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Proteomic analysis of human nasal mucosa: different expression profile in rhino-pathologic states

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Summary
Background. *Rhinitis comprises several diseases with varying causes and different clinical manifestations and pathological features, but treated as a single clinical disorder. As heterogeneous disease, proper differential diagnosis is useful to delineate appropriate therapeutic intervention. Comparative proteomic investigation was aimed to provide information for specific differentially expressed proteins in rhino pathologic state, that could be used for diagnostic purpose and therapeutic monitoring.* ***Methods.*** *Proteins extracted from nasal mucosa cells of patients with different features of rhinitis and from control subjects, were separated by 2-DE. Proteins differentially expressed were identified by mass spectrometry (MS).* ***Results.*** *Comparative proteomic analyses led to the identification of eighteen proteins differentially expressed in patients with rhinitis, mainly related to cell defense and innate and acquired immunity. From that, at least one protein can be a possible candidate as biomarker of disease.*

Introduction

The nasal mucosa tissue is a complex organ responsible for several functions of the nasal airway and, together with the osteo-meatal complex, is regarded as the critical area of the nasal cavity in the pathogenesis and treatment of various pathologic conditions. Rhinitis is one of the most common health care problems affecting a high percentage of the population and having a significant adverse effect on life quality and daily functioning (1). Historically treated as a single clinical disorder, rhinitis comprises several diseases with varying causes, each one characterized by distinct clinical manifestations and pathological features. These include allergic rhinitis affecting 30% of the population, non-allergic vasomotor rhinitis "cellular" (NARES, NARMA, NARESMA) affecting less than 15% of the population and nasal polyposis affecting 4% of the population (2-3). Many aspects of disease pathophysiology still remain unknown. Currently, clinical diagnosis and determination of the therapeutic effects mainly depend on the observation of nasal mucosal changes as clinical presentations, cellular and molecular characteristics. As it is a heterogeneous disease, proper differential diagnosis is required to delineate appropriate therapeutic intervention. This aspect includes methods for defining disease-specific differences. Proteomics is a modern approach aimed at decoding information con-

tained in genomic sequences in terms of protein structures and functions as well as in the control of biological processes and pathways. In the biomedical field, the comprehensive study of the cell proteome is providing insight into the molecular mechanisms of a variety of physiopathological processes and disease states. The potential of proteomics in identifying disease biomarkers is promising. However, this potential has yet to be explored across different subsets of patients with rhinitis. In this respect, a comprehensive analysis of protein components of nasal mucosa cells could be useful for evaluating the protein factors that are altered in pathologic states.
In the present study, comparative proteomics was applied to characterize the nasal mucosal proteome and identify protein differentially expressed in nasal mucosa cells of patients affected by different rhino-pathologic diseases.

Materials and methods

Demographics and sample collection

A case-control study was performed in the Rhinology Clinic of the Otolaryngology Unit of the University of Bari (Italy). Ethical approval and informed consent were obtained from participating institutions and individuals, respectively. In total eight volunteers, four patients with rhinitis and four control subjects were recruited for sample collection. The patient's history was taken to determine the presence of a familiar disease (atopy, asthma and ASA sensibility). The medical case history was also determined to gain suitable information on asthma, aspirin allergy, headache and/or facial pain, nasal obstruction, type of rhinorrhea, itch, sneezing, daytime or night-time cough, halitosis, postnasal drip and fever.

Skin prick test and nasal cytology

Allergic sensitization was assessed by skin prick test (4) using a definite panel of allergens (100 IR/mL), including house dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*); cat; dog; grass mix; *Compositae* mix; *Parietaria judaica*; birch, hazel and olive trees; *Alternaria tenuis*; *Cladosporium*; and *Aspergilli* mix) (Stallergenes, Milan, Italy).
Equivocal skin test results were further investigated by a CAP-RAST assay (Phadia, Uppsala, Sweden). Endoscopic examination was performed with a 3.4 mm diameter of flexible fiberscope ENT 2000 (Vision Sciences®, USA). Patients were rated according to the following objective criteria:

1. Presence of intranasal anatomic alterations (septal deviation, cartilaginous spurs, Concha bullosa, intranasal tumors).
2. Mucosal appearance (hyperemia, edema, atrophy, areas of de-epithelialization).
3. Type of secretions (serous, mucous, pus, hematic, exudate-clotted scabs) on the basis of the prick test and nasal cytology.

Nasal cytology was performed by anterior rhinoscopy. Nasal mucosa cells were collected from the middle portion of the inferior turbinate by a non-invasive scraping procedure (5). An aliquot of samples was placed on a glass slide, fixed by air-drying and then stained by the May-Grunwald Giemsa method (Carlo Erba, Milan, Italy). The slide was observed under a Nikon E600 light microscope (Nikon, Canada) equipped with a digital camera (Nikon "Coolpix 3:34") for the acquisition of microscopic images. Cell preparations were qualitatively evaluated to confirm the collection of a sufficient number of intact cells and the absence of evident contamination by blood cells caused by

Table 1 *- Clinical and cytological characteristics of the subjects examined.*

Control Subjects	Age	Sex	Prick test	Nasal Cytology	Diagnosis
Control 1	59	F	Neg.	Neg.	Negative
Control 2	24	F	Neg.	Neg.	Negative
Control 3	25	M	Neg.	Neg.	Negative
Control 4	55	M	Neg.	Neg.	Negative
Rhino-pathologic subjects					
Patient 1	32	F	Neg.	E++++/Mas+++++	NARESMA
Patient 2	23	F	Neg.	E+++	NARES
Patient 3	34	M	Parietaria. Cypress.	N+++ E+/Mast+	Allergic rhinitis
Patient 4	26	M	Olive, Cypress, Ep. Cat	N+++ E++++/Mast++++	Allergic rhinitis with Nasal polyposis

occasional bleeding. For the rhinocytogram analysis, 50 microscopic fields were read at a magnification of 1000 × to assess the presence of normal and abnormal cellular elements, along with microscopic features (spots, special inclusions, etc.) important for diagnosis. Cell counts, bacterial and fungal analysis, were carried out by a semi-quantitative grading (6). Based on prick test results and nasal cytology results, patients were subdivided into subjects with non-allergic and allergic rhinitis (**table 1**). Cellular forms were further subdivided based on their cytotype as follows: non-allergic rhinitis with eosinophils and mast cells (NARESMA; eosinophils > 20% and mast cells > 10%) (**figure 1**, Patient 1); non-allergic rhinitis with eosinophils (NARES; eosinophils > 20%), (**figure 1**, Patient 2); allergic rhinitis with neutrophil and eosinophils, (**figure 1**, Patient 3); allergic rhinitis with nasals polyposis, neutrophils eosinophils and mast cells, (**figure 1**, Patient 4).

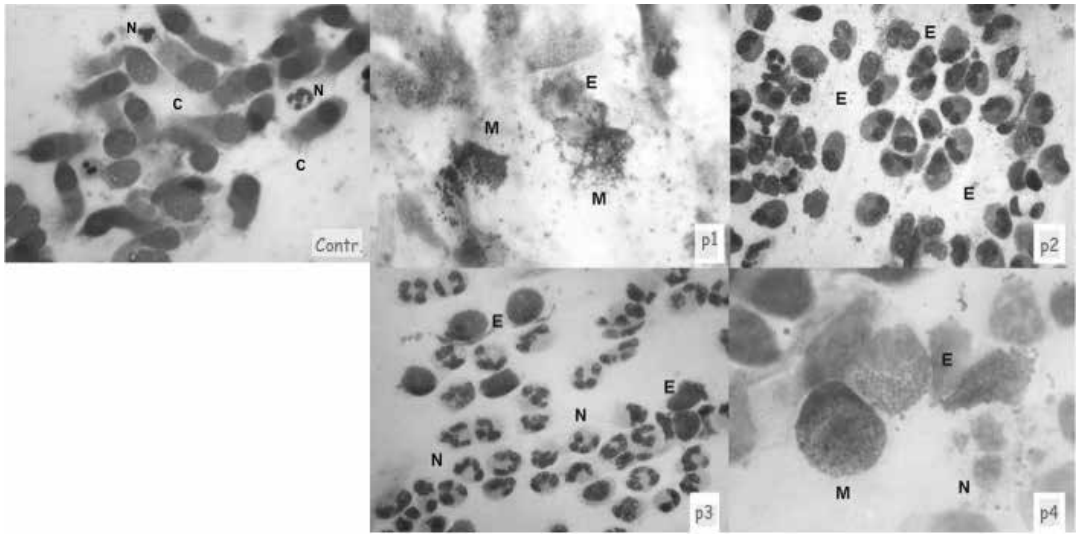


Figure 1 *- Nasal cytology: control normal subjects; Patient 1: non-allergic rhinitis with mast cells and eosinophils, (NARESMA); Patient. 2: non-allergic rhinitis with eosinophils (NARES); Patient. 3: allergic rhinitis; Patient. 4: allergic rhinitis with nasals polyposis. E: eosinophils; M: mast cells; N: neutrophils. May-Grünwald Giemsa staining, original magnification X 1000.*

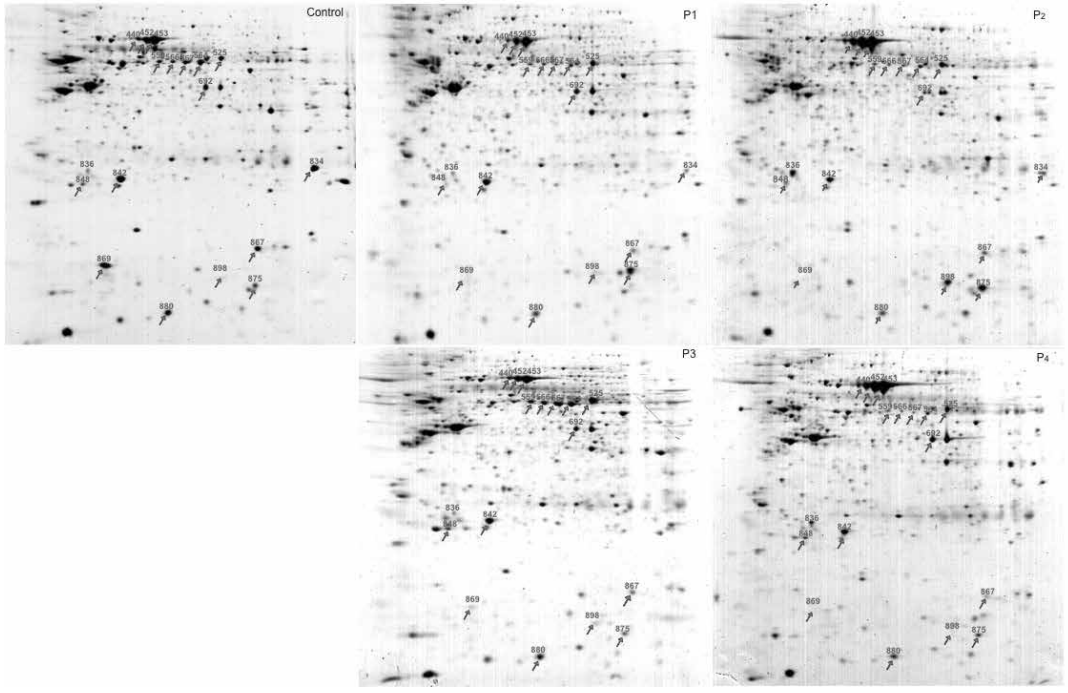


Figure 2 *- Overview of the 2-DE maps of control and patient subjects. Protein spots showing different intensity in the 2-DE maps of the four patients with respect to control subjects are indicated by match ID numbers.*

Figure 3 *- Quantitative analysis of spots showing different intensity. Each spot is indicated with a match ID.*

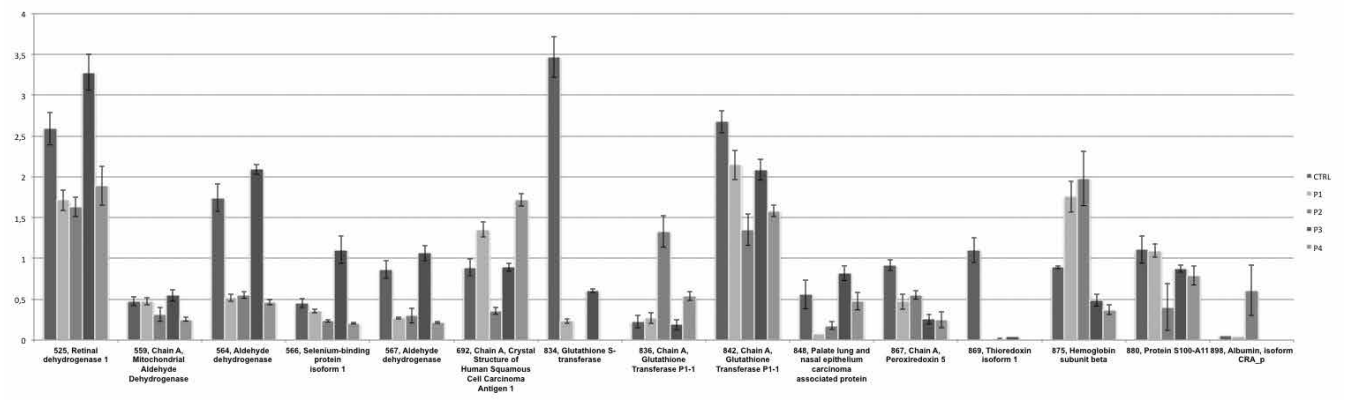
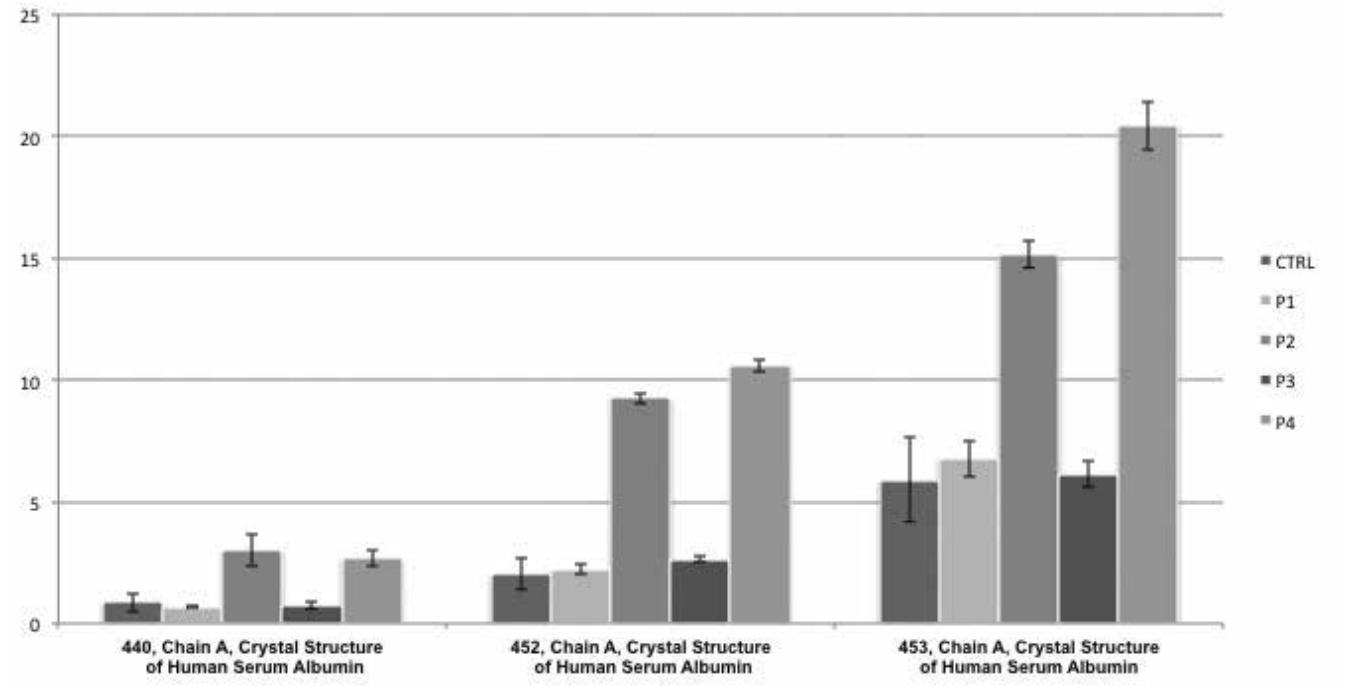


Figure 4 *- Quantitative analysis of spots containing Albumin. Each spot is indicated with a match ID.*



Protein extraction from nasal mucosa cells

Three samples of mucosa cells from each subject were collected separately during the pollen season. Immediately after scraping, recovered cells were washed with ice-cold PBS, pelleted and preserved at -80°C until analysis. To prepare soluble protein fractions, pelleted cells were thawed on ice and suspended in lysis buffer containing 7 M urea, 2 M thiourea, 4% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 50 mM 1,4-dithio-DL-threitol (DTT) and protease inhibitors cocktail (Sigma-Aldrich, St. Louis, MO, USA).

Cell lysis was achieved by sonication cycles on ice (10 × 10 s pulses with a 30 s interval between each ultrasonic cycle). The

sample was clarified by centrifugation at 13 000 rpm for 10 min at 4°C to remove cellular debris. Protein concentration was determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instruction (7). Soluble protein samples were stored at -80°C until use.

Two-dimensional gel electrophoresis (2-DE)

Proteins were separated by 2-DE essentially as previously described (8,9). 250 µg of each protein sample, diluted in the IPG strip rehydration buffer, containing 8 M urea, 2% (w/v) CHAPS, 2% (w/v) DTT, was loaded on a 24-cm IPG strip with a linear 3-10 pH gradient. Isoelectric focusing was carried out at 20°C using the Ettan IPGphor Isoelectric Focusing System (GE Healthcare, Amersham Biosciences AB, Uppsala, Sweden) to 70 kVh. After isoelectric focusing the IPG strips were equilibrated for 15 min in the sodium dodecyl sulphate (SDS) equilibration buffer (50 mM Tris/HCl, pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, containing 1% (w/v) DTT) and for further 15 min in the same equilibration buffer containing 2.5% (w/v) iodoacetamide and trace of bromophenol blue. The second-dimensional gel electrophoresis (SDS-PAGE) was carried out using the vertical slab separation unit Ettan Dalt II System (GE Healtcare). Homogeneous 12,5% polyacrylamide gel was used in a Laemmli buffer system (10) at a constant current of 15 mA gel^{-1} and at 10°C. The gels were stained using Brilliant blue G-colloidal concentrate (Sigma, St. Louis, MO, USA) (11).

Image analysis of protein patterns

The coomassie-stained 2-DE gels were scanned with an image scanner at 300 dpi resolution to acquire gel images. Image analysis was performed as reported (12), using Image Master 2-DE software v. 6.0 (GE Healthcare). Briefly, spot detection was carried out using the optimal values for spot intensity, spot area and saliency determined by applying real-time filters in order to minimize the detection of artefacts and to maximize the real spot detection. Three gels from each sample were used to create the five match sets with one gel included from each of three protein preparation repeats. Relative spot volume (% volume), i.e. digitized staining intensity integrated over the area of the individual spot divided by the sum of volume of all spots in the gel and multiplied by 100, was used for spot quantification (13). The match identification number (ID) was used to identify all spots in a match. Spots present in all the gels of the five classes and exhibiting an intensity difference between the five samples with a P value < 0.05, using the two-tailored Student's t-test, were considered to be differentially expressed.
Spots of interest were excised from 2-DE gels and in-gel digested using trypsin (14). Protein identification was achieved by Matrix Assisted Laser Desorption Ionization - Time of Flight - Mass Spectrometry (MALDI-TOF-MS) analyses performed on a Voyager DE PRO mass spectrometer (Applied Biosystems Foster City, CA) and database searches (12).

Results

Validation of nasal cell samples

Nasal mucosa cells samples from four controls and four rhino-pathologic patients with 34,75 years mean age (range 23-59 years) were obtained by a non-invasive nasal scraping procedure followed by their quality evaluation by cytology (**table 1**). Control samples showed a normal cytology, characterized by numerous well-formed ciliated cells and some rare neutrophils (**table 1** and **figure 1**, *Control*). The cytological analysis of nasal mucosa of the four rhino-pathologic patients showed a significant infiltration of inflammatory cells with variable percentages of neutrophils, eosinophil and mast cells (**figure 1**, *P1, P2, P3, P4* and **table 1**).

Proteome analysis of nasal mucosa

Comparative proteomic analysis of the nasal mucosa cells from controls and rhino-pathologic subjects was performed integrating 2-DE and mass spectrometric methodologies. Proteomic maps showed several hundreds of well-resolved protein spots distributed over a wide range of pI values and molecular masses (**figure 2**). The overall position and number of protein spots observed in the 2-DE maps were similar in the four control samples (476 ± 24, 458 ± 32, 470±19, 481± 57 for C1, C2, C3, and C4 respectively), and in the four patients samples (459 ± 37, 448 ± 43, 467 ± 83 and 489 ± 63 for P1, P2, P 3 and P4 respectively). For each subject, three biological samples were obtained and each sample was run in triplicate (figure 1-S and 2-S, in supplementary materials). The percent of matches between gels from the same class and from the five different classes was similar (around 65%). In order to analyze proteins differentially expressed in the four patient samples with respect to the control samples, only the matched spots present in all gels were investigated. Among this group of spots, only those showing a different intensity with a $P < 0.05$ were considered for further mass spectrometric analyses. Using this constrained statistical analysis, no significant variation of spot intensity was revealed comparing the 2-DE maps of the four-control subjects that can be therefore considered as homogeneous reference to pathological samples. Eighteen spots marked in **figure 2**, had different intensity in the 2-DE maps of the four patients samples compared to the control subjects. The data clearly indicated that differences in the proteome profile of the four patients samples were mostly affected by the specific pathology. Spots of interest

Table 2 *- Identification of proteins differentially expressed in nasal mucosa cells by PMF strategy. Functional classification according to Gene Ontology and regulation of protein expression level in the four patients.*

							2-DE maps Image analysis (fold change)[d]			
Spot ID[a]	**Protein Name**	**Gene**	**Biological process/ Molecular function**	**Accession number (NCBInr)**	**MW[b] (KDa)**	**pI[c]**	**P1**	**P2**	**P3**	**P4**
559	Chain A, Human Mitochondrial Aldehyde Dehydrogenase	ALDH2	carbohydrate metabolic process / aldehyde dehydrogenase (NAD) activity	gi|328877202	54875	5.69	(1,04)	(-1,2)	1,17	Down (-1,85)
564	Aldehyde dehydrogenase	ALDH3A1	cellular aldehyde metabolic process / aldehyde dehydrogenase (NAD) activity	gi|178375	50703	5.99	Down (-3,5)	Down (-3,1)	(1,2)	Down (-4,0)
567	Aldehyde dehydrogenase	ALDH3A1		gi|178375	50762	6,00	Down (-3,45)	Down (-2,87)	1,2	Down (-4,31)
440	Chain A, Crystal Structure of Human Serum Albumin	ALB	platelet degranulation transport response to stress response to nutrient blood coagulation / DNA binding antioxidant activity	gi|3212456	68425	5,60	(-1,29)	Up (3,49)	1,008	Up (3,14)
452	Chain A, Crystal Structure of Human Serum Albumin	ALB		gi|3212456	68425	5,61	(1,085)	Up (4,54)	(1,3)	Up (4,41)
453	Chain A, Crystal Structure of Human Serum Albumin	ALB		gi|3212456	68425	5.62	(-1,3)	Up (3,5)	(-1,1)	Up (3,1)
898	Albumin, isoform CRA_p	ALB		gi|119626079	23183	8.20	-- [e]	0.61±0.31[f]	--	--
875	Hemoglobin subunit beta	HBB	Oxygen transport / heme binding	gi|4504349	16102	6.75	UP (2,1)	UP (2,9)	(-1,6)	Down (-2.0)
867	Chain A, Human Peroxiredoxin 5	PRDX5	cellular response to reactive oxygen species / antioxidant activity	gi|46015018	18327	7.94	(-1,7)	(-1,7)	Down (-4,6)	Down (-3,9)
834	Glutathione S-transferase	GSTA2	glutathione metabolic process / glutathione transferase activity	gi|825605	25650	8.51	Down (-13,9)	--	Down (-6,5)	--
836 842	Chain A, Glutathione Transferase P1-1	GSTP1	cellular response to reactive oxygen species / glutathione transferase activity	gi|11514451	23394	5.74	Down (-3,5)	Down (-3,1)	(1,2)	Down (-4,0)
566	Selenium-binding protein1 isoform	SELENBP1	protein transport / selenium binding	gi|16306550	52928	5.93	(-1,1)	(-1,7)	(1,8)	Down (-2,0)
525	Retinal dehydrogenase 1	ALDH1A1	ethanol oxidation / Ras GTPase activator activity aldehyde dehydrogenase (NAD) activity	gi|21361176	55454	6.30	(-1,5)	(-1,58)	(1,26)	(1,37)
869	Thioredoxin isoform 1	TXN	cell redox homeostasis / peptide disulfide oxidoreductase activity	gi|50592994	12015	4.82	--	Down (-2,8)	--	--
880	Protein S100-A11	S100A11	negative regulation of DNA replication / calcium ion binding	gi|5032057	11847	5.56	(1,0)	Down (-2,5)	(-1,5)	(-1,5)
692	Squamous Cell Carcinoma Antigen	SERPINB4	immune response / serine-type endopeptidase inhibitor activity	gi|239552	44564	6.35	UP (1,8)	down (-2,0)	(1,3)	UP (2,4)
848	Palate lung and nasal epithelium carcinoma associated protein	BPIFA1	innate immune response / lipid binding	gi|114319015	25355	5.65	--	--	(1,4)	Down (-2,1)

[a]ID Spot Identification Number. [b]Theoretical molecular weight (kDa). [c]Theoretical isoelectric point.

[d]Changes in protein expression levels are reported as the ratio between the normalized protein spot volume from the 2-DE map of Patient (1-4) and that of *Control* ($V_{patient}/V_{Control}$) for up-regulated proteins while for down-regulated proteins as the negative reciprocal values ($-V_{patient}/V_{control}$). Only fold change > 2 are indicated as up or down regulation.

[e]The symbol -- indicates that spot containing this protein was not detected in the 2-DE maps of this patient.

[f]Spot containing this protein is only present in the 2-DE map of P2.

were identified by Peptide Mass Fingerprint (PMF) strategy and results are summarized in **table 2**. The differentially expressed proteins were classified on the basis of their biological and molecular functions by means of Gene Ontology and belonged to several functional categories as follows:
(i) proteins involved in cell detoxification and cell defense: glutathione S-transferase A-2 (GSTA-2), spot ID 834, under expressed in all the four rhino pathologic subjects, glutathione transferase P1-1 (GSTP-1) spot ID 842, under expressed in P1, P2 and P4, chain A, human peroxiredoxin-5 (PRDX5), spot ID 867, under expressed in all the four pathologic samples, and selenium binding protein (SELENBP-1), spot ID 566, under expressed in P2 and P4;
(ii) proteins related to immune responses: squamous cell carcinoma antigen (SERPINB4), spot ID 692, under expressed in P2 and over expressed in P1 and P4, and palate lung and nasal epithelium carcinoma associated protein (spot ID 848), PLUNK, under expressed in P1, P2 and P4;
(iii) Enzymes related to carbohydrate metabolism: chain A, human mitochondrial aldehyde dehydrogenase (ALDH2), spot ID 559, aldehyde dehydrogenase (ALDH3A1), which migrates as pearl chains, spots ID, 564, 567, these two protein spots were under expressed in the P1, P2 and P4;
(iv) oxidoreductase enzymes: retinal dehydrogenase (ALDH1A1), spot ID 525, under expressed in P1 and P2 and thioredoxin isoform 1 (TXN-1), spot ID 869, under expressed in all the four patients;
(v) a calcium ion binding protein S100-A11 (spot ID 880), under expressed in P2; and, finally, (vi) proteins arising from the systemic compartment: chain A, crystal structure of human serum albumin (ALB) Spot ID. 440, 452, 453, over expressed in P2 and P4 and haemoglobin subunit beta (HBB) spot ID 875, overexpressed in P1 and P2 and under expressed in P4. These results are shown in **figure 2**, **3** and **4** and summarized in **table 2**.

Discussion

The field of human proteomics has the potential to become a key tool in the characterization of disease biomarkers. In this report, we present a proteomic analysis of nasal mucosa in patients with different rhinitis. Comprehensive proteomic profiles of nasal mucosa cells showed high resolution and high reproducibility of the protein pattern, and allowed to obtain preliminary information about disease-related proteins.
Our results revealed that several proteins were differentially expressed in each rhino-pathologic state (**figure 2**, **3** and **4**). Eighteen proteins belonging to different functional categories were identified (**table 2**). The main functional groups included proteins related to cell defense as ALDH, GSTA-2, PRDX-5, SELENBP-1, and proteins associated with human immune response and inflammation marker as SERPINB4 and PLUNK. These proteins were expressed at high level in the normal nasal mucosa, in line with its role in the first-line defense against foreign particles as well as in the differentiation of immune responses (15). In patients affected by different kinds of rhinitis, the above mentioned proteins were differentially expressed. In particular ALDH, a carbohydrate metabolism related enzyme, was under expressed in patients P1, P2, and P4. ALDH plays a major role in the detoxification of exogenously and endogenously generated aldehydes and has protective effects on cells during environmental stress. The protective action of ALDH is important, as nasal mucosa is constantly exposed to stressors mainly in the form of cross-reacting substances. Our results could indicate a direct relation between eosinophil presence and under regulation of ALDH. It could be assumed that the pathologic state affects the expression level of this protein with a consequent decreased ability of mucosa cells to react to environmental stress. Also, the expression level of GSTA2 and GSTP1, which play an important role in protecting cells from cytotoxic reactive oxygen species and carcinogenic agents (16), and PRDX5, an antioxidant enzyme (17), were under regulated in the four patients. This feature could affect the cell ability to counteract stressor action. The SERPINB4 was expressed at high level in patients P1 and P4 and was down-regulated in patient P2. This protein belongs to the serine proteinase inhibitor family. SERPINB4 has been reported as serological marker for more advanced squamous cell tumors of the cervix, lung, and oropharynx (18,19). High expression of SERPINB4, as in patient P1 and P4, could suggest the onset of a pathological process of the high and lower airways respiratory epithelium. This finding might pave the way to further characterization of this protein as a marker of the respiratory epithelium pathology and for therapeutic monitoring. PLUNC is a protein specifically expressed in the upper airways and nasopharyngeal regions. It has been suggested to be involved in inflammatory responses to irritants and can play a role in innate immune responses (20). A decreased level of PLUNC was previously reported in the pooled nasal lavage fluid of current smokers when compared with non-smokers (21). Our results showed that this protein is expressed at very low level in the 2-DE maps of patients P1 and P2 while was detected in similar amount of the control subjects in patients P3 and P4. These distinct features could indicate a possible role of PLUNC in the pathogenesis of non allergic rhinitis, suggesting a decreased immune response to stressors and consequent increase of the inflammatory response of nasal mucosa cells in these patients. Two oxidoreductase enzymes were also differentially expressed: ALDH1A1, under expressed in patients P2, P3 and P4, and TXN-1, expressed at very lower amount in patient P2 and absent in the other three patients. ALDH1A1 can play a critical role in the maintenance the mucociliary phenotype of epithelial cells in the upper respiratory tract (22), while TXN-1 is a hydrogen donor for enzymes involved in reductive reactions and there-

fore can play a role in maintaining a reducing cell environment protecting from oxidative stress. (23). In addition, this protein has anti-apoptotic effect by binding to the apoptosis signal-regulating kinases (24). The expression level of TXN-1 is particularly important in the determination of the pathophysiologic state of the cell, therefore, it could be a potential marker for diagnostic purposes and therapeutic monitoring of the diseases. It is worth to note the different expression level of ALB in the four patients. In fact, this protein was over expressed in patient P2, and P4. Diffusion of albumin across the nasal mucosa has been shown in patients with rhinitis (25), and plasma proteins, mainly albumin, could be involved in the initial nasal process of polyposis (26).

In conclusion, our preliminary proteomic study revealed, for the first time, that rhinitis affected the expression levels of several nasal mucosa proteins, mainly involved in cell defense and immunological response. In addition, specific protein expression pattern was revealed for the different kind of pathologic states. Although additional studies are needed to assess the possibility to use some of these proteins as potential markers for diagnostic and therapeutic purposes, the present study further confirms the key role of proteomics in providing information for a better characterization of proteins specifically involved in each rhino-pathological state.

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References

1. Benninger MS, Ferguson BJ, Hadley JA, et al. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. Otolaryngol Head Neck Surg. 2003;129:S1-32.
2. Bousquet PJ, Demoly P, Devillier P, et al. Impact of allergic rhinitis symptoms on quality of life in primary care. Int Arch Allergy Immunol. 2012;160:393-400.
3. Gelardi M, Maselli Del Giudice A, Fiorella ML, et al. Non-allergic rhinitis with eosinophils and mast cells (NARESMA) constitutes a new severe nasal disorder. Int J Immunopathol Pharmacolol. 2008;23:325-31.
4. Academy of Allergology and Clinical Immunology: Skin tests used in type I allergy testing Position paper. Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology. Allergy. 1989;44:1-59.
5. Gelardi M. Atlas of nasal cytology. Torino, Italy: Centro Scientifico Editore, 2006.
6. Meltzer EO, Jalowayski AA. Nasal cytology in clinical practice. Am J Rhinol. 1988;2:47-54.
7. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.
8. Weiss W, Görg A. High-resolution two-dimensional electrophoresis. Methods Mol Biol. 2009;564:13-32.
9. Geiser L, Vaezzadeh AR, Deshusses JM, Hochstrasser DF. Shotgun proteomics: a qualitative approach applying isoelectric focusing on immobilized pH gradient and LC-MS/MS. Methods Mol Biol. 2011;681:449-58.
10. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227:680-5.
11. Neuhoff V, Taube AN, Ehrhardt WD. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nano grams sensitivity using Coomassie Brillant Blue G-250 and R-250. Electrophoresis. 1988:9:255-62.
12. Lippolis R, Gnoni A, Abbrescia A, et al. Comparative proteomic analysis of four Bacillus clausii strains: proteomic expression signature distinguishes protein profile of the strains. J. Proteomics. 2011;74:2846-55.
13. Appel RD, Hochstrasser DF. Computer analysis of 2-D images. Methods Mol Biol. 1999;112:363-81.
14. Shevchenko A, Wilm M, Vorm O, Mann M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem. 1996:68;850-8.
15. Casado B, Pannell LK, Iadarola P, Baraniuk JN. Identification of human nasal mucous proteins using proteomics. Proteomics. 2005;5:2949-59.
16. Bostwick DG, Alexander EE, Singh R, et al. Antioxidant enzyme expression and reactive oxygen species damage in prostatic intraepithelial neoplasia and cancer. Cancer. 2000;89:123-34.
17. Fujii J, Ikeda Y. Advances in our understanding of peroxiredoxin, a multifunctional, mammalian redox protein. Redox Rep. 2002;7:123-30.
18. Yasumatsu R, Nakashima T, Kuratomi Y, et al. Serum squamous cell carcinoma antigen is a useful biologic marker in patients with inverted papillomas of the sinonasal tract. Cancer. 2002;1:152-8.
19. Matoušek P, Zeleník K, Šafarčík K, et al. Squamous cell carcinoma antigen as a marker of sinonasal inverted papilloma. European Archives of Oto-Rhino-Laryngology. 2013;Jun19. (Epub ahead of print).
20. Bingle CD, Craven CJ. PLUNC: a novel family of candidate host defence proteins expressed in the upper airways and nasopharynx. Hum Mol Genet. 2002;11:937-43.
21. Ghafouri B, Stahlbom B, Tagesson C, Lindahl M. Newly identified proteins in human nasal lavage fluid from nonsmokers and smokers using two-dimensional gel electrophoresis and peptide mass fingerprinting. Proteomics. 2002;2:112-20.
22. Million K, Tournier F, Houcine O, et al. Effects of retinoic acid receptor-selective agonists on human nasal epithelial cell differentiation. Am J Respir Cell Mol Biol. 2001;25:744-50.
23. Arnér ESJ, Holmgren A. Physiological functions of thioredoxin and thioredoxinreductase. Eur J Biochem. 2000;267:6102-9.
24. Saitoh M, Nishitoh H, Fujii M, et al. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J. 1998;17:2569-606.
25. Buckle FG, Cohen AB. Nasal mucosal hyperpermeability to macromolecules in atopic rhinitis and extrinsic asthma. Journal of Allergy and Clinical Immunology. 1975;55:4:213-21.
26. Takabayashi T, Kato A, Peters AT, et al. Excessive fibrin deposition in nasal polyps caused by fibrinolytic impairment through reduction of tissue plasminogen activator expression. Am J Respir Crit Care Med. 2012;87:49-57.