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Epithelial proteome profiling suggests the essential role of interferon-inducible proteins in patients with allergic rhinitis

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GRAPHICAL ABSTRACT

Background: Seasonal allergic rhinitis (SAR) caused by intermittent exposure to seasonal pollen causes itching, nasal congestion, and repeated sneezing, with profound effects on quality of life, work productivity, and school performance. Although both the genotype and environmental factors can contribute to the immunologic basis of allergic reactions, the molecular underpinnings associated with the pathogenesis of allergic rhinitis are not entirely clear.

Methods: To address these questions, nasal epithelial brushings were collected from 29 patients with SAR and 31 control subjects during and after the pollen season. We then implemented an orbitrap-based, bottom-up, label-free quantitative proteomics approach, followed by multivariate analyses to identify differentially abundant (DA) proteins among the 4 sample groups.

Results: We identified a total of 133 DA proteins for which the most significantly overrepresented functional category was found to be interferon 1 signaling. Two proteins, cystatin 1 and myeloblastin, the former of which protects against protease activity of allergens and the latter with a role in epithelial barrier function, were DA in patients with SAR and control subjects, irrespective of season. Moreover, interferon-inducible protein with tetratricopeptide repeats 1, cystatin 1, and interferon-inducible protein with tetratricopeptide repeats 3 were found to be differentially regulated between patients with SAR and control subjects, with inverse abundance dynamics during the transition from fall to spring.

Conclusion: We identified type 1 interferon–regulated proteins as biomarkers in patients with SAR, potentially playing an important role in its pathogenesis. Moreover, when compared with patients with SAR, healthy subjects exhibit an antagonistic proteomic response across seasons, which might prove to be a therapeutic target for disease prevention. (J Allergy Clin Immunol 2017;140:1288-98.)
Allergic rhinitis is a chronic inflammatory disease of the upper airways that significantly reduces the quality of life of more than 500 million persons worldwide. It is characterized by rhinorrhea, nasal itching, obstruction, and sneezing and is often associated with detrimental effects on sleep, work, social life, and the ability to concentrate at school. In patients with seasonal allergic rhinitis (SAR), different allergens (pollen types) stimulate the production of corresponding allergen-specific IgE antibodies. Current diagnostic approaches rely heavily on questionnaires or measurements of IgE antibodies to specific allergens. Several genetic and environmental factors have been proposed to predispose to the pathogenesis of SAR, but a major challenge remains in that it partially shares biological mechanisms with asthma, chronic rhinosinusitis, food allergy, and atopic dermatitis.

Thus there is a need to elucidate which mechanisms are crucial in development of the disease and which are consequential to facilitate diagnosis, selection of treatment, and design of new treatment strategies. Because nasal mucus and nasal epithelium are the first barriers against allergens, nasal lavage fluid and nasal mucosal brushings have been used to investigate the underlying molecular signatures of SAR. By and large, these studies are sparse, and knowledge gaps still exist pertaining to the specific molecular mechanisms leading to disease onset and progression. In part, this can be attributed to the fact that thus far, especially at the level of disease mechanisms and identify biomarker candidates. In the long run, such an approach will facilitate development of optimized diagnostic and treatment strategies for SAR.

METHODS

Subjects and sampling

This study was approved by the ethics committee of Helsinki University Central Hospital (5/13/03/00/5). All participants provided written informed consent. At the first visit during the pollen season, participants completed an SAR symptom screening questionnaire and a 10-cm visual analog scale of nasal and ocular symptoms within 1 week. Also, skin prick tests (SPTs) to seasonal (birch, alder, meadow fescue, timothy, and mugwort) and perennial (cat, dog, and the house dust mites Dermatophagoides pteronyssinus and Dermatophagoides farinae) allergens and nasal brush samples (FLOQSwab; Copan Diagnostics, Murrieta, Calif) from the middle meatus of nasal cavity without anesthesia were performed. At the second visit after the pollen season, visual analog scales and nasal brush sampling were repeated. Inclusion criteria in the SAR group were a positive SPT response to seasonal allergen combined with relevant moderate-to-severe SAR symptoms according to Allergic Rhinitis and its Impact on Asthma classification during spring and no SAR symptoms or recent contact with allergens to which the subjects were sensitized on SPTs during the fall. The healthy control group had negative SPT responses and no SAR symptoms. Patients with perennial allergic rhinitis symptoms, smoking, antibiotic use during the study period, respiratory tract infections, or fever less than a month before sampling were excluded from the analysis. The study cohort comprised 60 subjects: 29 patients with SAR and 31 nonallergic control subjects. The characteristics of the study subjects are summarized in Table I.

Sample preparation for proteomics

The brush end of a nasal sample applicator was chopped into 1.5-mL tubes containing 900 μL of ice-cold 50 mmol/L ammonium bicarbonate (AMBIC) buffer. The tubes were gently mixed to release cells attached to the brush end of the swab, after which they were taken out with sterile forceps and discarded. All samples were stored at −80°C until required. Once thawed, nasal brushings in AMBIC were concentrated in 10K MWCO reverse spin columns (Amicon Ultra, Merck Millipore, Billerica, Mass). Sample lysis and homogenization were carried out by means of tip-sonicating samples on ice 2 times for 15 seconds with intermittent sonicator tip cooling. The lysates were then solubilized in 0.2% RapiGest SF (Waters, Milford, Mass) in 50 mmol/L AMBIC, pH 7.8. The protein concentration of each sample was determined by using a standard BCA protein assay kit (Thermo Scientific, Waltham, Mass). Ten micrograms of each sample was used (and the rest stored for immunoblot validation) to prepare tryptic peptides for sequencing by means of mass spectrometry (MS) and quantification by means of in-solution digestion of proteins. A detailed description of tryptic peptide preparation for label-free quantification is described in detail in the Methods section in this article’s Online Repository at www.jacionline.org.

Liquid chromatography–tandem mass spectrometry

Tryptic peptides were prepared, as described in the Methods section in this article’s Online Repository, for 10 control subjects and 10 patients with SAR within and outside the allergy season (40 samples in total). Samples were put in autosampler vials and loaded into a nanoLC (Easy nano1000) coupled to a Q Exactive Benchtop MS (Thermo Scientific). Chromatographic separation of peptides was carried out in commercially packed C18 columns (Acclaim PepMap [Thermo Fisher Scientific] C18, 2 μm, 100 Å, 75 μm × 15 cm). Peptides were loaded onto the column with buffer A (5% acetonitrile and 0.1% formic acid) and eluted with a 180-minute linear gradient from 5% to 30% buffer B (100% acetonitrile and 0.1% formic acid), with a single 30-minute wash run alternating between every sample injection. All 40 samples were run in triplicates, resulting in a total of 120 raw files. Mass spectra were acquired in

Key words: Seasonal allergic rhinitis, nasal epithelia, proteomics, interferon I signaling, biomarkers

Abbreviations used

AF: Allergic-Fall
AMBIC: Ammonium bicarbonate
AS: Allergic-Spring
BPIFB4: BPI fold–containing family B member 4
CF: Control-Fall
CS: Control-Spring
CST1: Cystatin 1
DA: Differentially abundant
FDR: False discovery rate
GBP: Guanylate-binding protein
IFT: Interferon-inducible protein with tetra-tricopeptide repeats
LC-MS/MS: Liquid chromatography–tandem mass spectrometry
MS: Mass spectrometry
MX1: Interferon-inducible GTP-binding protein
PRTN3: Myeloblastin
S100A7: S100 calcium-binding protein A7
SAR: Seasonal allergic rhinitis
SPT: Skin prick test
STATH: Statherin
TABLE I. Clinical characteristics of the study subjects

<table>
<thead>
<tr>
<th>Season</th>
<th>Patient groups</th>
<th>Control groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS score, total nasal symptoms (mm), mean (SD)</td>
<td>Patients with allergic rhinitis (n = 10)</td>
<td>Control subjects (n = 10)</td>
</tr>
<tr>
<td>Spring</td>
<td>47.0 (19.6)</td>
<td>27 (5.3)</td>
</tr>
<tr>
<td>Fall</td>
<td>4.6 (5.9)</td>
<td>2.1 (3.3)</td>
</tr>
<tr>
<td>VAS score, rhinorrhea (mm), mean (SD)</td>
<td>43.8 (25.2)</td>
<td>4.0 (5.4)</td>
</tr>
<tr>
<td>Spring</td>
<td>22.9 (13.5)</td>
<td>4.9 (7.1)</td>
</tr>
<tr>
<td>Fall</td>
<td>4.6 (14.1)</td>
<td>1.5 (3.1)</td>
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<tr>
<td>VAS score, nasal congestion (mm), mean (SD)</td>
<td>27.1 (17.5)</td>
<td>1.8 (3.2)</td>
</tr>
<tr>
<td>VAS score, nasal itching (mm), mean (SD)</td>
<td>33.0 (16.1)</td>
<td>2.8 (4.5)</td>
</tr>
<tr>
<td>VAS score, total eye symptoms (mm), mean (SD)</td>
<td>2.5/13/2013 to 19/6/2013</td>
<td>14/10/2013 to 23/10/2013</td>
</tr>
</tbody>
</table>

VAS, Visual analog scale.

Data processing

Proteome identification and quantification from liquid chromatography–tandem mass spectrometry (LC-MS/MS) data were carried out with a label-free algorithm implemented in MaxQuant software (version 1.5.0.30). Default parameters were used for peptide and protein identification, and for enzyme specificity, trypsin allowing for N-terminal cleavage to proline was chosen. An MS/MS spectra search was carried out against the UniprotKB template). Default parameters were used for peptide and protein identification, and for enzyme specificity, trypsin allowing for N-terminal cleavage to proline was chosen. An MS/MS spectra search was carried out against the UniprotKB template). Default parameters were used for peptide and protein identification, and for enzyme specificity, trypsin allowing for N-terminal cleavage to proline was chosen. An MS/MS spectra search was carried out against the UniprotKB template).

For proteomic data obtained on the same LC-MS/MS platform, the false discovery rate (FDR) for the “match between runs” algorithm was 0.12% and 0.44% for identification transfer across 3 and 11 cell lines, respectively. Thus we can expect a “match between runs” FDR of about 5% for these 40 nasal brush samples. We elaborate on the relevance and implications of this in silico pooling approach within the context of our data in the Discussion section in this article’s Online Repository.

Data analysis

Differential abundance analysis and hierarchical clustering were carried out with Perseus data analysis software. Label-free quantification data were imported into Perseus (version 1.5.6) from MaxQuant output files. All intensity values were log2 transformed, and protein identifications classified as only identified by site, reverse, potential contaminant were filtered out from the main data frame. Additionally, only protein identifications with nonzero intensity values in all 3 replicate in silico pools in at least 1 group (be it AS, CS, AF, or CF) were used for differential abundance analysis. By using the imputation function in Perseus, the remaining zero-intensity values were replaced with random numbers from a normal distribution reflective of intensity values for low-abundance measurements.

Multiple hypothesis testing was performed with a Benjamini-Hochberg FDR of 0.05 to determine which protein groups were differentially abundant (DA) among the 4 groups. Protein groups with a fold change in abundance of around 2-fold or more between selected contrast sets were identified by using a standard t test with an FDR of 0.05 and an artificial within-group variance, S0, of 0.2.

Pathway analysis

“Gene” set and functional enrichment analyses were all carried out with Web-based bioinformatics tools (Genemania [http://genemania.org/][22] and EnrichR [http://amp.pharm.mssm.edu/Enrichr/][23]), as indicated in the figure legends, where relevant.

Western blot validation

For validation of LC-MS/MS quantitation, we chose 1 protein that was significantly different between patients with SAR and control subjects only within the allergy season (interferon-inducible GTP-binding protein [MX1], P20591) and 1 protein that was significantly different between patients and
control subjects both inside and outside of the allergy season (myeloblastin [PRTN3], F5H8B6). Antibodies to MX1 (ab95926) and PRTN3 (ab133613) were purchased from Abcam (Cambridge, United Kingdom). Sample preparation for Western blotting was carried out according to standard procedures (see the Methods section in this article’s Online Repository for details) in all 60 subjects: 29 patients with SAR and 31 control subjects. Band intensity quantitation was carried out with ImageJ software (http://imagej.nih.gov/ij/). The band intensity for every sample was normalized to its total protein concentration and expressed relative to the intensity ratio of the pooled sample.

FIG 1. Heat map of DA proteins across all groups. Hierarchical clustering of the 133 proteins found to be DA (FDR < 0.05) between the AS, AF, CS, and CF groups reveals the molecular profile of each group is unique, with the technical replicates being closest together.
RESULTS
Protein concentration
No significant differences in nasal brush protein concentrations were observed in patients and control subjects between seasons or within the same season between patients and control subjects. Mean protein concentrations were 6.5 ± 2.0 μg/μL (AS, n = 29), 7.0 ± 2.4 μg/μL (CS, n = 31), 7.5 ± 2.2 μg/μL (AF, n = 29), and 8.2 ± 2.7 μg/μL (CF, n = 31).

Quantitative proteome differences
A total of 3207 proteins were identified. After filtering out contaminants and proteins without triplicate intensity values in at least 1 group, a total of 2198 unique proteins (see Table E2 in this article’s Online Repository at www.jacionline.org), corresponding to 2024 protein groups in the AS group, 2090 in the CS group, 2066 in the AF group, and 2107 in the CF group, were retained for differential abundance analysis. One hundred thirty-three proteins were found to be DA (ANOVA; Benjamini-Hochberg FDR, 0.05) across these 4 groups (see Table E3 in this article’s Online Repository at www.jacionline.org). Hierarchical clustering of these DA proteins separates all 4 sample groups, with the technical replicates being closest together (Fig 1). A 2-sample t test with a fold change cutoff at approximately 2 was used to highlight proteins that characterized the SAR and control proteomes within (spring) and outside (fall) the pollen season. As expected, the highest and lowest numbers of DA proteins were observed in the AS/CS (43 proteins) and AF/CF (10 proteins) contrasts, respectively. Eighteen proteins were found to be DA in the AF/AS contrast. The CF/CS contrast, with 21 DA proteins, had the second largest number of DA proteins. The gene names and

<table>
<thead>
<tr>
<th>Table II. Gene names and fold change of DA proteins</th>
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<tbody>
<tr>
<td><strong>AF/CF</strong></td>
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<tr>
<td>Gene name</td>
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<tr>
<td>ARPP19</td>
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<tr>
<td>DPYS</td>
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<tr>
<td>C6</td>
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<td>PRTN3</td>
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<td>PRDM1</td>
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<tr>
<td>SCGB2A1</td>
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<tr>
<td>IFIT1</td>
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<tr>
<td>SNRPE</td>
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<td>GBP5</td>
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<td>STATH</td>
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<td>ATG7</td>
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<td>ATP1B3</td>
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<tr>
<td>LACRT</td>
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| Gene names in boldface represent those common to the contrast sets specified in Fig 2.
fold changes of all DA proteins in specified contrast sets are listed in Table II. The ratio of upregulated versus downregulated proteins for each contrast set are highlighted by volcano plots in Fig 2.

To observe the distribution of DA proteins between seasons and between patients with SAR and control subjects, we used Venn diagrams to compare DA proteins for specified contrast sets (Fig 2, B). Only 2 proteins (PRTN3 and cystatin 1 [CST1]) were DA between seasons in patients with SAR and control subjects. However, although they are upregulated during the pollen season and downregulated outside the pollen season in patients with SAR, the reverse is true in control subjects.

FIG 2. Volcano plots (A) and overlapping DA proteins (B) in specified contrast sets are shown. The x-axis and y-axis of the volcano plot depict the log2 fold change and the −log of the t test P value, respectively.

Normalised abundance of CST1, IFIT1 and IFIT3 proteins

FIG 3. Z score-normalised intensities of DA proteins common between the AS/AF and CS/CF contrast sets. IFIT1, IFIT3, and CST1 were found to be DA between seasons in patients with SAR and control subjects. However, although they are upregulated during the pollen season and downregulated outside the pollen season in patients with SAR, the reverse is true in control subjects.

Pathway analysis

Functional enrichment analysis of DA proteins from all contrast sets are depicted in Fig 4. With the exception of the AF/CF contrast, type 1 interferon signaling is among the top 3 most significantly enriched functions. Outside the pollen season (AF/CF contrast), the most enriched pathways were glycosaminoglycan binding, the cytoplasmic membrane-bound vesicle lumen, and secretory granules. In the AS/CS and AS/AF contrast sets, the response to type I interferon and type I interferon pathways have the most intense interactions. In line with this, gene clusters corresponding to the top enriched GO biological pathways reveal that MX1, ISG20, IFIT1 to IFIT3, HLA-C, and GBP2 for AS/CS contrast and MX1 and IFIT1 to IFIT3 for AS/AF contrast are major proteins involved in type I interferon and defense response associated pathways (Fig 5).

Western blot validation

Results of quantitative Western blotting with antibodies to MX1 and PRTN3 on the same MS-quantified samples (10 patients with SAR and 10 control subjects), as well as an additional 40 subjects (19 patients with SAR and 21 control subjects), were consistent with LC-MS/MS data (Fig 6).
DISCUSSION

Because the nasal epithelium acts as a barrier to the external environment and serves as a relay between foreign agents and the immune system (reviewed by Lambrecht and Hammad), it has been routinely sampled to uncover the immunologic basis of allergic respiratory diseases. In this study nasal mucosal cells, which are predominantly (80% to 95%) epithelial, were sampled within and outside the pollen season in a well-defined SAR and control cohort. The total number of 2198 unique proteins quantified in this study represents the most comprehensive nasal brush proteome to date. Analysis of proteome abundance dynamics in the contrast sets provides mechanistic insight that potentially reflects genetic predisposition to sensitization, proteome remodeling that protects against...
sensitization during the transition from fall to spring, and biomarkers of SAR pathophysiology.

Technical limitations are still the major hurdle with regard to sequencing low-level proteins in highly complex samples. A combination of state-of-the-art instrumentation and computational tools can be harnessed to improve MS-based protein identification. The orbitrap-based, data-dependent, label-free quantitative strategy we used in this study is appealing for its dynamic range of quantitation (in the range of 5-6 orders of magnitude) and the fact that it is better suited for discovery because no predetermined peptide lists are required, as opposed to other targeted approaches. However, the stochastic precursor selection associated with data-dependent label-free quantification hampers its effectiveness, especially in highly complex and heterogeneous biological samples. Sample pooling is most often carried out before LC-MS/MS quantitation, which, although having the advantage of requiring less instrument time and decreasing biological variance between “treatments,” has the limitation of potentially masking some proteins that are detectable in individual samples. In our approach to pool samples after LC-MS/MS detection (in silico), individual sample identity is still conserved without increasing biological variance across independent pools. Therefore in silico pooling combined with protein-specific validation is a quantitatively effective strategy to improve proteome coverage in complex samples in discovery proteomics.

We hypothesized that the proteins differentiating between patients with SAR and healthy volunteers, irrespective of the season, could be reflective of underlying genetic differences between these groups. The 2 proteins in our data set that fall within this category (common between the AF/CF and AS/CS groups) are PRTN3 and CST1. Expression of PRTN3, a serine protease implicated in maintenance of endothelial barrier function, was found to be downregulated in patients with SAR compared with control subjects both within and outside the pollen season. Incidentally, increased epithelial permeability is a key pathophysiological feature of allergic rhinitis.

CST1, the second protein from our data in this category, is a member of the cystatin superfamily of cysteine protease inhibitors, which are thought to play a protective role against cysteine proteases of host or foreign origin. In our data CST1 was upregulated and then downregulated during and outside the pollen season, respectively, in patients with SAR. This is in line with microarray studies in a Japanese SAR cohort, wherein the CST1 gene was found to be upregulated in symptomatic patients with SAR exposed to Japanese cedar pollen.
FIG 6. Western blot validation of MX1 and PRTN3. A, LC-MS/MS quantified Log₂ intensities from in silico pools of 10 subjects across 3 replicates for both MX1 and PRTN3. B, Technical validation of MS quantitation by means of Western blotting. Data points are normalized Western blot intensities (means ± SEMs) individually measured from the same 10 subjects per group used for MS analysis. C, Functional validation of MX1 and PRTN3 abundance levels by means of Western blot analysis of the 20 MS-quantified samples plus an additional 40 samples, making up a total of 60 subjects. Error bars are means ± SEMs of 29 patients with SAR or 31 control subjects. In both Fig 6, B and C, Western blot intensities are normalized to total protein content in each sample and band intensity of a pooled sample loaded in each gel. *P < .05, **P < .01, and ***P < .001.
upregulated CST1 transcripts in nasal and bronchial epithelia were recently patented as one of several IL13-dependent biomarkers that can be used to identify asthmatic patients with a high risk of disease exacerbation. In the same study, by using unsupervised clustering, genes with a positive correlation with IL13 levels (among which CST1 showed the second strongest positive correlation) were found to distinguish asthmatic patients with self-reported rhinitis.

The second category of DA proteins are those common to both the AS/AF and CS/CF contrasts but with an antagonistic expression profile in patients with SAR and control subjects. In our data set we identified 3 proteins in this category: IFIT1, IFIT3, and CST1. Given that these proteins are among those undergoing the biggest changes in abundance between seasons in patients with SAR, we can deduce that their antagonistic abundance profile observed in control subjects is likely reflective of a distorted protective mechanism in patients with SAR. These antagonistic abundance dynamics (Fig 3) of the nasal epithelial proteome are in line with what was observed for secreted nasal proteins in patients with SAR versus control subjects within and outside the pollen season.

The third category of proteins highlighted in this study are candidate biomarkers for diagnosis of SAR. Ideally, their abundance profiles should be directly related to symptom onset and offset, and therefore these are the DA proteins that are common between the AS/AF and AS/CS contrasts. From these 2 contrast sets, we observed an upregulation of MX1 (3- to 4-fold), BPIFB4 (4 fold), IFIT1 (5- to 6-fold), IFIT2 (6- to 7-fold), IFIT3 (7- to 10-fold), CST1 (5- to 6-fold), and GBP5 (4- to 7-fold) and a downregulation of S100A7 (3-fold) in patients with SAR during the allergy season (Table II). All 8 of these proteins are involved in immune response and immunomodulatory activities. Specifically, upregulation of CST1, IFIT1, IFIT2, IFIT3, GBP5, and MX1 has been shown to mediate/enhance antiviral defense mechanisms in vitro and in vivo.

Because the majority of these candidate SAR biomarkers are involved in protection against viral infection, it is not surprising that on a systemic level, interferon 1 signaling was the most significantly affected endogenous pathway. It seems that as a result of nasal epithelial barrier dysfunction and dysregulated protective mechanisms, an exaggerated antiviral-type inflammatory response is triggered in patients with SAR on seasonal exposure to environmental stimuli. These antiviral immune responses might contribute to symptoms and airway hyperreactivity by causing an influx of inflammatory cells that negatively affect airway physiology. In fact, within the context of inflammatory disease, allergic sensitization, viral infections, or both are recognized risk factors of asthma exacerbations in older children and adults with asthma. Thus in the same way it is plausible that in patients with SAR, a dysfunctional and overactivated antiviral response during allergen exposure leads to abnormal production of interferon-inducible proteins. These in turn result in overproduction of proinflammatory cytokines and chemokines, finally leading to exaggerated inflammatory reactions in the nasal mucosa that contribute to the disease phenotype. It is also possible that allergic sensitization increases the susceptibility to viral infection or vice versa.

In conclusion, nasal epithelia continues to be a minimally invasive and highly informative avenue to study SAR. In this study we have provided the most comprehensive nasal brush proteome to date, which serves as an important data source to explore the molecular signatures and underlying pathologic mechanisms of SAR.

Our study identified season-independent differences in the nasal epithelial proteome (PRTN3 and CST1) between patients with SAR and healthy control subjects reflective of potential molecular mechanisms underlying SAR. We also demonstrated that, when compared with that of patients with SAR, the nasal epithelium of healthy subjects undergoes antagonistic proteome remodeling (IFIT1, IFIT3, and CST1) during onset of the pollen season. Therefore, after additional validation, downregulation of PRTN3 and upregulation of CST1 could be used as biomarkers of SAR outside the allergy season, and upregulation of IFIT1 and IFIT3 could be used to confirm SAR development during the allergy season. Finally, SAR pathophysiology appears to be principally mediated through an exacerbated expression of interferon inducible proteins which leads to the aggravated nasal inflammation. Together, our data indicate that type 1 interferon–regulated proteins can play a prominent role in the pathogenesis of SAR, and further studies exploring their diagnostic or therapeutic potential for SAR are warranted.

We thank Sauli Savukoski, Outi Fischer, Suvi-Päivikki Salo, Niina Ahonen, and Sari Tillander for their assistance with sample collection, processing, and storage. Special thanks go to Dario Greco for valuable discussions on -omics data analysis and Niina Ahonen for Western blot sample preparation. Additional image attribution for graphical abstract: Fall by Kooteo (own work; CC BY-SA 4.0’ http://creativecommons.org/licenses/by-sa/4.0/), through Wikimedia Commons. Spring by Benjamin Gimmel (GFDLCC-BY-SA-3.0; http://creativecommons.org/licenses/by-sa/3.0/), through Wikimedia Commons. Nasal Swab: authors’ own image.

Key messages
- Candidate biomarkers of SAR were identified.
- Interferon-inducible proteins seem to be key players in the pathogenesis of SAR.
- The proteome of healthy subjects exhibits an antagonistic response to seasonal changes that might serve to protect against pollen sensitization.

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