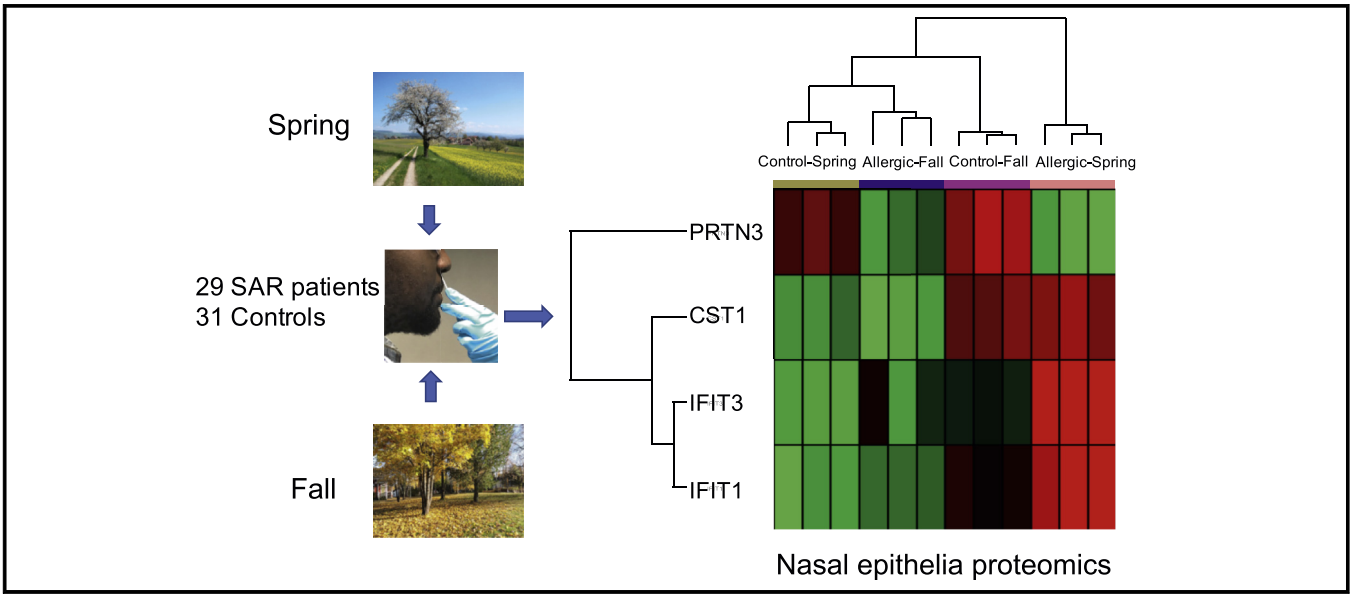
Epithelial proteome profiling suggests the essential role of interferon-inducible proteins in patients with allergic rhinitis

Joseph Ndika, PhD,[a] Liisa Airaksinen, MD, PhD,[b] Hille Suojalehto, MD, PhD,[b] Piia Karisola, PhD,[a] Nanna Fyhrquist, PhD,[a] Anne Puustinen, PhD,[c] and Harri Alenius, PhD[a,d] *Helsinki, Finland, and Stockholm, Sweden*

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.)

From [a]the Department of Bacteriology and Immunology, Medicum, University of Helsinki; [b]Occupational Medicine, Finnish Institute of Occupational Health, Helsinki; [c]the Department of Clinical Chemistry, Helsinki University Hospital and University of Helsinki; and [d]the Institute of Environmental Medicine, Karolinska Institutet, Stockholm.

Supported by Allergiasäätiö (Allergy Research Foundation: Alenius, H; 2013).

Disclosure of potential conflict of interest: J. Ndika personally received a grant from Allergiasäätiö for this work and his salary was covered by a grant received by H. Alenius. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication November 23, 2016; accepted for publication May 9, 2017.

Available online June 19, 2017.

Corresponding author: Harri Alenius, PhD, Institute of Environmental Medicine (IMM), Karolinska Institutet, C6, Systems Toxicology, Box 210 171 77, Stockholm, Sweden. E-mail: Harri.Alenius@ki.se.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2017.05.040

Key words: *Seasonal allergic rhinitis, nasal epithelia, proteomics, interferon 1 signaling, biomarkers*

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.[1] 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.[2] Several genetic and environmental factors have been proposed to predispose to the pathogenesis of SAR,[3-5] but a major challenge remains in that it partially shares biological mechanisms with asthma, chronic rhinosinusitis, food allergy, and atopic dermatitis.[1,2,6-8]

Thus there is a high 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[9-13] and nasal mucosal brushings[14-16] 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 the proteome, only a single study has assessed more than 1 time point.[12] In this regard we have implemented a proteomic assessment of nasal brushings within and outside the pollen season in patients with SAR and healthy control subjects to unravel the molecular basis of SAR.

This is the first study to investigate differences in protein abundance within cells of the nasal mucosa during and after the pollen season between patients with SAR and healthy control subjects, the goal of which is to shed more light on the underlying disease mechanisms and identify biomarker candidates. In the long run, such an approach will facilitate development of optimized diagnostic and treatment strategies for SAR.

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
IFIT:	Interferon-inducible protein with tetratricopeptide 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

METHODS

Subjects and sampling

This study was approved by the ethics committee of Helsinki University Central Hospital (5/13/03/00/3). 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[1] 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