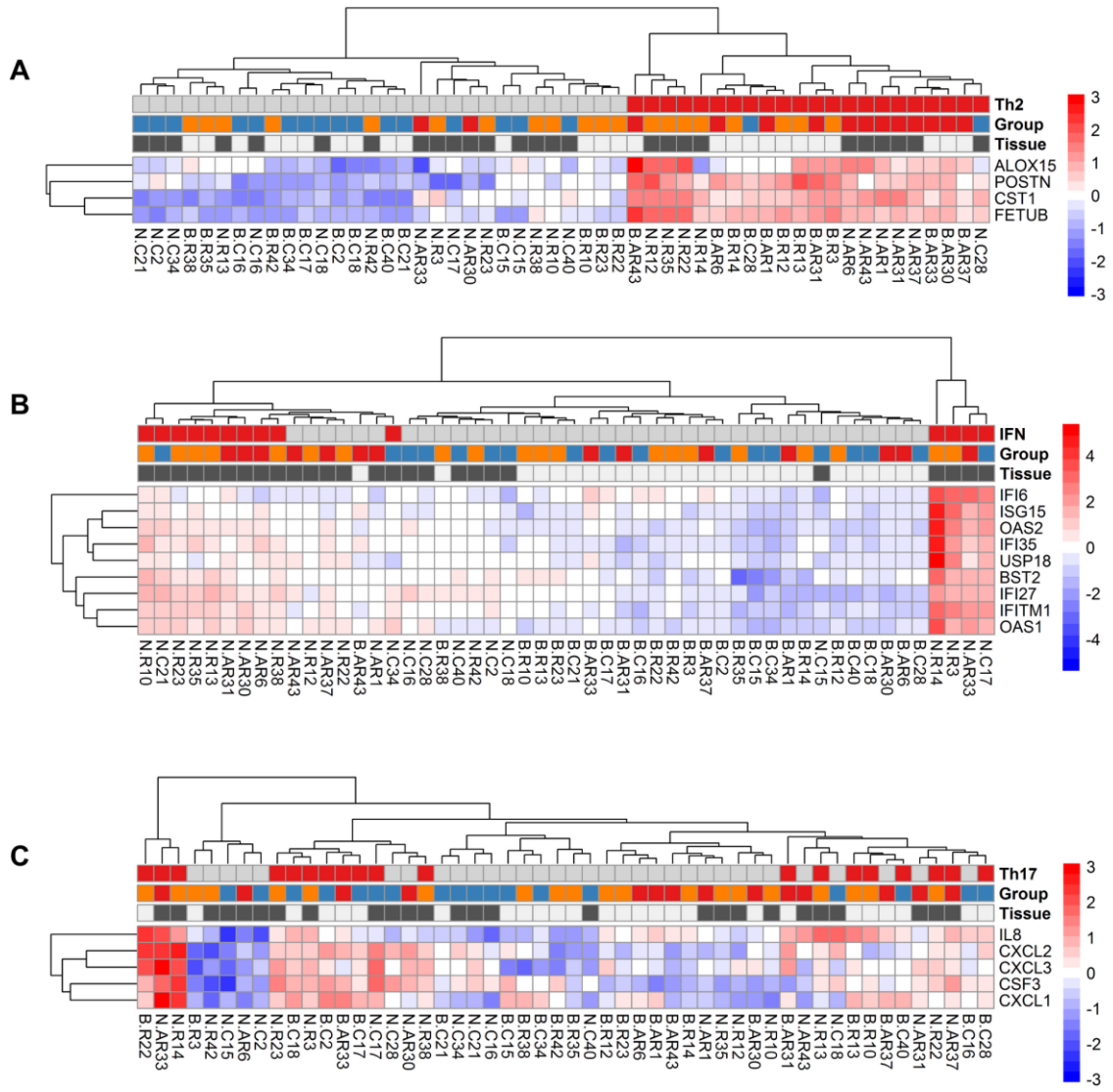
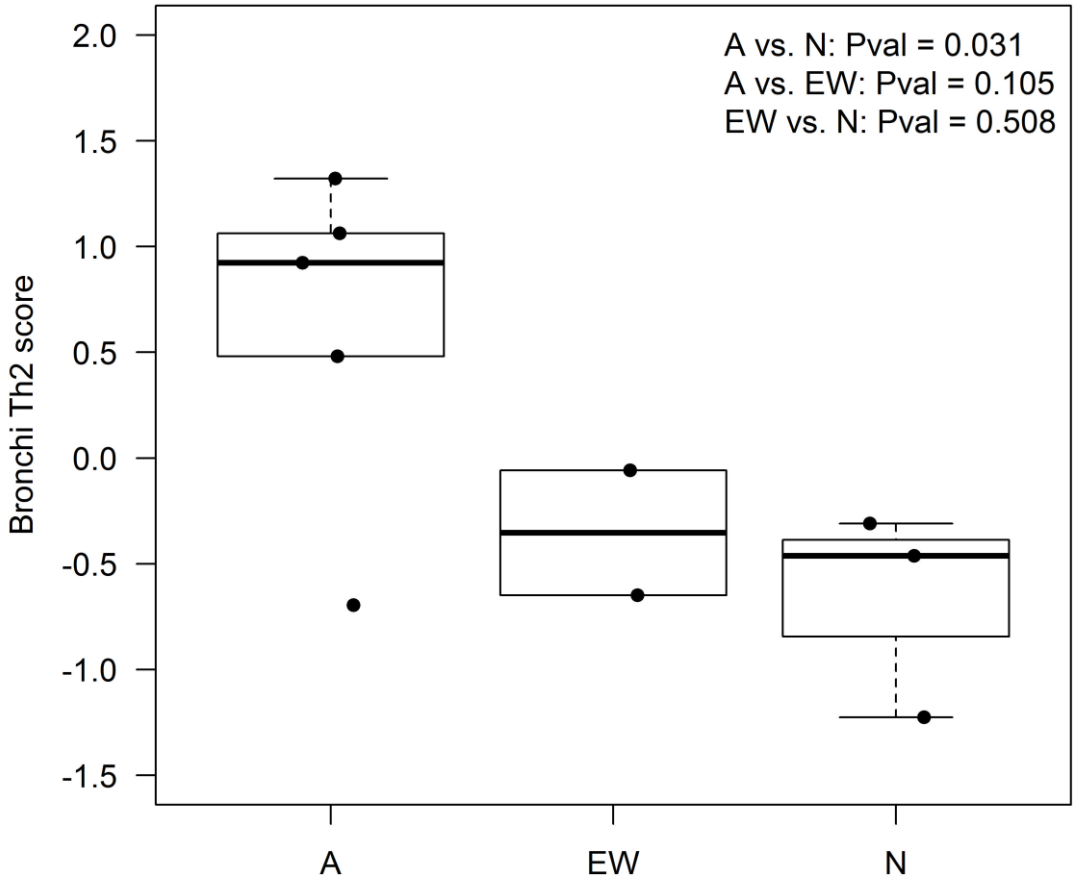
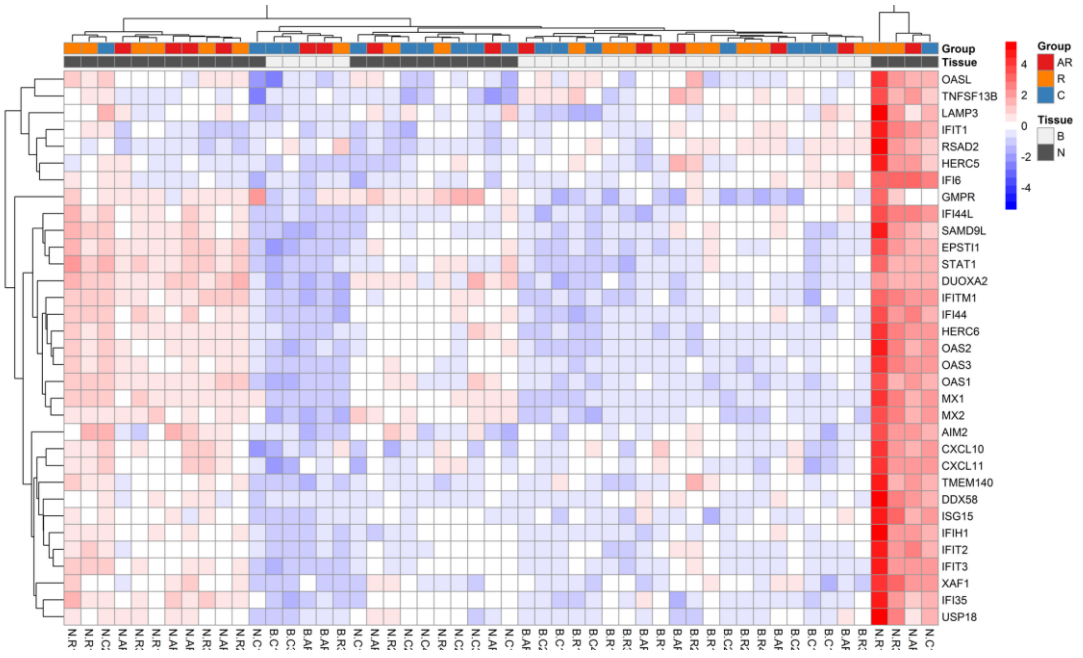
BC16
BR42
BC40


CH25H
DPP4
KCNJ16
GADD45G
ITLN1
CISH
CST1
FETUB
ALOX15
POSTN
SERPINB10
FCGBP
TSPAN8
CDH26
CLCA1
KCNS3
ANO1
SH2D1B
CA2
SLC39A8
CD36
SLC26A4
SERPINB2
SPDEF
NOS2
SLC6A14
GGH
LOXL4
1
0.5
0
-0.5


A
Th2
Group
Tissue
ALOX15
POSTN
CST1
FETUB
3
2
1
0
-1
-2
-3
B
IFN
Group
Tissue
IFI6
ISG15
OAS2
IFI35
USP18
BST2
IFI27
IFITM1
OAS1
4
2
0
-2
-4
C
Th17
Group
Tissue
IL8
CXCL2
CXCL3
CSF3
CXCL1
3
2
1
0
-1
-2
-3


A vs. N: Pval = 0.031
A vs. EW: Pval = 0.105
EW vs. N: Pval = 0.508
Bronchi Th2 score
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
A
EW
N


Group
Tissue
OASL
TNFSF13B
LAMP3
IFIT1
RSAD2
HERC5
IFI6
GMPR
IFI44L
SAMD9L
EPSTI1
STAT1
DUOXA2
IFITM1
IFI44
HERC6
OAS2
OAS3
OAS1
MX1
MX2
AIM2
CXCL10
CXCL11
TMEM140
DDX58
ISG15
IFIH1
IFIT2
IFIT3
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IFI35
USP18
Group
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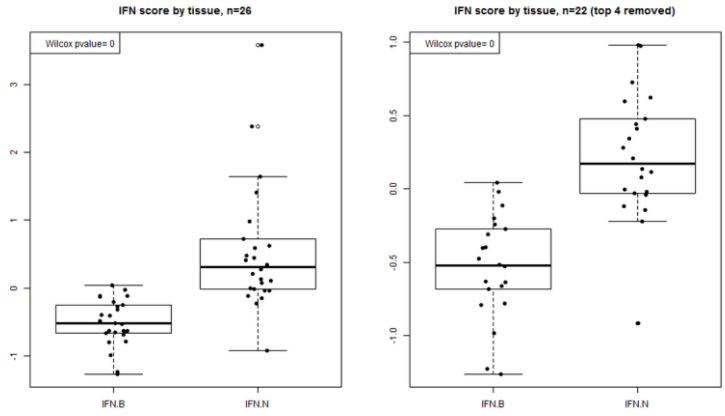
R
C
Tissue
B
N
4
2
0
-2
-4


IFN score by tissue, n=26
Wilcox pvalue= 0
IFN.B
IFN.N
IFN score by tissue, n=22 (top 4 removed)
Wilcox pvalue= 0
IFN.B
IFN.N

Supplementary data

Detailed Material and Methods

1- Subjects and samples

- Subjects

We collected nasal and bronchial brushings from 39 subjects: 12 with both allergic rhinitis and asthma, 14 with allergic rhinitis only, and 13 healthy controls.

A power calculation was performed to determine the sample size using the results of our previous work published in the ERJ[1]. Briefly a power of 90% to detect a log2 fold change >1 at a FDR of 5% for Th2 marker genes would be achieved with experimental of groups of 6-7 patients.

Allergic rhinitis was defined according to 2010 ARIA (*Allergic Rhinitis and its Impact on Asthma*) recommendations[2]. Asthma was defined according to 2012 GINA (Global Initiative for Asthma)[3] and 2007 EPR3 (Expert Panel Report)[4] recommendations.

Three experimental groups were defined: (i) Group AR: allergic rhinitis with asthma. The inclusion criteria were: allergic rhinitis defined with clinical ARIA criteria; mild to moderate persistent controlled asthma defined with clinical GINA and EPR3 criteria; (ii) Group R: isolated allergic rhinitis. The inclusion criteria were: allergic rhinitis defined with clinical ARIA criteria; no asthma defined with clinical GINA and EPR3 criteria; (iii) Group C: healthy controls. The inclusion criteria were: no clinical criteria of allergic rhinitis according to ARIA recommendation, no clinical criteria for asthma according to GINA and EPR recommendations.

Our noninclusion criteria were: rhinitis or bronchial infection during the previous 6 weeks; asthma exacerbation during the previous 6 weeks; chronic disease that could modify the results or expose to risk; intranasal, inhaled or oral corticosteroids during the previous 6 weeks; current tobacco use; smoking cessation for less than 12 months; cumulative smoking

above five pack-years; intellectually disabled; vulnerable person; pregnancy or breast feeding; woman not using contraception.

Our exclusion criteria were: respiratory functional test criteria: severe obstructive pulmonary disease with VEMS < 30% predictive value, severe obstructive pulmonary disease not significantly reversible, obstructive pulmonary disease not corresponding to asthma; serious adverse event; withdrawal of informed consent; violation of protocol; pregnancy.

- Clinical work-up:

We organized a preselection visit and two hospital visits for participants.

The preselection visit consisted of the collection of clinical data and performance of cutaneous allergy tests. Data were collected via allergy interview and physical examination.

Patients' nasal symptoms were investigated through a five-item (PAREO) score derived from [5], which consisted of three levels (0 = no complaint, 1 = light-to-moderate complaints, 2 = high level of complaints) about five clinical parameters: itchy nose (P), anosmia (A), rhinorrhea (R), sneezing (E) and nasal obstruction (O). The total nasal symptom score was calculated by summing the scores of each symptom. The SNOT-22 score[6] was also determined.

All patients were required to discontinue antihistamine medication for at least 5 days before the skin prick test (SPT). The SPT, a total of 18 common standardized allergen extracts (SPT: Alyostal Stallergenesgreer, London, UK and Lancet, Stallerpoint®) was performed on the medial side of the forearm. In our study, the allergens used were house-dust mite (*Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides farinae* (Df)), cockroach (German cockroach), molds (*Alternaria alternata*, Aspergillus mix), animal dander (cat, dog), latex, a five-grasses mixture (*Dactilis glomerata, Phleum pratense, Anthoxanthum odoratum, Lolium perenne*, *Poa pratensis*), *Parietaria officinalis*, *Plantago lanceolata*, sweet wormwood, ambrosia, olive, oak, cypresses, plane tree, trees mixture (alder, birch, hornbean,

hazel). Histamine (1% histamine phosphate) and 0.9% saline were used as positive and negative controls, respectively.

The number of sensitized allergens was calculated as the sum of positive results for the list of allergens given above. An allergen was interpreted as positive if the largest wheal induced was ≥ 3 mm in diameter as recommended[7].

The results were communicated to the participant at the time of testing.

The investigator checked the inclusion and noninclusion criteria and proposed to suitable participants to enter the study.

The first visit included the practice of functional respiratory tests in accordance with ATS (American Thoracic Society)/ERS (European Respiratory Society) recommendations[8]. This visit was realized within two months of the preselection visit.

At the second visit, bronchial endoscopy was performed. This visit was realized within three weeks of visit one.

The project received the approval of the CPP Sud Méditerranée V Ethics Committee (ref 13.032) on July 23, 2013 and all volunteers gave their written informed consent.

- Brushings

We performed a same-day upper and lower endoscopy sampling during the same procedure first the nasal epithelium and then bronchial epithelium.

The brush was passed down the working channel of a bronchoscope and kept concealed within the bronchoscope tip. For nasal brushings: the bronchoscope was passed through a nostril and directed and positioned, either to the medial part of the middle third (the anterior third being squamous) of the inferior turbinate, or to the medial part of the medium turbinate (if the inferior was scarred). For bronchial brushings: the bronchoscope was passed down through the nasopharynx and positioned at the entrance of the right main bronchus. In cases

where the brush was passed down the working channel after aspiration of mucus, the working channel was flushed carefully with sterile saline in order not to plug the brush with mucus.

The cytology brush was advanced, unsheathed and directed until resistance was encountered, either on the medial part of the turbinate or on the lateral wall of right main bronchus.

About twenty rotational movements were performed to sample the cells. The cytology brush was then retracted to just beyond the tip of the bronchoscope without being resheathed (to avoid massive loss of cells when the bristles of the brush directly slid along the interior wall of the sheath at retraction). The bronchoscope and the retracted brush were withdrawn together. The cytology brush was then pushed out from the bronchoscope and transferred into 1.5 ml 4°C Hank's Buffered Salt Solution (HBSS) culture medium (Invitrogen, Carlsbad, CA 92008, United States) in a sterile 15 mL conical polypropylene Falcon tube and its handle cut 3 cm over the top of the tube. The brush was first stirred manually for about one minute and then resheathed-unsheathed about five times in order to remove cells. This procedure was repeated once more for each sampling site with a second cytological brush deposited in the same conical tube. Samples for each site were then split into two parts: 750 µL were used for cell counting and flow cytometry analyses, 750 µL for RNA extraction. The part used for RNA extraction was mixed with 2.25 mL Trizol LS and the tube was vortexed for one minute. The two 15 mL conical polypropylene Falcon tubes were transported on ice to the laboratory.

2- Cell counting

Thoma cell counting (cf http://insilico.ehu.es/counting_chamber/thoma.php)

The large central square of a Thoma cell (1 mm^2, entirely visible with a 10X objective) is divided into 16 medium squares (each one entirely visible with a 40X objective). The height of a cell suspension inserted under a coverslip is equal to 0.1 mm. The volume of the largest square thus corresponds to 10^{-4} ml. The cellular concentration in the sample is equal to f x N x

10^4 cell/ml, where f is the dilution factor introduced at the addition of the Trypan blue, and N is the number of cells detected in the large square.

3- RNA extraction

Total RNA was isolated from brushings using using Trizol LS (Invitrogen), miRNeasy Mini kit (Qiagen, France), according to the manufacturer's instructions. RNA quantity was estimated by spectrophotometry with a Nanodrop ND-1000 UV spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). RNA quality was checked by electrophoresis on a Bioanalyser 2100 (Agilent Technologies, Santa Clara, CA 95051, USA) with an RNA Pico LabChip® (Agilent Technologies, Santa Clara, CA 95051, USA).

4- RNAseq analysis

Mapping was performed with STAR_2.4.0a versus hg19 followed by read counting using featureCounts (subread-1.4.6-p1-Linux-x86_64) with "--primary -g gene_name -p -s 1 -M " options. Quality control of RNAseq count data was assessed using in-house R scripts. Normalization was performed using the voomWithQualityWeights function from the Bioconductor package *limma*[8]. Differential gene expression was assessed using linear models. A paired design was used for the direct comparison between nasal and bronchial tissues. To compare the different combinations of tissue types and clinical conditions, we used a model including a term for batch and sex. For each comparison, differential expression was assessed using moderated *t*-statistics[9]. P values were adjusted for multiple testing using the Benjamini and Hochberg method. Heatmaps were generated using the R package *pheatmap*. Hierarchical clustering of nasal and bronchial samples based on the 63 most differentially expressed genes

was performed using Euclidean distance and complete linkage. Clustering of samples based on the Th2, Th17 or IFN gene signature was performed using 1- abs (Spearman correlation) as distance for genes, Euclidean distance for samples, and complete linkage. RNAseq data are archived in GEO under reference GSE101720. Molecular function and biological networks analysis were performed using Ingenuity Pathway Analysis software (http://www.ingenuity.com/).

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