Doctoral Thesis

Nasal Mucus Proteome and its Involvement in Allergic Rhinitis

submitted by

Dr.med.univ.

Peter Valentin TOMAZIC

for the Academic Degree of

Doctor of Philosophy

(PhD)

at the

Medical University of Graz

ENT-University Hospital

Department of General Otorhinolaryngology, Head and Neck Surgery, Graz, Austria

under the Supervision of

Prof.Dr. Doris Lang-Loidolt

Assoc.Prof.Dr. Ruth Birner-Grünberger

Prof.Dr. Akos Heinemann

2016

Statutory Declaration

“Declaration:
I hereby declare that this thesis is my own original work and that I have fully acknowledged
by name all of those individuals and organisations that have contributed to the research for
this thesis. Due acknowledgement has been made in the text to all other material used.
Throughout this thesis and in all related publications I followed the “Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz“.

Graz, June 1st, 2016 *Dr. Peter Valentin TOMAZIC*

Foreword

It is an honor for me to be part of such an excellent research group in times like these.

My deepest gratitude goes to Prof. Doris Lang-Loidolt and Prof. Ruth Birner-Grünberger without whom this project would not have been possible. I would also like to express my gratitude to Prof. Akos Heinemann for his continuous advice, his instructions and for believing in this project.

I also want to thank my teacher Prof. Heinz Stammberger who opened the way into Rhinology for me.

Last but not least I want to thank my parents, my family, friends and colleagues who always supported me with patience and gave me a helping hand whenever I needed it.

Acknowledgements

I want to thank Mrs. Anita Leitner, Mrs. Britta Obrist, Mrs. Barbara Darnhofer and Mr. Claus Gerstenberger for their support throughout the entire project.

Table of Contents

Abbreviations

AUC, areas under the curve

CID, collision induced dissociation

DTT, dithiotheitrol

Fw-rev, Forward-reverse score

IAA, iodoacetamide

ICR, ion cyclotron resonance

LC-MS/MS, liquid chromatography-tandem mass spectrometry

nano-HPLC, nano flow-high performance liquid chromatography

SC, spectral count

SD, standard deviation

SEM, standard error of mean

%SPI, Scored Peak Intensity Percent

SPT, skin prick test

Abstract (German)

Das Nasenschleimproteom und dessen Bedeutung für die allergische Rhinitis

Hintergrund*:* Nasenschleim ist die erste Abwehrbarriere gegen Aeroallergene. Das Nasenschleimproteom und seine Funktionen wurden bis dato nicht ausreichend untersucht.

Ziel der Studie: Die Rolle des Nasenschleims in der Pathophysiologie der Allergischen Rhinitits wurde mit proteomischen Methoden an Allergikern und gesunden Probanden sowohl innerhalb als auch außerhalb der Pollensaison analysiert.

Methoden: Nasenschleim wurde mittels eines speziellen Saugers entnommen, tryptisch verdaut und der LC MS/MS Massenspektrometrie zugeführt. Proteine wurden anhand von Datenbanken (SwissProt) analysiert und mittels Proteindatenbanken und der existierenden Literatur annotiert. Eine “Gene enrichment“ Analyse wurde mittels Cytsoscape/BINGO Software durchgeführt. Die Proteine wurden ferner mittels Spectral Counting oder Areas under der Curve quantifiziert und die Ergebnisse von distinkten Proteinen mittels Western Bot bestätigt.

Ergebnisse: Gesamt wurden 430 Proteine identifiziert wobei 372 bei Allergikern und 366 bei gesunden Probanden gefunden wurden. Zehn Proteine waren bei Allergikern in der Saison im Vergleich mit gesunden Probanden signifikant hochreguliert: Complement C4-B (C4B), alpha-1-acid glycoprotein 2 (ORM2), und phospholipid transfer protein (PLTP), welche bei gesunden Probanden nicht vorkamen sowie alpha-2-macroglobulin (A2M, 13,2-fach erhöht), apolipoprotein A-II (APOA2, 9,4-fach), vitamin D-binding protein (GC, 4,6-fach), complement C3 (C3, 3,6-fach), apolipoprotein A-I (APOA1, 3,6-fach), BPI fold-containing family B member 2 (BPIFB2, 2,9-fach) and clusterin (CLU, 2,6-fach).

Schlussfolgerung: Entgegen der Symptomexpression zeigen Allergiker eine erhöhte Immunantwort auf Proteomebene auch außerhalb der Pollensaison. In Kombination mit reduzierten Abwehrmechanismen und einer erhöhten Entzündungsreaktion innerhalb der Saison, zeigt das Nasensekret von Allergikern eine reduzierte Plastizität und ist daher nicht fähig adäquat auf natürliche Allergenprovokation zu reagieren wie es Gesunde tun.

Abstract

Background*:* Nasal mucus is the first line defense barrier against (aero-) allergens. Its proteome and function is not clearly investigated yet.

Objective: The role of nasal mucus in pathophysiology of allergic rhinitis was investigated by analyzing its proteome in allergic rhinitis patients and healthy controls in as well as out of pollen season.

Methods: Nasal mucus was collected with a suction device, tryptically digested and analyzed by LC-MS/MS. Proteins were identified by searching the SwissProt database and annotated by collecting gene ontology data from databases and existing literature. Gene enrichment analysis was performed by Cytoscape/BINGO software tools. Proteins were quantified using spectral counting or areas under the curve and selected proteins were confirmed by Western blotting.

Results: In total 430 proteins were identified where 430 were present in allergic rhinitis patients and 372 proteins were found in healthy controls. In pollen season 10 proteins were significantly more abundant in allergic rhinitis patients than in healthy controls. These were complement C4-B (C4B), alpha-1-acid glycoprotein 2 (ORM2), and phospholipid transfer protein (PLTP), which were not detected in HC at all; as well as alpha-2-macroglobulin (A2M, 13.2-fold), apolipoprotein A-II (APOA2, 9.4-fold), vitamin D-binding protein (GC, 4.6-fold), complement C3 (C3, 3.6-fold), apolipoprotein A-I (APOA1, 3.6-fold), BPI fold-containing family B member 2 (BPIFB2, 2.9-fold) and clusterin (CLU, 2.6-fold).

Conclusion: Contrary to their symptom pattern, allergic rhinitis patients show an increased inflammatory response in their nasal mucus proteome even out of pollen season. In combination with reduced defense mechanisms and an increase in inflammation in season, the nasal mucus proteome of allergic subjects reflects a decreased plasticity and thus inadequate reaction to allergen stress as compared to healthy controls.

1. Introduction

1.1 The nasal mucosa: embryological and histological basics

The nose and paranasal sinuses originate from following embryonic layers: ectoderm, mesoderm and neuro-ectoderm. At 4 weeks' gestation time the primary stomodeum, a depression below the developing brain starts to indent to mark the beginning of facial development. The nasal placodes, from which the nose and nasal cavity starts to develop, is located above the mandibular prominence and superior-medial to the maxillary prominence which later form the upper and lower jaws. During the fifth week the nasal placodes form horse-shoe like processes which later form the external nose. Between those two processes nasal pits are formed which have an inverting development to form the later lumen of the nasal cavity. The medial processes of the nasal placode develop further into the nasal septum after fusion with parts of the primordial palate. Ectodermal strands grow towards the future nasal cavity, detach from the surface and after canalization form the nasolacrimal duct and sac. From week eight to twenty-four the nasal turbinates as well as the infundibular structures develop at the lateral nasal wall. The turbinates primarily are bulges at the lateral wall wherein cartilage subsequently grows and later ossifies. Already at an early stage, the turbinates have similar orientation to the end stage in adults. After week twenty-four the development of the nasal wall is almost finished and ossification processes have progressed. From epithelial indentions the paranasal sinuses develop subsequently (1,2).

The nose harbors two different types of epithelium; the olfactory- and the respiratory epithelium. The olfactory epithelium is by far smaller with a mean area of 500 mm² (3). In contrast to the respiratory epithelium which is pink, it is of brownish color and extends 8 to 10 mm downwards from the nasal roof to each side of the nasal septum and the superior (and supreme) turbinates. It is a pseudostratified columnar epithelium of approximately 60 µm thickness consisting of supporting cells, basal cells and olfactory cells (3). The olfactory cells end proximally in a thin axon-like filament forming bundles

with neighboring cells which are macroscopically depicted as olfactory fibres that end in the olfactory bulb of the corresponding nerve. The cells together with the nerve are responsible for the sense of smell. The conduction of this sensory stimulus is chemical. Odorants reach the olfactory mucosa via inhalation of air. Glands within the epithelium (Bowman glands) keep them soluble and clean the receptor site for possible continuous stimulation. Since many odorants are more soluble in lipid environment than aqueous environment the olfactory epithelium and its cilia contain lipids. Once the odor molecule reaches the epithelium, a receptor ligand-cascade through G-proteins is initiated causing a receptor potential which is conducted to the brain. Here the information is further processed. There are a large number of odor receptors in the epithelium for differentiation of an innumerable amount of environmental odors (3,4).

The respiratory mucosa lines the remaining areas of the nasal cavity and paranasal sinuses. It consists of a pseudostratified columnar ciliated epithelium made up of basal cells, columnar cells and goblet cells resting on a basal membrane. In the submucosa one can find seromucinous glands predominantly present in the nasal turbinate and septal mucosa. Despite histological similarities the mucosa differs between the nasal cavity and the sinuses. Sinus mucosa is usually thinner than the rest of the nose. The epithelium is lower, containing fewer goblet cells and hardly any seromucinous glands. The basal membrane is less pronounced and the major cells are columnar ciliated cells that measure 5 µm on average carrying 100 to 150 cilia. The average thickness of sinus mucosa is between 0.2 and 0.8 mm. The epithelium measures 25 to 50 µm. Seromucinous glands are rare in the sinuses compared to the nasal cavity with an average of 200 compared to 36.000 in the nose proper. The density of glands is around 7 to 10 glands per mm². A similar trend is present for the goblet cells which underlines the fact that mucus production is strikingly higher in the nose proper than in the paranasal sinuses (5).

1.2 Allergic Rhinitis

The terms “allergy” dates back to v. Pirquet who coined it in 1906 after having discovered that antibodies can cause or suppress disease. Allergic Rhinitis is a disease of the nose which is induced after allergen exposure by class E immunoglobulins causing inflammation of the nasal mucosa. Thus allergic rhinitis may be considered a type I allergic reaction. Type II is mediated by natural killer cells, type III by antibody-antigen complexes and type IV by antigen-specific T-cells. The allergens per se are harmless, but a “wrong” immune response leading to IgE production is responsible for the symptoms. Allergens can be variable from grass or tree pollen, animal secretions (urine, saliva, sweat etc.) and skin scales, house dust mite excreta and fungi. The majority of allergens are proteins with a molecular weight between 5000 and 100,000. They carry a number of epitopes that mediate their antigenicity. Allergens are named according to the first three letters of their genus followed by the first letter of their species in latin and an arabic number ascending in order of their discovery/description. An example would be an antigen from Alternaria alternata which would be named as *“Alt a 1”*.

Defined in 1929 the major symptoms of allergic rhinitis are: sneezing, nasal obstruction and nasal discharge (6). According to the allergen exposure allergic rhinitis can be subdivided according to occurrence of symptoms into a seasonal allergy particularly starting in spring when pollen are expelled in the air by trees and plants and persistent or perennial allergy when allergen exposure occurs throughout the year like with house dust mites or animals (6). A common elderly name for allergic rhinitis thus was “hayfever”. Another classification differentiates between intermittent rhinitis where symptoms are low and do not impair the quality of life and are present less than 4 days per week or less than 4 weeks per year, and persistent rhinitis where symptoms are severe and incriminating and are present more than 4 days a week or longer than 4 weeks per year (7).

Allergic rhinitis is a global health issue and affects all ethnical groups, and men and women of all ages. Patients are affected in their professional and social life and health care costs are tremendous particularly when including co-morbidities.

Since the nose is the primary airway tract organ important for filtering the inhaled air, it is exposed to airborne particles more than any other organ. A characteristic feature of allergic disease and inflammation is a local accumulation of immune cells like B- and T-lymphocytes, eosinophils and mast cells. Mast cells, Basophils and Eosinophilic granulocytes are the effector cells in allergic diseases. Mast cells originate in the bone marrow as immature mononuclear cells. Their role in allergic disease is marked by the high affinity IgE-receptor at their cell surface. For activation 2 IgE molecules need to be cross-linked by a binding antigen to the immunoglobulin receptor. The consequence is a tremendous release of histamine. Other mast cell products are proteases like chymase and tryptase. Released mediators are IL-3 and TNF-alpha as well as IL-1, IL-4, IL-6, IL-8 and IL-10, and lipids such as leukotriene C4 and prostaglandin D2. These mediators are involved in the mediation of allergic symptoms.

Basophils as well as mast cells have a high affinity IgE receptor and their major mediator is histamine. For immigration of basophils into inflamed tissue C5a, GM-CSF, IL-3 or IL-8 are important. Through vascular adhesion molecules basophils can easily leave the blood stream and enter into the tissue.

Eosinophils do not have a high affinity IgE receptor as mast cells or basophils, but are activated through Il-5 produced by allergen-activated T-cells. Other mediators like C5a, C3a, MCP-3 and 4, or eotaxin have a strong chemo attractant effect on eosinophils. Vascular adhesion molecules like ICAM-1 or VCAM-1 mediate the emigration of eosinophils from plasma to tissue. They are characterized by their toxic products like major basic protein, eosinophilic cationic protein and others.

In allergic patients allergens are presented by antigen presenting cells through their MHC II receptor to T-cell receptors activating T_H-2 cells. These cells produce IL-4 which stimulates B-cells to produce IgE instead of IgG in B-cells, a process called “immunoglobulin switch” (8). Apart from IL-4 an important cytokine for IgE production is interleukin-13. Their counter part is interferon-gamma (9,10). The circulating IgE

antibodies, due to their binding affinity to FcεI receptors, attach to mast cells and basophils, and if they are cross-linked by bound allergen, this leads to the release of histamine, which is the most important mediator in allergic rhinitis concerning symptoms. Histamine directly promotes vasodilatation, edema, and plasma exudation in nasal mucosa and indirectly –through stimulation of neural endings- sneezing and itching (6,8,9).

Since IgE is crucial for the development of allergic symptoms, the adaptive immune system is the key player in the development of allergic disease; however, the innate immune system with its remarkable number of cytokines and chemokines has important modulatory effects in allergic rhinitis where its ultimate role is still not completely understood (10).

1.2.1 Diagnosis of allergic rhinitis

In addition to obtaining a detailed patient history and evaluating the symptoms, basically two ways of diagnosing allergy are possible: *in-vivo* or *in-vitro* testing. *In vivo* tests are directly targeted to the effector organ and work by provocation in a standardized way. The most established provocation test is skin prick test. For allergic rhinitis intranasal or conjunctival tests are possible, but are less practical and reserved for special indications or research. Nowadays, *in-vitro* tests to determine specific IgE are Carrier-Polymer-System Fluorescence- Enzyme-Immunoassays (CAP-FEIA) that replaced radio-allergo-sorbent-assays (RAST) or radio-immuno-sorbent-assays (RIST). Additionally, total serum IgE is determined. Component-resolved diagnosis focuses on specific epitopes to which the patient is sensitized that may also be minor allergens. Another potential diagnostic method is the basophil activation test for inconclusive IgE tests or for special allergens where serum antibodies are not commercially available (11,12).

Patient history	Symptoms Seasonal / perennial
Skin tests	Skin Prick Test Intracutaneous test Scratch test
Blood tests	CAP-FEIA Component resolved diagnosis Basophil activation test

Table 1: Diagnostic tools for allergic rhinitis.

1.2.2 Therapy of allergic rhinitis

There are two pillars of treatment strategies for allergic rhinitis, i.e. a causal therapy and symptomatic therapies. The causal therapies are targeted towards avoidance of allergen exposure or specific immunotherapies. Specific immunotherapy aims for a re-orientation of the immune system so that allergens are not considered “harmful” anymore and consequently mediator release, cell activation etc. is reduced leading to less or –in the best way- no symptoms. The principle is the standardized application of distinct concentrations of allergen over a long period of time to patients. The immunological concept of the effect of immunotherapy lies in a re-organization of T-cells. This can either be a new induction of Th-1 cells, a selective proliferation of existing Th-1 cells or a deactivation of Th-2 cells. Th-2 cells become anergic, thus they do not react to allergen exposure. Another mechanism is a change of chemokine environment form IL-4 and IL-5 to IFN-gamma. This leads to a significant deactivation of eosinophils and reduction of IgE production in plasma cells. In simple words the stepwise increasing, controlled challenge with allergen should teach the immune system to consider them as harmless, which they are in fact. Newer concepts of immunotherapy include sublingual application of allergen instead of injections and the effect of allergoids that are recombinant

allergens only for the allergenic effector site of the allergen. Further controlled trials need to be performed to elucidate these aspects (9).

Symptomatic treatment regimens, such as antihistamines, corticosteroids or mast cell stabilizers, are based on immune suppression or blocking mediators of allergic inflammation,. The pathophysiological background of allergen sensitization thus is not treated.

Newer therapies like omalizumab, an anti-IgE antibody, and mepolizumab, an anti-IL-5 antibody, need to be determined in future studies with special focus on allergic rhinitis (13,14).

Allergen avoidance	Primary prevention Secondary prevention Tertiary prevention
Specific Immunotherapy (IT)	Subcutaneous IT Sublingual IT Component resolved IT
Symptomatic treatment	Antihistamines Corticosteroids Mast cell stabilizers (Antileukotrienes) Anti IgE antibodies (under evaluation) Anti IL-5 antibodies (under evaluation)

Table 2: Treatment modalities for allergic rhinitis.

1.3 Nasal mucus

Nasal mucus is the first line defense barrier against a variety of inhaled pathogens. With around 12.000 L of air inhaled daily the airways are confronted with 25 million particles an hour that need to be filtered and/or transported away (15-17). The reaction of the epithelium to this challenge is the production of nasal mucus (and also mucus in the lower airways) which is a viscoelastic gel covering the epithelial surface as a film. Some of its functions are a physical barrier against particles, irritants, microbes, viruses, but also food and liquids. These pathogens are trapped in the mucus and are transported away through mucociliary clearance of the beating ciliated epithelium underneath the mucus layer. Furthermore the epithelium is protected from dehydration, inhaled air is warmed and humidified and pH value is buffered in the airways. Mucus also contains anti-microbial agents as well as immunoglobulin for innate and adaptive immune response. Toxic gases are neutralized and the mucosa is insulated.

Nasal mucus consists of a variety of lipids, glycoconjugates, cells, cellular debris and proteins. They work as enzymes or enzyme inhibitors, antioxidants, antibacterial agents and mediators. Well described and abundant proteins in nasal mucus are mainly antimicrobial proteins like lysozyme and lactotransferrin as well secretory IgA, IgE, IgG and albumin. Other proteins present are kallikrein, antiproteases, ß-glucuronidase, α-galactosidase and others described in elderly, mainly ELISA-based studies (18,19). Proteins stem from epithelial cells, goblet cells of sero-mucous glands in the submucosal tissue. Another important contributor to protein composition of the mucus are exudated proteins from plasma which is regarded as a key defense mechanism (15,18,20,21). Especially in pathological conditions like allergic rhinitis, lysozyme and lactotransferrin were shown to be up-regulated after provocation with allergen (18). These older studies already underlined the importance of the nasal mucus proteome in disease, but were restricted to distinct proteins or small groups of proteins. Cellular debris can be detected as DNA or nucleic products such as histones (15).

The mucus itself comprises two phases, a more viscous gel-layer above and a less viscous sol-layer below which is in close contact to the cilia of the epithelium. Trough

metachronous and synchronous movement of the cilia the mucus is moved in a way that the cilia beat within the sol-phase and the momentum is conducted to the gel-phase. Under pathological condition where the mucus is thickened and the sol-phase is reduced at the expense of the sol-phase the ciliary beat is stopped and thus the mucociliary clearance is hampered (15,22). Thus, a correct balance between the two phases is mandatory in a healthy condition. Trapped pathogens are transported into the upper digestive tract and are swallowed and degraded in the stomach or are coughed or sneezed out.

Despite its important role in defending the nose and the entire organism against various pathogens nasal mucus has been granted less attention than the nasal epithelium. Potential therapeutic targets like surfactant protein (SP-A) or Clara cell protein (CC10) have been addressed in mouse models or *in vitro* models by analyzing their expression in the epithelium (23,24). Particularly, nasal mucus proteins, major constituent of this body fluid and functional units, have not been studied that extensively. Proper viscoelasticity and fluidic properties of the mucus is mainly accounted to glycoproteins called mucins. Mucins have been implicated in many airway diseases and were mainly studied in the epithelium, despite the fact that they exert their functions and also harmful potential in the mucus of the upper and lower airways (25). Mucins comprise up to 2% of the net weight of nasal mucus. They, too, are produced in goblet cells and submucosal glands. They are glycoproteins with a linear peptide backbone encoded by specific MUC genes. They often have multiple protein domains that are sited of extensive O-glycan attachment (15,26). Since 1992, twenty human MUC genes have been identified. In the respiratory tract only nine of these are expressed; MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8, MUC11 and MUC 13 (15,17). In healthy individuals MUC5B is mainly expressed in submucosal glands whereas MUC5AC expression is exclusively present in goblet cells. The composition of nasal mucus is dependent on the relative concentrations of these two mucins from glandular sources. For the regulation of MUC5B secretion from submucosal glands *Lin et al.* (27) showed that 2-ABP can reduce MUC5B secretion whereas IL-33 enhances its secretion into the nasal mucus of mice under allergic rhinitis conditions. 2-ABP could also reduce IL-4, IL-5

and IL-13 in nasal mucus as well as in the epithelium, however production of IL-33 was not influenced.

Mucin secretion from goblet cells is differentiated by two mechanisms: a basal secretion which is unregulated and occurs at lower levels, and a regulated secretion stimulated by extracellular triggers (15). Inflammatory stimuli in allergic disease via Th2 cytokines like IL-4, IL-9 and IL-13 trigger mucin production. IL-13 together with STAT6 furthermore causes mucous metaplasia in airway epithelium which is also important in allergic rhinitis since a higher viscosity of nasal mucus leads to an impairment of mucociliary clearance. The trapped mucus enhances symptoms like nasal blockage and harbors the potential of superinfections and subsequent chronic rhinosinusitis(17). Mucins are stored in intracellular granules after synthesis at the endoplasmatic reticulum and glycosylation at the Golgi apparatus. For exocytosis the granules are moved to the apical cell surface which is dependent on myristoylated alanine-rich C-kinase substrate (MARCKS). After phosphorylation of MARCKS actin/myosin contracts and the granules fuse with the plasma membrane (15). The importance of goblet cells and mucin production is evident in pathological conditions like COPD (chronic obstructive pulmonary disease), asthma and CF (cystic fibrosis) where goblet cell hyperplasia is predominant. This hyperplasia comes from differentiation of basal progenitor cells into goblet cells. Apart from mucus overproduction as part of the disease pathology goblet cell hyperplasia leads to a reduction of Clara cells which are the main precursors of goblet cell differentiation. Since Clara cells produce important immunomodulatory, anti-inflammatory and anti-bacterial mediators, their reduction further aggravates disease progress and clinical course (15).

In the past, many studies focused on immunoglobulins in nasal secretions, mainly IgE. These studies focused on the local production of IgE and its relevance in nasal mucus. Since plasma levels did not correlate with the levels measured in nasal mucus the presence of IgE in nasal mucus cannot be explained by simple plasma exudation. Moreover, in pollen season and/or after provocation IgE antibodies in nasal secretions were significantly higher in allergic rhinitis patients than in healthy controls (28-35). A clear diagnostic or therapeutic benefit from these findings could not be deducted. Other important immunoglobulins such as secretory IgA (S-IgA) are secreted into body fluids in the upper respiratory, urogenital and gastrointestinal tract. They are also present in

nasal mucus. They bind to antigens entering the mucus and thus reduce their absorption. Immunoglobulin-deficient subjects show a greater absorption which becomes apparent in food allergy. IgA can exert anti-inflammatory properties and encase microorganisms which then cannot bind to the epithelium, a mechanism which can be augmented by mucins. Through clumping of bacteria which is antibody and glycan-mediated their mucosal clearance is enhanced. Furthermore IgA can enhance the activity of protective proteins like lactoferrin. IgG on the other hand may activate the complement system promoting inflammation and subsequent epithelial damage and dysintegrity (36).

Another focus was laid on the investigation of kallikrein and kinins in nasal mucus as mediators of the allergic reaction. After provocation these molecules were significantly elevated along with histamine in nasal secretions. These studies were performed in the course of the up-coming 1st generation antihistamines and proved that topical application of the latter could ameliorate allergic symptoms and decrease these mediators (37-39). Baraniuk later suggested that the muscarinic receptor antagonist ipratropium bromide could reduce glandular secretion into nasal mucus via the bradykinin system (40). However, histamine release and activation of the kallikrein-kinin system is a primary consequence of IgE cross-linking and mast cell activation, but does not necessarily reflect which processes are going on in the mucus during allergen challenge. As of today many studies focus on symptomatic therapy and its influence on nasal secretions but, because of therapeutic failures, targeted therapies towards mucus proteins should be favored (41). The association of eosinophils to allergic rhinitis gave rise to investigating its major protein component eosinophilic cationic protein (ECP) in nasal mucus. Since eosinophilic infiltration of the mucosa determines inflammation, secretion of ECP into the mucus could reflect disease severity and regulate the course of disease since ECP was significantly elevated in allergic mucus (42-46). As potent toxin this protein is also involved in epithelial disruption acting from outside-in, i.e. from the mucus towards the mucosa. An association to elevated eosinophilic cationic protein (ECP) and IL-5 was found by Kramer *et al*. (47) by using ELISA. Anti-IL-5 is thus a promising therapeutic agent, but large randomized placebo controlled trials for allergic

rhinitis are lacking as of today (14,48). Similar findings are true for major basic protein (MBP), another highly abundant eosinophil protein (49).

Tonnesen *et al.* (50) studied substance P and vasoactive intestinal polypeptide (VIP) in nasal mucus. As elevated inflammatory mediators in nasal secretions of allergic rhinitis, they suggested that antagonists could positively influence the disease. Raphael *et al.* (51) and Ichikawa *et al*. (52) focused on protein sources in allergic nasal mucus. Depending on allergen challenge proteins are produced in local mucosal glands and are secreted by means of plasma exudation. In spite of this fact albumin, lactoferrin, lysozyme, tryptase, substance P, immunoglobulins, prostaglandins, complement, cytokines and leukotrienes in the mucus could act as potential markers of allergic disease and/or disease modifiers (19,51-59). Albumin is a plasma protein and its main functions are the maintenance the colloid osmotic pressure in the vessels and transport of hormones and fatty acids. Its presence in nasal mucus is due to increased plasma exudation and thus it is regarded as response to inflammatory stimuli. Whether albumin itself has immune defense properties is unclear as of today (21,60). Local glandular production in the nose is possible, however these amounts would be neglectable and no clear evidence has been presented to it.

Lactoferrin or lactotransferrin is an abundant protein in nasal mucus mainly produced in nasal submucosal glands where it is stored and released on demand. It is a 78 kD protein that exerts bacteriostatic and bactericidal functions which was first described in 1966 in bovine milk. The antimicrobial effect is mainly achieved through iron binding capacities. Iron is essential for bacterial growth and is taken up by the microorganisms. Furthermore, it blocks the formation of complement factor C3 convertase and regulates granulocyte production influencing granulocyte colony-stimulating factor (GCSF). Its iron-binding properties are comparable to that of transferrin in serum. Not only in nasal secretions, but also in other body fluid one can find lactoferrin abundantly: tears, bronchial mucus, uro-genital secretions, pancreas, liver and gastrointestinal tract as well as in specific granules of neutrophils. The concentration of lactoferrin in nasal secretions is around 1µg/ml, which can be dramatically increased upon provocation with metacholine or histamine. The local production and secretion of this protein upon stimulation make it an important defense protein in the nasal mucus (18). Lysozyme was

first described by Flemming in 1922 when he discovered bactericidal capacities of nasal secretion in patients with rhinitis after incubation with bacterial cultures. It has a molecular weight of around 14 kD. Contrary to lactoferrin, lysozyme does not bind iron but can enzymatically degrade bacterial cell walls. It is found in kidneys, stomach glands, small intestine, Paneth cells, lacrimal and parotid glands as well as other salivary glands, neutrophils and macrophages. In the lungs Lysozyme even accounts for 5-6% of total proteins. As well as lactoferrin, it is produced in the local nasal mucosal glands and its secretion can be enhanced by external stimuli (18).

To study the nasal mucus proteome it is important to consider plasma exudation as potential source of proteins biasing their abundances and to exclude potential triggers like capsaicin (61).

Pollen proteases can degrade tight junctions (62) and subsequently harm the nasal epithelium. One hypothesis of this study is that an imbalance of nasal mucus antiproteases could favor protease activity and influence the disease. Hamaguchi *et al*. (37) found that protease activity in acute sinusitis was high, potentially hampering the healing process, whereas in allergic rhinitis protease activity in mucus was weak. However, they only focused on functional activity of cathepsin B and L, both cysteine proteases, but the identification of distinct antiproteases and their potential functional defects were not addressed. Belkowski *et al.* (63) investigated secretory leukocyte protease inhibitor (SLPI) which is cleaved by chymase. SLPI as well as elafin are potent serine protease inhibitors where SLPI inhibits cathepsin G and elastase whereas elafin additionally inhibits proteinase 3 but not cathepsin G. Cathepsin G is a serine protease working bactericidal through hydrolytic degradation of bacteria and extracellular matrix components. This mechanism cannot be completely hampered by protease inhibitors (64,65). SLPI and elafin were found in epithelium and mucus of the upper respiratory tract. They have antibacterial and antifungal properties and are up-regulated during inflammatory processes. The ratio between the native and the cleaved form of SLPI reflects disease activity, but its involvement in the pathophysiology of the disease has not been well elucidated. Bryborn *et al.* (66) and Kvarnhammar *et al.* (67) focused on psoriasin by means of 2-dimensional gel electrophoresis and mass spectrometry as well as ELISA respectively. Bryborn postulated that this chemoattractant protein could be a

potential biomarker in allergic mucus, whereas Kvarnhammar suggested a compromised antimicrobial defense since the protein was diminished in an allergic milieu and could not exert its immunomodulatory properties.

The clear advantage of proteomic techniques using mass spectrometry is to identify the entire proteins in a body compartment (*i.e.* the proteome) given the possibility to allocate their origin and function without defining the targets beforehand as in Western blotting or other gel-based techniques. The nasal mucus proteome can serve as a reservoir for biomarkers facilitating differential diagnosis for allergic rhinitis, like vasomotor rhinitis, or as diagnostic adjuncts in allergic diagnostics (68-70) . Iguchi *et al.* found a 26kD protein as marker to differentiate between allergic rhinitis and vasomotor rhinitis using SDS-PAGE, however, the identification of the protein was missing (68). Casado *et al.* proved the feasibility of proteomic approaches to studying the nasal mucus in a healthy collective. The present study is the first to investigate the entire nasal mucus proteome comparing healthy individuals to allergic rhinitis patients.

1.3.1 Potential disease modifying proteins

Some proteins have been well described in upper airway secretions with a potential of influencing diseases like asthma. Their role in allergic nasal mucus needs to be further determined.

1.3.1.1 Defensins

Defensins are antimicrobial peptides with a triple-stranded ß-hairpin structure, six conserved disulfide linked cysteine residues and are positively charged existing in two main families in humans, six alpha and four beta defensins (64,71). They have

antimicrobial, antiviral and antifungal properties, modify cell migration and maturation and trigger histamine and prostaglandin D_2 release from mast cells (64).

1.3.1.2 Azurocidin

Azurocidin is a bactericidal and antiviral protein and has no proteolytic activity despite being a serine protease. Its antiviral potential stems from a disruption of the envelope or the capsid and is not dependent on disulfide bonds. Furthermore, it is released early from secretory granules of neutrophils and has regulatory effects on recruitment and activation of monocytes (64).

1.3.1.3 Bactericidal/permeability increasing protein (BPI) and the Palate, lung and nasal epithelium clone PLUNC family

BPI is present in neutrophils, eosinophils and also epithelial cells of the oral cavity. It is active against gram-negative bacteria and neutralizes lipopolysaccharide (LPS). As referred to in its name, BPI increases permeability of bacterial cell membranes causing hydrolysis of bacterial phospholipids. Because of its structural similarities the PLUNC (palate, lung and nasal epithelium clone) protein belongs to the BPI protein family. In humans it is expressed by major salivary glands and mucosal glands in the upper respiratory tract including the nose. The PLUNC proteins are indirectly antimicrobial through binding to bacteria and LPS, but the exact mechanisms are still unknown (64,72-74).

1.4 Proteomics

Proteomics was introduced and defined in 1995 as characterization of the entire protein content of cells, tissues, body fluids or even entire organisms on a large scale (75,76). Basically two definitions of proteomic analyses exist, one that only deals with proteins as gene products and the second that also involves the genetic background i.e. genomics and/or transcriptomics on, for instance, mRNA based analysis. The goal of proteomics is to identify proteins, their functions, interactions, localizations, origins, modifications and involvement in disease. Proteomics as a relatively new field has ancestors in proteins studies dating back to 1975 when 2D gel-electrophoresis came up. With this technology proteins could be separated on the gel according to their molecular mass and movement in the electric field, however visualization of proteins alone did not lead to identification (75). The first technology for proteins sequencing was the Edman degradation for analyzing amino acids and peptides constituting a protein. The advent of mass spectrometry was the breakthrough of protein identification and thus the proteomic field (75-77).

The advantage of proteomic analysis is to obtain information of the end product of genes, which pure genomic studies cannot entirely fulfill. Proteins are the actors in living organisms and only their understanding can provide understanding of biological function. Gene expression is important, however, their complex composition of exons and introns cannot make the end product fully predictable. The same is true for RNA analysis, which does not entirely reflect protein expression. More so, proteins also undergo posttranslational modification impacting on their function, which is not deductible from RNAs. Furthermore, protein function is also defined by 3D structure and one protein may have a variety of functions in an organism. Protein modification *e.g.* phosphorylation and impact on function can be studies by proteomic approaches comparing different proteomes simultaneously. Protein localization is also an important factor in disease since wrong localization of proteins can be the cause of the disease affecting protein channels, protein complexes etc. The interaction between proteins is another important issue. Particularly in cell cycle signaling is important. The up- or down-

regulation of proteins can decide over cell death or proliferation, which again is mandatory in cancer research and understanding mechanism of disease onset (75).

Basically a proteomic experiment is performed in three steps: i) separation and isolation of proteins of a specific specimen, ii) obtaining structural information for identification, and iii) characterization and database search to obtain information about the proteins and put them into, *e.g.* a clinical context.

1.4.1 Gel electrophoresis

Since complex protein mixtures are obtained from biological specimen it is important to separate the distinct proteins from each other and visualize them. Gel electrophoresis in polyacrylamide gel (PAGE) is very common. Proteins are solubilized in sodium dodecyl sulfate. In 1D electrophoresis proteins are separated in one lane, in 2D electrophoresis a second dimension is included thus proteins are separated according to their mass and their charge. This is of advantage in complex protein mixtures where only one property would account for more distinct proteins which would inadequately be separated and thus overlooked. Another development in electrophoresis was difference gel electrophoresis (DIGE). Here fluorescent tags are applied on two protein samples with two different dyes. After separation the two gels can be superimposed and differences between the samples become evident (75). Problems of gel electrophoresis are the time-consuming process, complex proteomes are not sufficiently separated by a single gel, and abundant proteins might camouflage low-abundant proteins, which are lost (75).

Nevertheless, gel electrophoresis is very common, but newer technologies aim at by-passing the step of electrophoresis in proteomic analysis. Protein digestion into peptides by tryptase and separation by high-performance liquid chromatography (HPLC) as performed in the present study are more practicable (75,76).

1.4.2 Mass spectrometry

After sample preparation by liquid chromatography, mass spectrometry (LC MS/MS) provides structural information like peptide masses and amino acid sequences, which can be used to search databases and identify proteins in the experiment. In order to analyze samples they need to be charged and dry, this process of ionization can be performed in two ways: electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) (75). In ESI the liquid sample flows from a micro capillary to the mass spectrometer with a potential difference leading to evaporation and formations of ions. An improvement was the nanospray ionization where flow rates are decreased, so the amount of sample needed is reduces and time for analysis is increased. This approach was used in the present study (Figure 1). In MALDI the sample is put onto a matrix and irradiated by a laser. The matrix absorbs the laser and the energy of the laser promotes the formation of molecular ions. The last step of mass spectrometry is mass analysis. The mass analyzer resolves the ions according to their mass and charge. Basically, three types of mass analyzers exist: quadrupole mass analyzers, time of flight mass analyzers and ion trap analyzers, or combination of these with a Fourier transform ion cyclotron resonance (75,76).

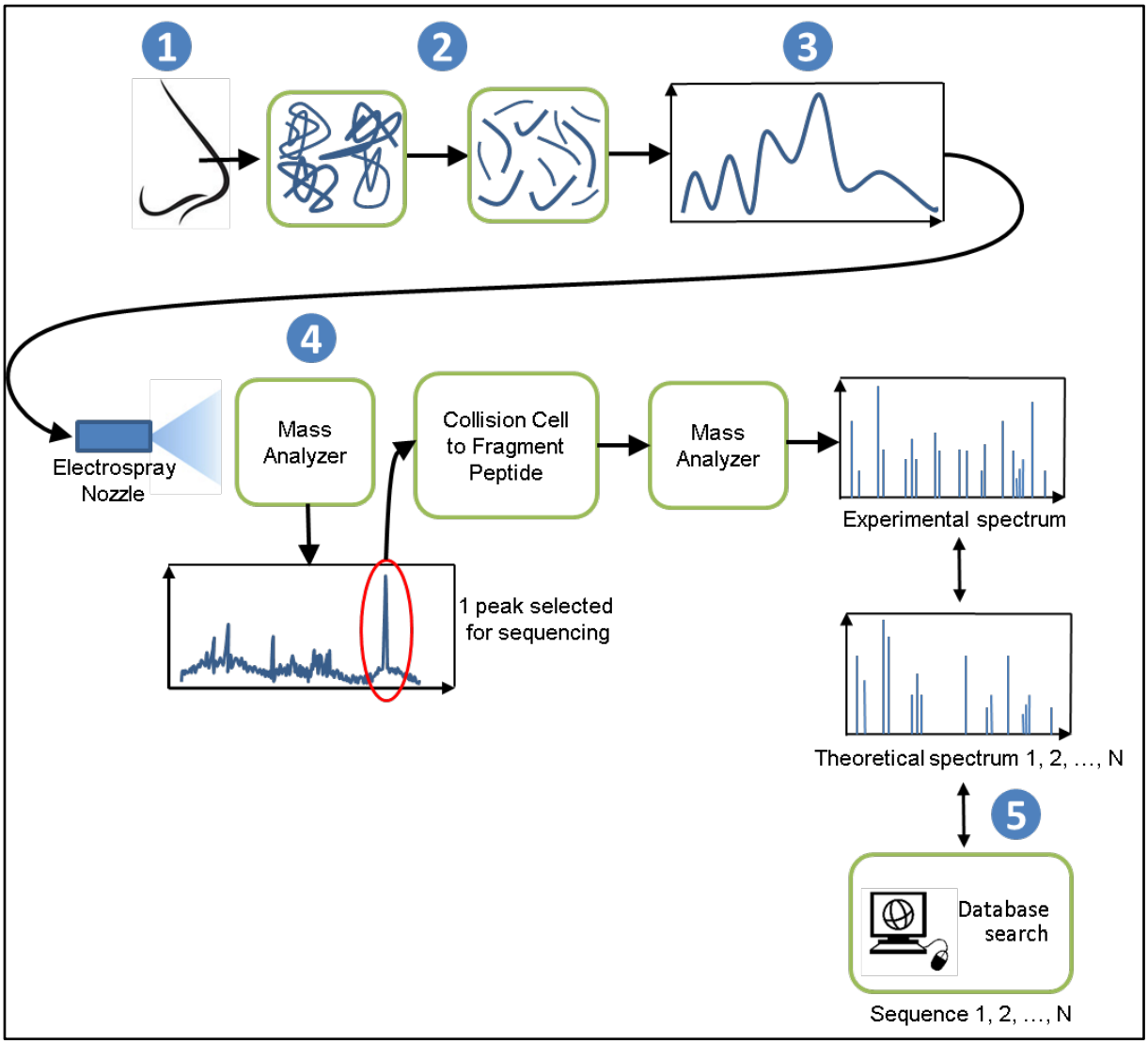

Figure 1: Schematic display of proteomic workflow, 1=mucus collection, 2=protein digestion, 3=chromatographic separation, 4=mass spectrometric analysis (LC MS/MS), 5=adjustment of experimental to theoretical spectra and database search.

1.4.2.1 Label-free quantitative mass spectrometry

Quantitative proteomics can be separated into isotope labeling or label-free techniques. Labeling involves modification of peptides with stable isotopes. Approaches used are iTRAQ, TMT, ICAT, SILAC, ICPL and many others. In isobaric tags for relative and absolute quantitation (iTRAQ) the peptides from digested proteins are covalently labeled with commercially available tags of varying mass at their N-terminus and side chain amines. Due to the different masses of the tags they are fragmented and show specific mass spectra that allow identification of the attached protein. Up to 8 samples can be labeled and quantified simultaneously. Tandem mass tags (TMT) are isobaric isotopes similar to iTRAQ, here up to six samples can be labeled. Isotope-coded affinity tag (ICAT) isotope labeling of either hydrogen or deuterium is used to differentiate between two samples (*e.g.* disease *vs.* healthy). Three elements are crucial for this technique: a reactive group to label an amino acid side chain, an isotopically coded linker to differentiate the two groups into light hydrogen and heavy deuterium, and an affinity tag like biotin for the isolation of labeled proteins. Compared to iTRAQ the protein coverage in ICAT is less complete compared to the original proteins sequence. Stable isotope labeling by/with amino acids in cell culture (SILAC) is similar to ICAT, the labels are not radioactive and samples (*e.g.* cells) are cultured in two different media with normal amino acids and with labelled amino acids. Isotope Coded Protein Labels (ICPL) further allows to detect protein isoforms, posttranslational modifications and splicing variants. The quantitation is considered exact, however, special software and larger sample concentrations are required. Higher costs and incomplete labeling are downsides of this technique requiring reliable label-free techniques (77,78).

Label-free quantitation can be divided into area under the curve (AUC) and spectral counting.

1.4.2.1.1 Area under the curve

This involves the measurement of chromatographic peak areas that are linearly proportional to the concentration of a given peptide. This is based on the finding that an ion with a specific mass to charge ratio (m/z) is detected at a certain time with certain intensity. Signal intensity from ESI correlates with ion concentration (77,78).

1.4.2.1.2 Spectral counting

It was observed that more abundant peptides will produce a higher amount of MS/MS spectra. The spectral counts, therefore, are proportional to the protein amount. Spectral counts can be used to calculate abundances as in the protein abundance index (PAI), which is defined as the number of observed peptides divided by the number of observable tryptic peptides for each protein within a given mass range of the mass spectrometer. This was later modified to the emPAI, which is the exponential form of the PAI minus one (78). This is directly proportional to the protein content of a given sample. Correlations were found to be high between the calculated values and the effective protein amount.

1.5 Aims of the Study

The aim of this study was to obtain a large spectrum of proteins present in nasal mucus and identify potential key proteins involved in the pathophysiology of allergic rhinitis, since nasal mucus is considered to be the first line defense barrier of the nasal mucosa. As of today nasal mucus proteins have only been studied in a small number. The proteomic approach offers to analyze the entire proteins of a respective body compartment and see the changes in the proteome between groups or under different environmental circumstances (*e.g.* in pollen season *vs.* out of pollen season). In allergic rhinitis patients some of these proteins might be harmful being involved in inflammatory responses, whereas other proteins could reduce immune response and deactivate harmful pollen content like proteases as defense mechanisms. Targeting these proteins for interventions might offer new therapeutic strategies on a mucus level as it is the first line defense barrier of the nasal mucosa.

The study consists of two chapters. In chapter one the feasibility and preliminary data were analysed as a proof of concept. The proteomic data from mass spectrometric analysis was validated by Western blotting. Here patients were recruited throughout the year regardless of seasonal differences in natural pollen exposure. In the second chapter patients were recruited in or out of season respectively and followed-up at a second time point in and out of season opposite to their first visit to obtain changes in the proteome over one year with and without natural pollen exposure.

2. Material and Methods

2.1 Patients

For chapter one fifty-eight individuals (31 male, 27 female) were included in this study. Mean age was 35 years (SD: 8.9 years) comprising 29 (50%) allergic rhinitis patients and 29 (50%) healthy controls. For chapter two, twenty-two individuals (7 male, 15 female) with a mean age of 33 years (SD: 9.7 years) were included in the study group comprising 10 (45%) allergic rhinitis patients and 12 (55%) healthy controls. Allergy status was verified by skin prick tests (SPT, Allergopharma GmbH & Co. KG, Reinbek, Germany) and specific IgE (ImmunoCAP, Thermo Fisher Scientific Inc., Vienna, Austria) respectively (Table E1 and Table E2 in the Appendix). Patients sensitized to house dust mite or animals only were excluded to avoid bias due to small sample size. Thus only patients reporting symptoms during pollen season were considered for evaluation. Patients with acute and/or chronic sinusitis as defined by the EPOS (79) guidelines were excluded from the study. Furthermore patients with malignant tumors, any other infectious or cardiopulmonary disease, or those treated with systemic or topical drugs like antihistamines, corticosteroids, antibiotics, antifungals or any other immunemodulatory drugs in the four weeks prior to the study were excluded. Informed consent was obtained from all patients (allergic rhinitis patients and healthy controls) before entering the study. The study was approved by the institutional review board of the Medical University Graz (20-045 ex 08/09 and 26-080 ex 13/14).

2.2 Sample collection

To collect nasal mucus a special suction device was used (Sinus Secretion Collector, Medtronic Xomed Inc., Jacksonville, Florida, USA). Without previous interventions (decongestants, local anesthetics) untreated mucus was obtained under endoscopic control from the nasal cavity and middle meatus with meticulous care taken not to touch the mucosa. Then, mucus was deep-frozen at -80° Celsius before processing for LC-MS/MS mass spectrometry.

2.3 Sample preparation

After defrosting 500 µl phosphate buffered saline were added to the samples, thoroughly mixed and centrifuged at 12,000 rpm for 5 min to remove insoluble particles. Protein content was estimated by Bradford assay (Bio-Rad, Vienna, Austria). Fifty µg of protein was precipitated with >10 volumes of acetone at -20°C overnight. Protein was collected by centrifugation at 10,000 rpm for 10 min, resolubilised in 35 µl 6 M urea, reduced with 5 mM DTT for 20 min by shaking at 550 rpm at 56°C and alkylated with 10 mM IAA by shaking at 550 rpm at RT for 15 min. The samples were diluted to 0.9 M urea with 155 µl 100 mM ammonium bicarbonate. Protein was digested with 1 µg trypsin by shaking at 550 rpm over night at 37°C. Samples were acidified with 6 µl 5 % formic acid. Completion of digest was controlled by 4-12% SDS-PAGE of 2 µg aliquots of digested versus undigested samples and silver staining 2.5 µg of protein digest was filled up to 50 µl with 0.1% formic acid for proteomic analysis.

2.4 Mass spectrometric analysis

40 µl, i.e. 2 µg proteins, were separated by nano-HPLC on an Agilent (Vienna, Austria) 1200 system equipped with a Zorbax 300SB-C18, 5µm, 5 x 0.3mm enrichment column and a Zorbax 300SB-C18, 3.5 µm, 150 x 0.075 mm nanocolumn. Samples were injected and concentrated on the enrichment column for 6 min using 0.1 % formic acid as isocratic solvent at a flow rate of 20 µL/min. The column was then switched into the nanoflow circuit, and the sample was loaded on the nanocolumn at a flow rate of 300 nL/min and separated using the following gradient: solvent A: water, 0.1% formic acid; solvent B: acetonitril/water 80/20, 0.1% formic acid; 0-10min: 10% B; 10-130min 10-60% B, 130-132min 60-95% B, 132-140min 95% B, 140-140.01 min 95-10%. The sample was ionized in the nanospray source equipped with nanospray tips (PicoTipTM Stock# FS360-75-15-D-20, Coating: 1P-4P, 15+/- 1µm Emitter, New Objective) and analyzed in a Thermo Scientific (Vienna, Austria) LTQ-FT mass spectrometer in positive ion mode by alternating full scan MS (m/z 400 to 2000) in the ICR cell and MS/MS by CID of the 5 most intense peaks in the ion trap with dynamic exclusion enabled (for a duration of 10s).

2.4.1 Mass spectrometric data analysis

The LC-MS/MS data were analyzed by searching the human SwissProt public database (downloaded on March 10th 2012) with Spectrum Mill Rev. A.03.03.078 (Agilent, Vienna, Austria) and Proteome Discoverer 1.3 (Thermo Scientific, Vienna, Austria) using Mascot 2.2 (MatrixScience, London, UK). Detailed settings: Enzyme: trypsin, max. missed cleavage sites: 2, carbamidomethylation on lysine as fixed modification, oxidised methionine as variable modification, maximum precursor charge 3; precursor mass tolerance +/- 5 ppm, product mass tolerance +/- 0.7 Da; acceptance parameters were 2 or more identified distinct peptides after automatic validation (Mascot: decoy search, FDR < 5%; Spectrum Mill: for precursor charge of 2: score threshold was 6.0, %SPI

threshold was 60.0, Fwd-Rev score threshold was 2.0 and rank 1-2 score threshold was 2.0, for precursor charge of 1: score threshold was 6.0, %SPI threshold was 70.0, Fwd-Rev score threshold was 2.0 and rank 1-2 score threshold was 2.0, for precursor charge of 3: score threshold was 8.0, %SPI threshold was 70.0, Fwd-Rev score threshold was 2.0 and rank 1-2 score threshold was 2.0).

2.4.2 Proteomic analysis

Sample preparation, LC-MS/MS analysis and LC-MS/MS data was performed and reported according to MIAPE (minimum information about a proteomic experiment) (80). Spectral counting of the total peptides identified (i.e. number of MS/MS spectra matched to a protein) was used to compare relative protein abundances of the same protein between groups (81,81,82,82) and normalized areas under the curves for group and seasonal differences. Identified proteins were annotated using data from Uniprot (www.uniprot.org), PANTHER classification system (www.pantherdb.org) and DAVID (DAVID Bioinformatics Resources 6.7, National Institute of Allergy and Infectious Diseases (NIAID), NIH, USA; http://david.abcc.ncifcrf.gov/). Enrichment analysis was performed with BINGO 2.44 (83) in Cytoscape 2.81 software (www.cytoscape.org) (83).

2.4.3 Statistical analysis

For statistical analysis of group differences in chapter one, spectral counts for proteins with a mean spectral count (SC) of equal or greater than four in either group were accepted (12). For chapter two areas under the curve (AUCs) (i.e. mean areas of extracted ion chromatograms of the individual peptides matched to a protein) normalized on the total AUC of all proteins in each sample were used to compare relative protein abundances of the same protein between groups and seasons given a smaller sample size (17). SCs and AUCs are presented as means with standard deviation (SD) or

standard error of mean (SEM). Mann Whitney U test was used to identify significantly altered proteins between groups with SPSS 21.0 software (Chicago, Illinois, USA). A p-value of <0.05 was considered significant.

For statistical analysis of enrichment data created with BINGO/ Cytoscape hypergeometric tests were performed and corrected by Benjamini & Hochberg False Discovery Rate (FDR) correction at a significance level of 0.05.

2.5 Western blot analysis

20 µg of lysed proteins from different nasal mucus samples were separated by 4-20% denaturing reducing SDS-PAGE (Bio-Rad, Vienna, Austria). Separated proteins were transferred onto nitrocellulose membrane by semi-dry blotting for 1 hour at 180 mA. Total transferred protein was detected with Pierce MemCode (Thermo Scientific, Vienna, Austria) reversible stain and imaged on a ChemDocXRS (BioRAD, Vienna, Austria). Membranes were blocked in blocking buffer (5 % skim milk in TBST buffer (20 mM Tris-HCl, 137 mM NaCl, 0.1 % Tween 20, pH 6.7) for 1 h at room temperature, and then incubated with primary antibodies overnight at 4ºC. Primary antibodies specific to α-1-antitrypsin (Abcam, Cambridge, UK, product number 9400), α-2-macroglobulin (Abcam, Cambridge, UK, product number 58703), haptoglobin (Abcam, Cambridge, UK, product number 13429), complement C3 (Abcam, Cambridge, UK, product number 97462) and apolipoprotein A-2 (Abcam, Cambridge, UK, product number 24241) were used for protein detection. After washing with TBST the membrane was incubated with secondary antibodies (goat anti mouse or anti rabbit IgG-HRP conjugates, Abcam, Cambridge, UK) for 1 h at RT. Immunocomplexes were visualized with Pierce ECL chemiluminescent substrate (Thermo Scientific, Vienna, Austria). Densitometric evaluation was performed with Image Lab 4.1 (Bio-Rad, Vienna, Austria). Volumes of protein bands were determined by global background subtraction and normalised on total protein detected in the lane by MemCode. Means of 5 patients in each group and SEM were calculated.

3. Results

Please note that parts of this chapter have been published in Tomazic P.V. *et al.*, Journal of Allergy and Clinical Immunology, 2014 (84), Tomazic P.V. *et al.*, Laryngoscope, 2015 (85) and Tomazic P.V. *et al.*, Journal of Proteomics, 2016 (86).

3.1 Chapter 1

The mean mucus protein concentration was 3.34 mg/ml (SD 4.1) in allergic rhinitis patients (AR) and 2.88 mg/ml (SD 3.7) in healthy controls (HC) not reaching significance (p=0.57). In shotgun proteomics the total sample is digested by a protease, typically trypsin, and the resultant peptides are separated by liquid chromatography and sequenced by tandem mass spectrometry. Proteins are identified by matching experimental and theoretical peptide spectra and statistically validated. The number of identified peptides which are unique (i.e. specific) for a protein, and the protein sequence coverage reflect the reliability of the identification. Using this approach, 267 proteins were identified in nasal mucus over all 29 AR patients and 29 HC by shotgun proteomics (please see corresponding Tables in Appendix).

To compare relative levels of individual proteins between the two groups we applied spectral counting, which is an identity-based label-free quantitation method and, therefore, well suited to analyze large sample numbers (81,81,82,82). The spectral count (SC) is the total number of detected peptide spectra matched to each protein. A mean SC of 1165 (SD 201) was measured in mucus of AR patients (n=29) and 1058 (SD 300) in HC (n=29) respectively when injecting the same amount of digested protein (2µg) into the mass spectrometer.

The number of detected peptides depends on 1) the number of possible peptides and thus on individual protein size and sequence as well as their hydrophobicity and on the used analytical set up, and 2) on their ionization properties and thus on the peptide sequence and the sample matrix. Accordingly, while relative comparison of individual protein amounts between samples is reliably performed in highly similar samples by spectral counting, absolute quantitation of individual proteins within one sample would require alternative methods using internal standards. However, this is beyond the scope of this study, where we aimed at identifying relative differences in mucus proteome abundance between rhinitis allergic patients and HC.

The control nasal mucus proteome comprises 247 proteins, which were identified with at least 2 unique peptides over all 29 HC (Table E3). The most abundant proteins with a mean SC>20 were albumin (ALB), lactotransferrin (LTF), Ig alpha-1 chain C region (IGHA1), polymeric immunoglobulin receptor (PIGR). Prolactin-inducible protein (PIP), Ig kappa chain C region (IGKC), BPI fold-containing family B member 1 (BPIFB1), lysozyme C (LYZ), and Ig lambda-2 chain C region (IGLC2). The found albumin originates from plasma. Immunglobulin A consisting of the heavy alpha chain and the light kappa and/or lambda chains is secreted locally into the nasal mucosa by transport across the endothelial cells via binding to Polymeric immunoglobulin receptor. The BPI fold containing family B member is also known as Long palate, lung and nasal epithelium carcinoma-associated protein 1 and originates from nasal epithelium too while prolactin-inducible protein is produced by salivary glands and lactotransferrin and lysozyme by submucosal glands, respectively.

Then we compared the control nasal mucus proteome to the AR proteome. Here, 222 of the 267 in total identified proteins (83 %) were present in both AR patients and HC whereas 20 of 267 (7.5%) were exclusively found in AR patients and 25 of 267 (9.5%) exclusively in HC (Table E3).

The fifty-one most abundant proteins with a mean SC of ≥4 in either group are depicted in Figure 2 and compared between AR and controls by spectral counting (Figure 2). Of the most abundant proteins, albumin, Ig A (heavy and light chains) and BPIFB1 were

slightly increased in AR, while LTF, LYZ and PIGR were slightly decreased, but none of these changes was statistically significant.

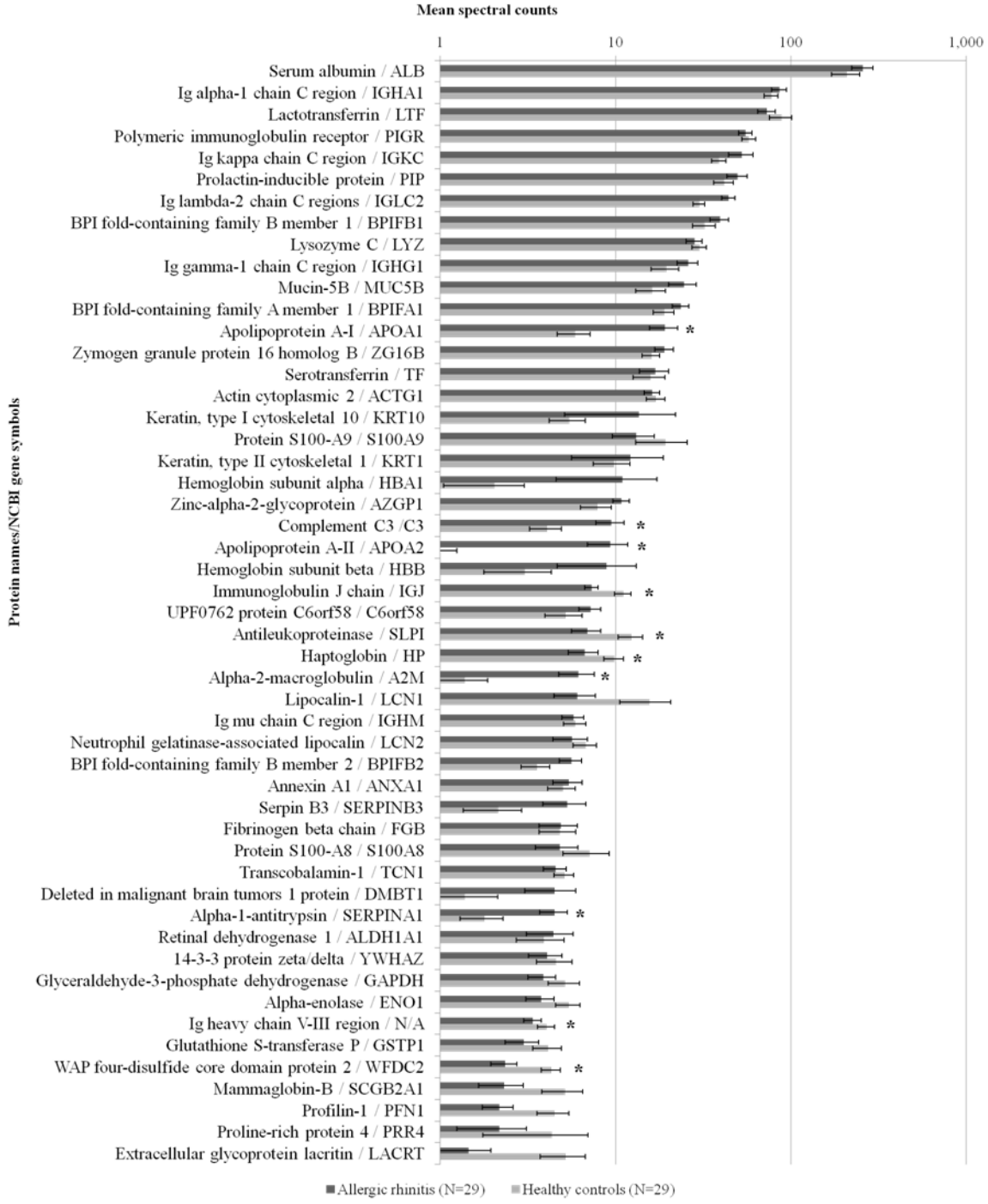

Figure 2: Differences in mean spectral counts (logarithmic scale) between allergic rhinitis patients and healthy controls for proteins (N=51) with a spectral count (SC) of equal or higher than 4 in either group. Proteins marked with an asterisk are significantly different ($P<0.05$, Mann Whitney U-Test).

3.1.1 Significantly more abundant proteins and enriched biological processes in allergic rhinitis patients

With a mean SC ≥4, following 5 proteins were significantly more abundant in allergic rhinitis patients: apolipoprotein A-2/APOA2 (9.7 fold), alpha-2-macroglobulin/A2M (4.5 fold), apolipoprotein A-1/APOA1 (3.2 fold), alpha-1-antitrypsin/SERPINA1 (2.5 fold), and complement C3/C3 (2.3 fold) (Table 3).

Acc. No.	NCBI Gene symbols	P-value	SC Mean"AR"	SEM "AR"	P. in "N" AR	SC Mean "HC"	SEM "HC"	P. in "N" HC	Ratio AR/HC
P02652	APOA2	0.000	9	2.4	24/29	1	0.3	12/29	9.68
P01023	A2M	0.002	6	1.4	20/29	1	0.5	11/29	4.45
P02647	APOA1	0.001	19	3.5	26/29	6	1.3	20/29	3.25
P01009	SERPINA1	0.003	4	0.8	24/29	2	0.5	16/29	2.50
P01024	C3	0.026	9	1.7	25/29	4	0.8	21/29	2.32
P01766	Ig heavy chain V-III region BRO	0.042	3	0.4	28/29	4	0.5	27/29	0.84
P00738	HP	0.008	7	1.3	29/29	10	1.3	29/29	0.68
P01591	IGJ	0.010	7	0.7	29/29	11	1.2	29/29	0.66
P03973	SLPI	0.030	7	1.3	27/29	12	2.0	27/29	0.56
Q14508	WFDC2	0.004	2	0.4	25/29	4	0.5	28/29	0.54

Table 3: Significantly different proteins between allergic rhinitis patients (AR) and healthy controls (HC), SC=spectral count, SEM=standard error of mean, AR/HC=ratio of mean SC (not rounded) of allergic rhinitis patients vs. healthy controls, P. in “N” AR or HC=presence of protein in number of patients or controls/total number of probands, Acc.No.=accession number obtained from UniProt Database.

Enrichment analysis obtained by BINGO software (GOSlim_generic) revealed 12 enriched biological processes in AR patients as compared to the total human proteome, 8 of which were also increased in HC. The remaining four were exclusively found in AR patients: lipid metabolic process, transport, symbiosis encompassing mutualism through parasitism and response to external stimulus (Figure 3a).

3.1.2. Significantly less abundant proteins and depleted biological processes in allergic rhinitis patients

Considering a mean SC ≥4, following 5 proteins were significantly decreased in AR patients compared to a higher abundance in HC: antileukoproteinase/SLPI (0.6 fold), WAP four-disulfide core domain protein/WFDC2 (0.5 fold), haptoglobin/HP (0.7 fold), IgJ chain/IGJ (0.7 fold) and Ig hc V-III region BRO (0.8 fold) (Table 3). Six biological processes were significantly reduced and not present in AR patients compared to HC: carbohydrate metabolic processes, generation of precursor metabolites and energy, organelle organization, cytoskeleton organization, cell differentiation and cellular component organization (Figure 3b).

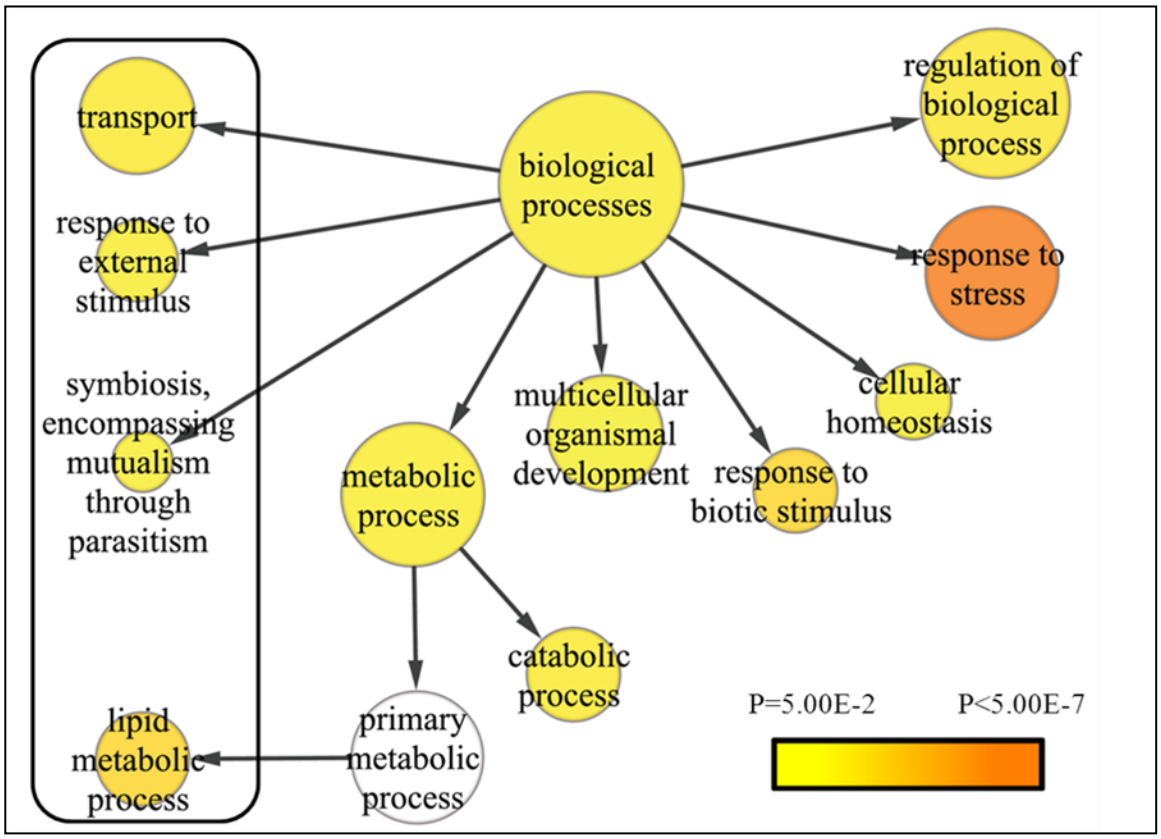

Figure 3a: Enrichment analysis of biological processes, obtained by BINGO software, of significantly enriched biological processes of proteins present in allergic rhinitis patients (N=12) compared to total human proteome. Nodes surrounded by black rectangle indicate biological processes exclusively found in allergic rhinitis patients (N=4) compared to healthy controls. Colour bar in the right lower quadrant indicates level of significance from low (yellow) to high (orange). Statistical analysis was performed with a hypergeometrical test. A p-value of <0.05 was considered significant.

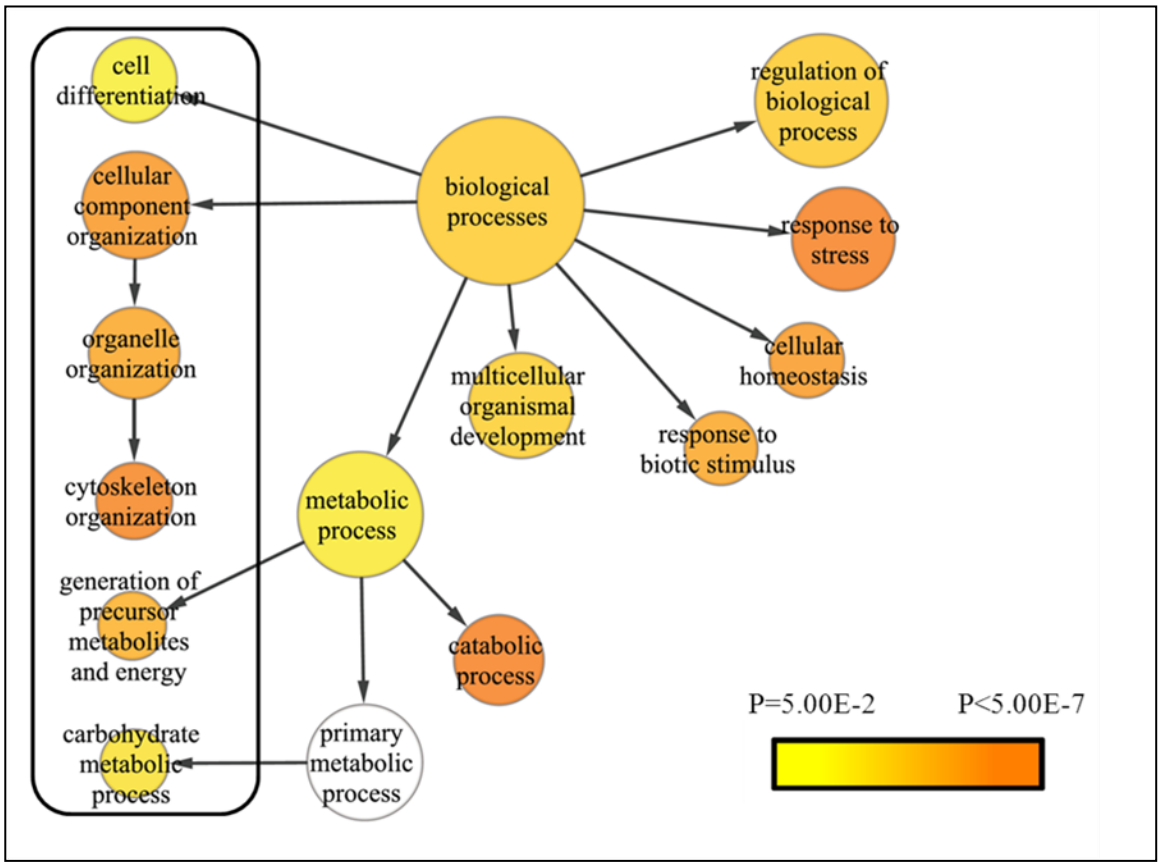

Figure 3b: Enrichment analysis of biological processes, obtained by BINGO software, of significantly enriched biological processes of proteins present in healthy controls (N=14) compared to total human proteome. Nodes surrounded by black rectangle indicate biological processes exclusively found in healthy controls (N=6) compared to allergic rhinitis patients. Colour bar in the right lower quadrant indicates level of significance. Statistical analysis was performed with a hypergeometrical test. A p-value of <0.05 was considered significant.

3.1.3. Western Blot Analysis

Five significantly altered proteins, for which commercial antibodies were available, were additionally assessed by Western blotting in nasal mucosa of five AR patients and five HC, respectively. The detected bands for APOA2 (12 kDa), A2M (160 kDa), SERPINA1 (55 kDa) and C3 (110 kDa) were found to be increased while HP (50 kDa) was decreased in allergic subjects confirming mass-spectrometric findings (Figure 3).

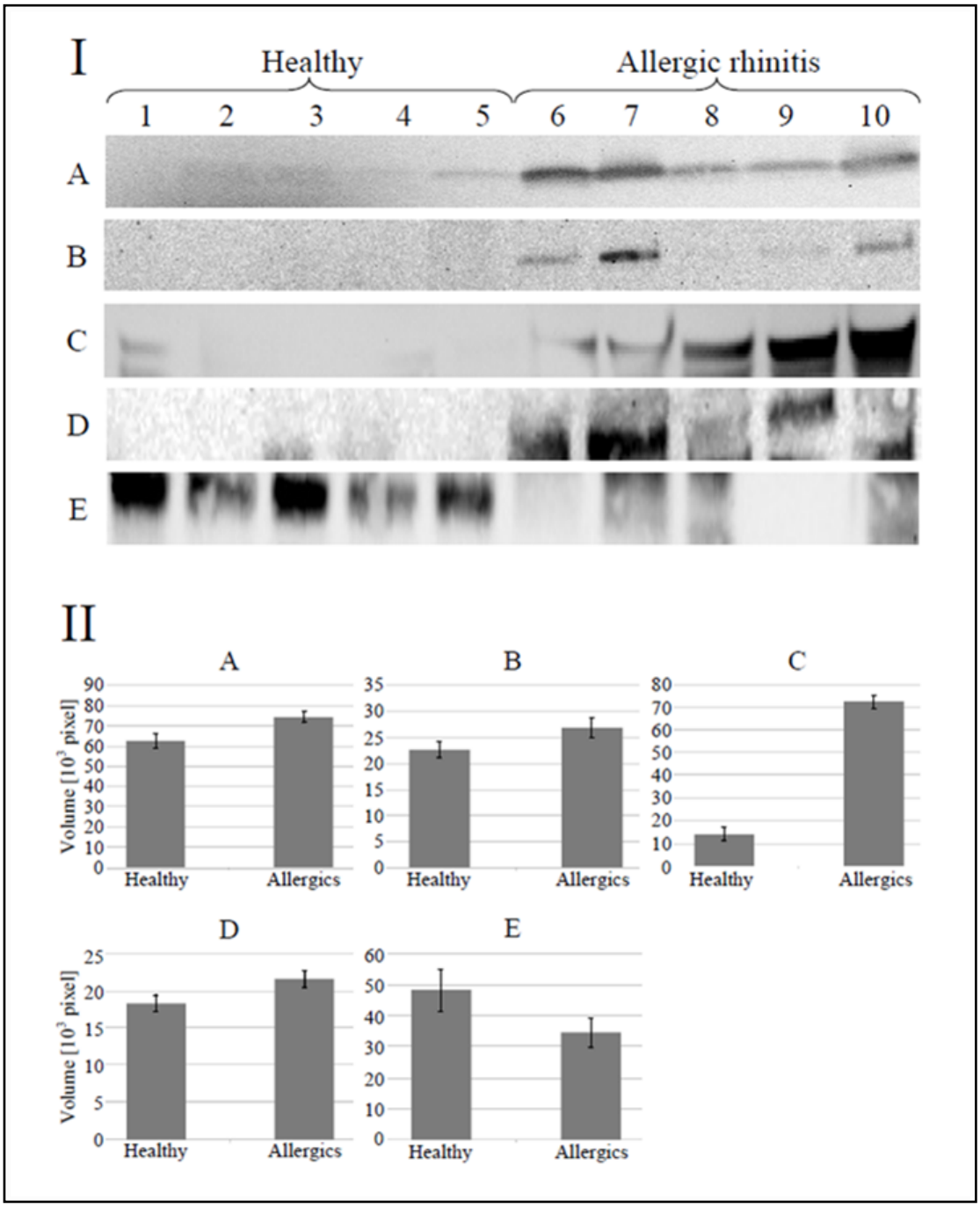

Figure 4: Western blot analysis of selected proteins. Five proteins APOA2 (A), A2M (B), SERPINA1 (C), C3 (D) and HP (E) were analysed. Panel I shows the respective immunoblots of 5 healthy controls and 5 allergic rhinitis patients. Estimated molecular weights in comparison to a protein standard were 12 kDa for APOA2, 160 kDa for A2M, 55 kDa for SERPINA1, 120 kDa for C3 and 50 kDa for HP. Panel II shows the densitometric analysis of the immunoblots. Means and SEM are compared in healthy versus allergic patients for each protein.

3.1 Chapter 2

The mean total protein concentration over all samples was 0.58 mg/ml (SD: 0.66). Allergic rhinitis patients (AR) had a higher mean protein concentration ((0.78 mg/ml (SD: 0.87)) than healthy controls ((0.42 mg/ml (SD: 0.35)). However, this difference was not significant (p=0.377). Overall 430 different proteins were detected in both groups, while 327 were detected in AR and 366 in HC respectively. Of these 203 proteins (47.2%) were newly identified as nasal mucus proteins (Table E4). The most abundant proteins were serum albumin (ALB), lysozyme C (LYZ), Ig alpha-1 chain C region (IGHA1) and Ig alpha-2 chain C region (IGHA2) independent of disease and season. Statherin (STATH) significantly showed the highest diametric abundance change with respect to group and seasonal differences (Figure 5).

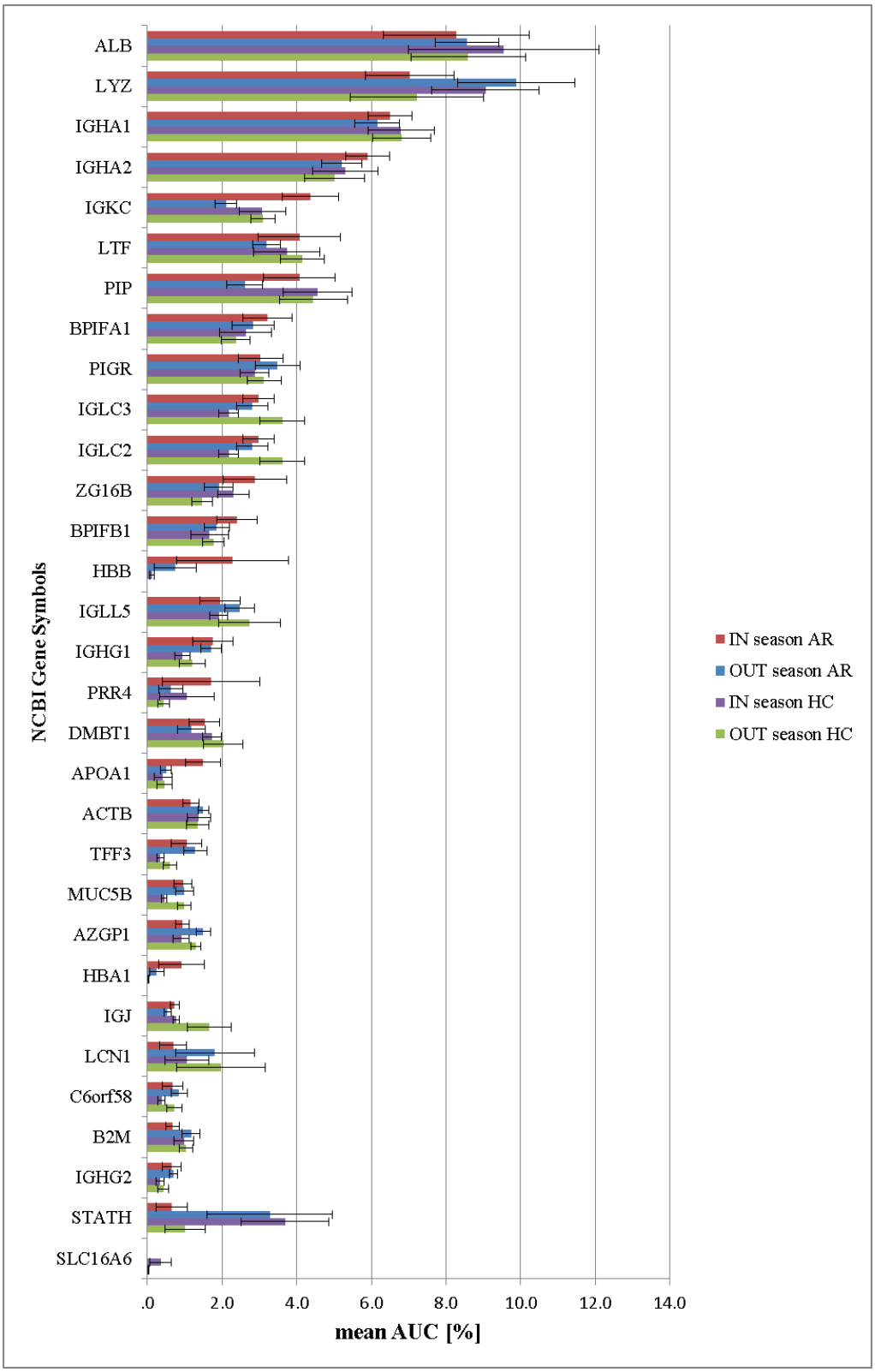

Figure 5: Differences in mean normalized areas under the curve between allergic rhinitis patients and healthy controls in and out of season for the 30 most abundant proteins.

In AR patients 280 proteins were detected in season and 259 out of season. Of these, 211 proteins were identified in and out of pollen season, 69 solely in season (with 36 exclusively identified in AR) and 48 solely out of season (with 18 exclusively in AR). In HC 328 proteins were detected in season, and 242 out of season. Of these 204 proteins were found in and out of season, 124 solely in season (with 65 exclusively in HC) and 38 solely out of season (with 22 exclusively in HC). The difference in abundance between the groups for proteins present only either in or out of season was highly significant (p=0.004) (Figure 6).

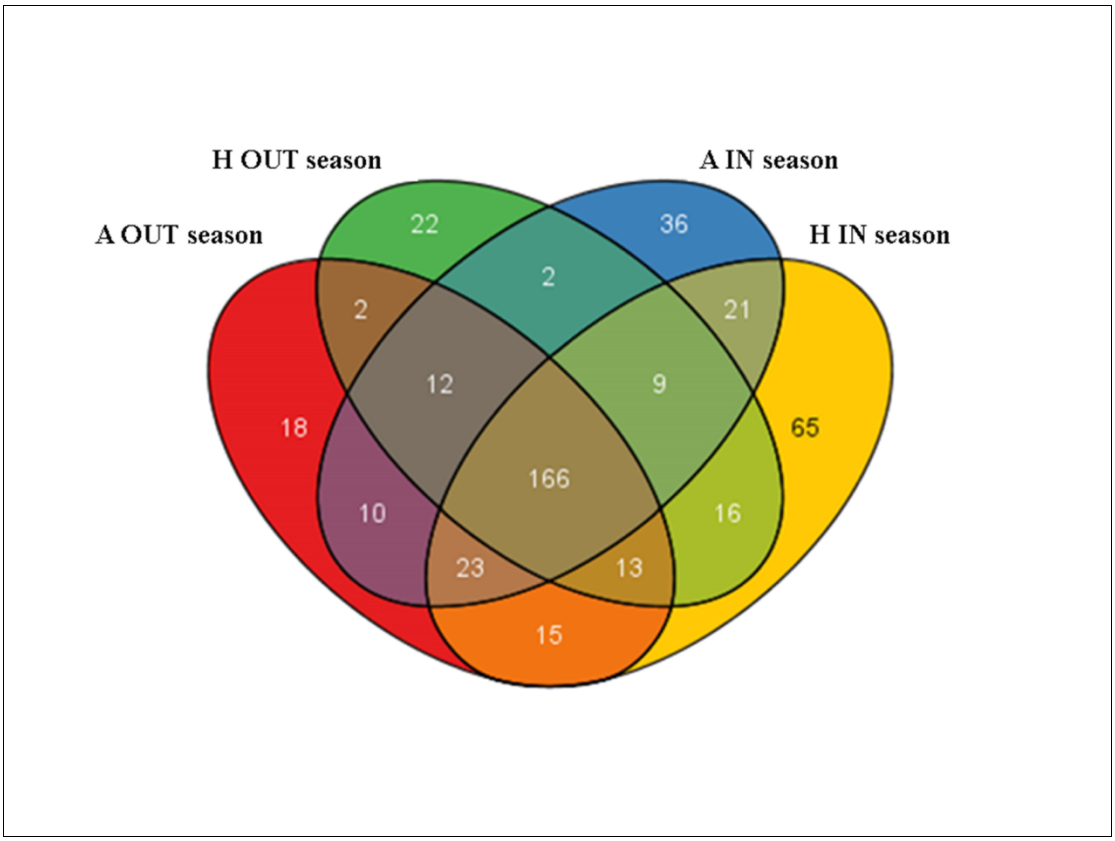

Figure 6: Venn diagram of protein distribution (number of proteins) between groups and seasons.

3.2.1. Seasonal differences in allergic rhinitis patients

In AR patients eight proteins showed significantly different abundances in season as compared to out of pollen season. These were clusterin (CLU), Ig kappa chain C region (IGKC), glutathione s-transferase P (GSTP1), neutrophil elastase (ELANE), histone H2B type 1-K (HIST1H2BK), protein S100-A8 (S100A8), protein S100-A12 (S100A12) and rho GDP-dissociation inhibitor 2 (ARHGDIB). Of these CLU and IGKC were significantly more abundant in season (2.2 and 2.1-fold respectively). GSTP1 (0.5-fold), ELANE (0.4-fold), HIST1H2BK (0.3-fold), S100A8 (0.2-fold), S100A12 (0.2-fold) and ARHGDIB (0.1-fold) were significantly less abundant in season (Table 4).

3.2.2. Seasonal differences in healthy controls

In HC 12 proteins showed significantly different abundances between the seasons. These were polyubiquitin-C (UBC), tubulin alpha-1B (TUBA1B), hemoglobin beta (HBB), fatty acid-binding protein, epidermal (FABP5), Ig kappa chain V-I region DEE (N/A), clusterin (CLU), thioredoxin (TXN), beta-microseminoprotein (MSMB), Ig heavy chain V-III region BRO, mucin-5B (MUC5B), antileukoproteinase (SLPI) and protein S100-P (S100P). Of these UBC, TUBA1B, HBB and FABP5 were only present in pollen season. Ig kappa chain V-I region DEE (5.3-fold), CLU (5.0-fold), TXN (4.3-fold), MSMB (3.2-fold) and Ig heavy chain V-III region BRO (2.7-fold) were significantly more abundant in

season as compared to out of pollen season. MUC5B (0.5-fold), SLPI (0.2-fold) and S100P (0.2-fold) were significantly less abundant in season (Table 4).

				Timepoint						
				IN season			OUT season			
Group	Acc. No.	NCBI Gene names (primary)	p-value	mean AUC [%]	SEM AUC [%]	P. in "N"	mean AUC [%]	SEM AUC [%]	P. in "N"	Ratio IN vs. OUT season
Allergic	P10909	CLU	0.027	0.22	0.03	10/10	0.10	0.04	5/10	2.2
	P01834	IGKC	0.008	4.36	0.76	10/10	2.11	0.29	10/10	2.1
	P09211	GSTP1	0.037	0.17	0.07	5/10	0.37	0.06	1/10	0.5
	P08246	ELANE	0.039	0.07	0.04	3/10	0.19	0.04	8/10	0.4
	O60814	HIST1H2BK	0.029	0.21	0.08	6/10	0.65	0.21	1/10	0.3
	P05109	S100A8	0.047	0.09	0.03	5/10	0.38	0.17	9/10	0.2
	P80511	S100A12	0.045	0.04	0.03	2/10	0.19	0.06	7/10	0.2
	P52566	ARHGDIB	0.034	0.02	0.02	1/10	0.15	0.04	6/10	0.1
Healthy	P0CG48	UBC	0.014	0.11	0.05	6/12	0.00	0.00	0/12	N/A
	P68363	TUBA1B	0.014	0.18	0.09	6/12	0.00	0.00	0/12	N/A
	P68871	HBB	0.037	0.13	0.05	5/12	0.00	0.00	0/12	N/A
	Q01469	FABP5	0.005	0.07	0.02	7/12	0.00	0.00	0/12	N/A
	P01597	N/A	0.021	0.44	0.13	7/12	0.08	0.06	2/12	5.3
	P10909	CLU	0.029	0.08	0.02	8/12	0.02	0.01	2/12	5.0
	P10599	TXN	0.006	0.14	0.04	10/12	0.03	0.02	3/12	4.3
	P08118	MSMB	0.027	0.16	0.05	7/12	0.05	0.05	1/12	3.2
	P01766	N/A	0.000	0.59	0.08	12/12	0.22	0.06	8/12	2.7
	Q9HC84	MUC5B	0.042	0.45	0.07	11/12	0.98	0.18	12/12	0.5
	P03973	SLPI	0.005	0.58	0.15	10/12	2.44	0.57	11/12	0.2
	P25815	S100P	0.008	0.02	0.02	1/12	0.10	0.03	8/12	0.2

Table 4: Significant proteins for seasonal differences in allergic rhinitis patients and healthy controls. Acc.No.=accession number obtained from UniProt Database: AUC=mean normalized area under the curve; SEM=standard error of mean; P. in “N” =presence of protein in number of patients or controls/total number of probands in and out of pollen season.

3.2.3. In season differences between allergic rhinitis patients and healthy controls

In pollen season 10 proteins were significantly more abundant in allergic rhinitis patients than in healthy controls. These were complement C4-B (C4B), alpha-1-acid glycoprotein 2 (ORM2), and phospholipid transfer protein (PLTP), which were not detected in HC at all; as well as alpha-2-macroglobulin (A2M, 13.2-fold), apolipoprotein A-II (APOA2, 9.4-fold), vitamin D-binding protein (GC, 4.6-fold), complement C3 (C3, 3.6-fold), apolipoprotein A-I (APOA1, 3.6-fold), BPI fold-containing family B member 2 (BPIFB2, 2.9-fold) and clusterin (CLU, 2.6-fold) (Table 5).

Acc. No.	NCBI Gene names	p-value	mean AUC [%] AR	SEM AUC [%]	P. in "N" AR	mean AUC [%] HC	Sem AUC [%]	P. in "N" HC	Ratio AR vs. HC IN season
P55058	PLTP	0.029	0.1	.1	4/10	0.0	0.0	0/12	N/A
P19652	ORM2	0.010	0.1	.0	5/10	0.0	0.0	0/12	N/A
P0C0L5	C4B	0.010	0.1	.0	5/10	0.0	0.0	0/12	N/A
P01023	A2M	0.002	0.2	.1	7/10	0.0	.0	2/12	13.2
P02652	APOA2	0.001	0.5	.2	9/10	0.1	.0	3/12	9.4
P02774	GC	0.034	0.2	.1	7/10	0.1	.0	6/12	4.6
P01024	C3	0.043	0.3	.1	7/10	0.1	.0	6/12	3.6
P02647	APOA1	0.011	1.5	.5	10/10	0.4	.2	10/12	3.6
Q8N4F0	BPIFB2	0.017	0.4	.1	10/10	0.1	.0	8/12	2.9
P10909	CLU	0.002	0.2	.0	10/10	0.1	.0	8/12	2.6

Table 5: Significantly different proteins in season between allergic rhinitis patients and healthy controls: AR=Allergic rhinitis patients, HC=Healthy controls; Acc.No.=accession number obtained from UniProt Database; AUC=mean normalized area under the curve; SEM=standard error of mean; P. in “N” AR or HC=presence of protein in number of patients or controls/total number of probands.

3.2.4. Seasonal differences in biological processes of allergic rhinitis patients' and healthy controls' proteome

In total 22 biological processes (BP) were enriched in the overall nasal mucus proteome of the whole study group compared to the total human proteome. In allergic rhinitis patients nine BPs were up-regulated in season (Figure 7a) while 14 were up-regulated out of season (Figure 7b). In healthy controls 21 BPs were up-regulated in season (Figure 8a) and 4 up-regulated out of pollen season (Figure 8b) respectively. The following BPs were exclusively found in healthy controls: anatomical structure morphogenesis, transport, cell differentiation, death as well as cell death. Vice versa, the only BP not found in HC regardless of season enriched in allergic rhinitis patients was behaviour.

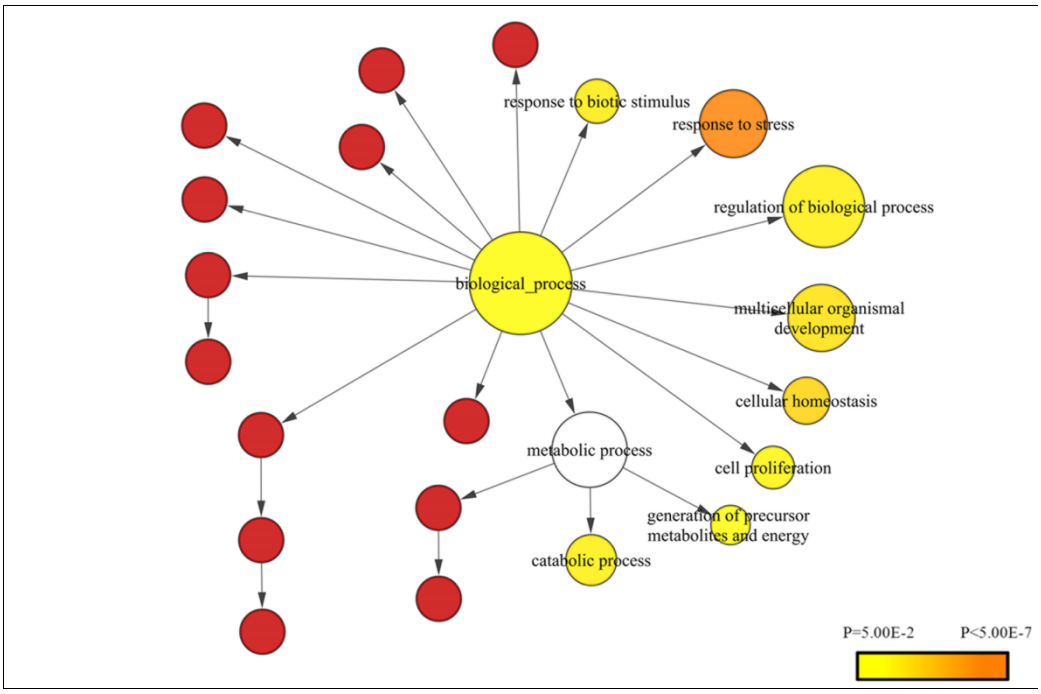

Figure7a: Enrichment analysis of biological processes, obtained by BINGO software. Significantly enriched biological processes of proteins present in allergic rhinitis patients (N=17) compared to total human proteome, 9 of these up-regulated in season. Colour bar in the right lower quadrant indicates level of significance from low (yellow) to high (orange). Statistical analysis was performed with a hypergeometrical test. A p-value of <0.05 was considered significant.

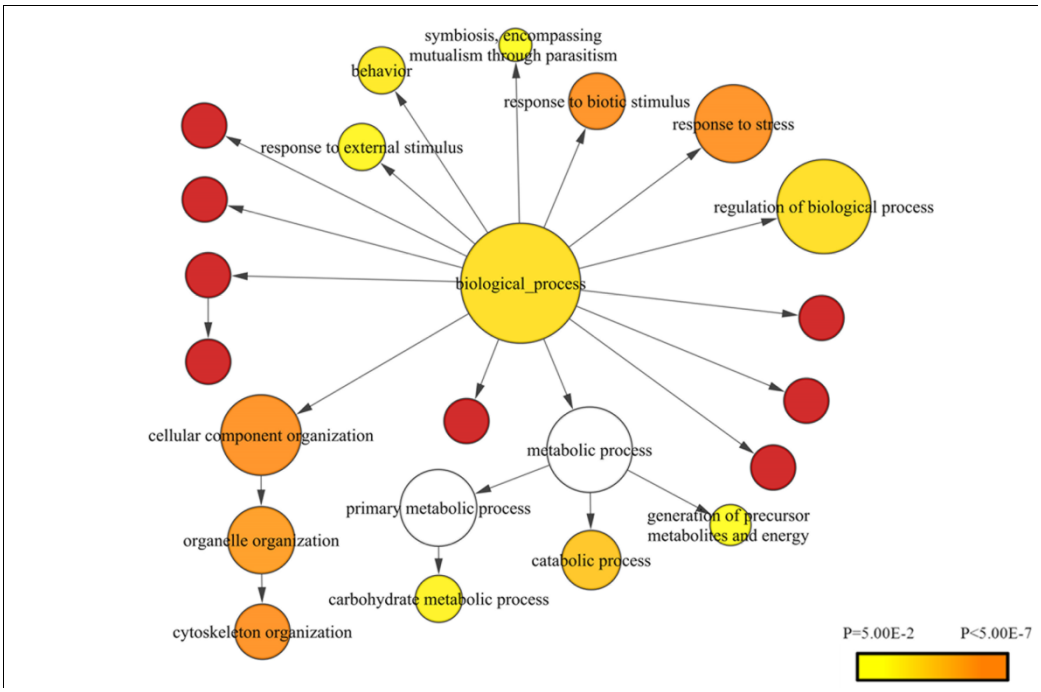

Figure 7b: Enrichment analysis of biological processes, obtained by BINGO software. Significantly enriched biological processes of proteins present in allergic rhinitis patients (N=17) compared to total human proteome of which 14 were up-regulated out of season. Red nodes indicate silenced biological processes. Colour bar in the right lower quadrant indicates level of significance from low (yellow) to high (orange). Statistical analysis was performed with a hypergeometrical test. A p-value of <0.05 was considered significant.

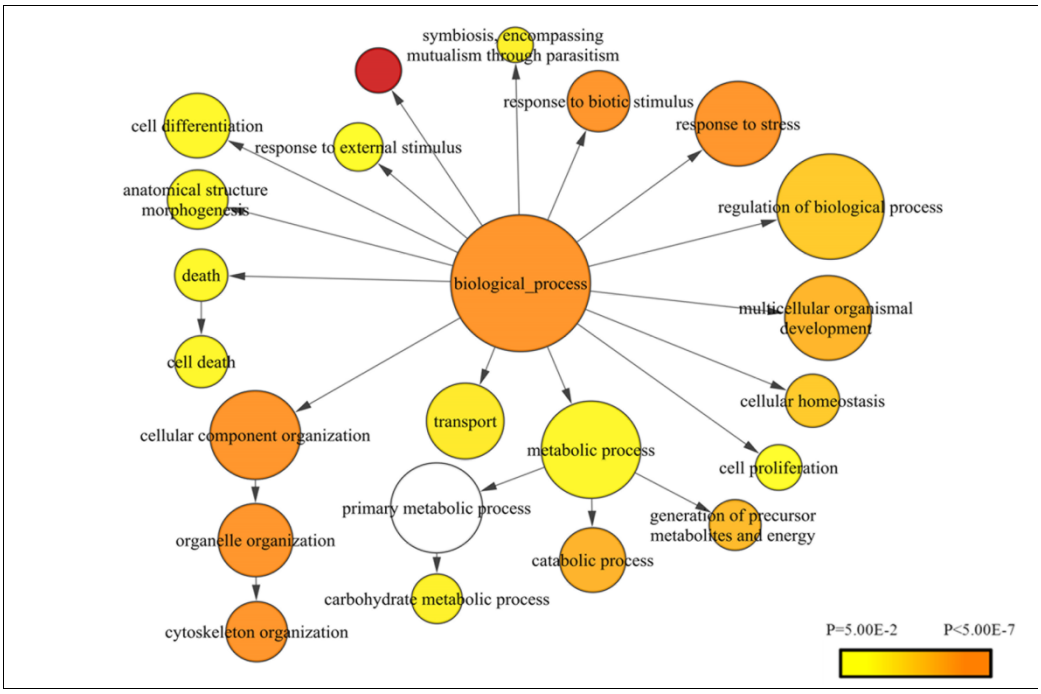

Figure 8a: Enrichment analysis of biological processes, obtained by BINGO software, of significantly enriched biological processes of proteins present in healthy controls (N=21) compared to total human proteome, 21 of these up-regulated in season. Red nodes indicate silenced biological processes. Colour bar in the right lower quadrant indicates level of significance from low (yellow) to high (orange). Statistical analysis was performed with a hypergeometrical test. A p-value of <0.05 was considered significant.

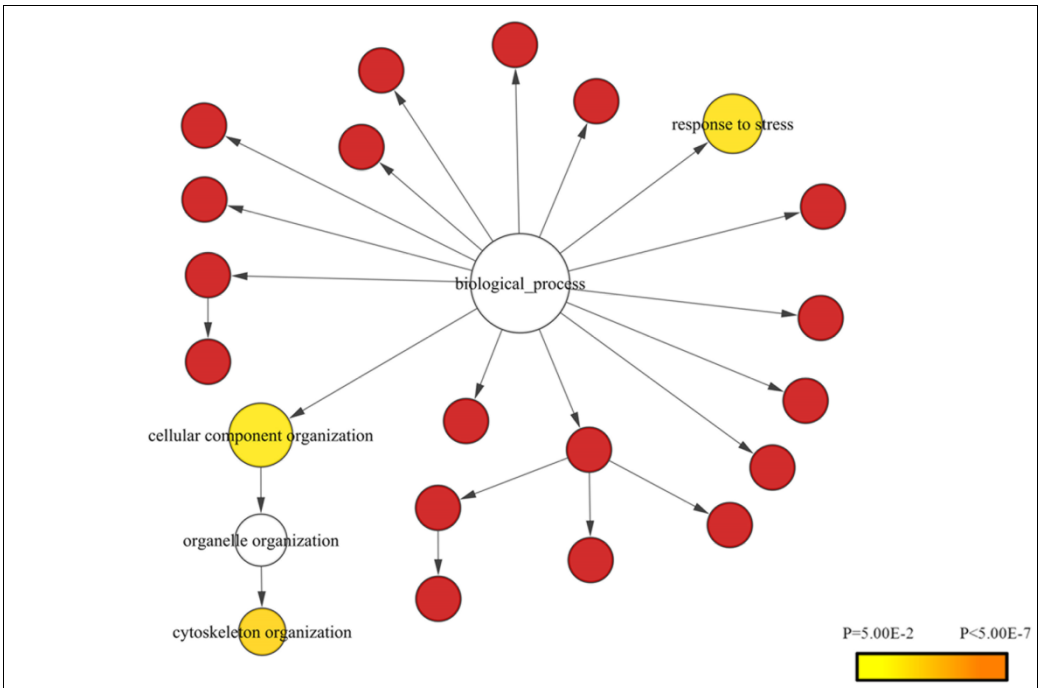

Figure 8b: Enrichment analysis of biological processes, obtained by BINGO software, of significantly enriched biological processes of proteins present in healthy controls (N=21) compared to total human proteome. Of these 4 were up-regulated out of season. Red nodes indicate silenced biological processes. Colour bar in the right lower quadrant indicates level of significance from low (yellow) to high (orange). Statistical analysis was performed with a hypergeometrical test. A p-value of <0.05 was considered significant.

4. Discussion

Nasal mucus acts as a barrier against external pathogens and has antioxidant, antiprotease and antimicrobial activities (87). Major components of nasal mucus are mucins and around 20 mucin genes have been identified in humans (17). Their function is not entirely understood, but they are involved in antimicrobial and anti-inflammatory responses as well as in impaired mucociliary clearance in disease through overproduction. MUC5, MUC5B, MUC7 and MUC8 are usually expressed in the upper and lower respiratory tract (16,88). MUC5B and MUC7 were identified in the present study. MUC5B was found to be more abundant in allergic rhinitis mucus but this difference did not reach statistical significance. Albumin/ALB was the most abundant protein that we detected. Lysozyme/LYZ and lactotransferrin/LTF, two well-described antimicrobial agents of the innate immune system in the nasal mucus also were among the most abundantly expressed proteins in the present study (Figures 2 and 5). Raphael *et al.* (18) found that LYZ and LTF are produced locally in the submucosal nasal glands. Their high abundance comes from cholinergic stimuli, contrary to albumin where higher concentrations are found after histamine challenge causing increased vascular permeability. Since abundances of LYZ and LTF did not significantly differ between allergic rhinitis patients and healthy controls we conclude that their secretion is not influenced by the disease. Their role in innate immune response is well known; however, the role that they play in chronic inflammatory responses and the adaptive immune system remains unclear (64).

These examples demonstrate that proteins comprising the mucus mediate its functions. Moreover, the most abundant proteins found in the present study correlate to previously published data underlining the feasibility of mass spectrometric approaches. Changes in the mucus proteome may favor disintegration of mucosal barrier function leading to direct challenge of the epithelium with various noxa. Few publications exist about the nasal mucus proteome despite that fact that it can be obtained in a non-invasive manner which makes it an ideal source for biomarker research. In one of the first studies on this topic *Casado et al.* identified 111 proteins (20). Mortstedt *et al.* (89) compiled a list of 244

relevant proteins in nasal lavage fluid. In the present study a total of 430 proteins were identified in and out of pollen season in allergic rhinitis patients and healthy controls (Table E4).

Allergic rhinitis patients showed significantly fewer proteins present exclusively in season compared to healthy controls (68 vs. 124) suggesting a higher diversity in expression of proteins in healthy controls as a reaction to pollen exposure; particularly since the number of proteins exclusively present in and out of season did not change markedly in allergic rhinitis patients (68 vs. 48) as compared to healthy controls (124 vs. 38) the data reflect a higher plasticity of the healthy compared to the allergic mucus proteome (Figure 6). Similarly, allergic rhinitis patients showed two significantly more abundant proteins in season and six significantly more abundant proteins out of seasons, while healthy controls had 9 significantly more abundant proteins in season and three out of season, respectively. Thus, in total 8 proteins were significantly altered in dependence of the season in allergic rhinitis as compared to 12 in healthy controls, which is again an indication of the reduced plasticity of the allergic rhinitis mucus proteome.

In season, clusterin/CLU and Ig kappa chain C region/IGKC were significantly more abundant in allergic rhinitis patients (Table 4). CLU exists in two isoforms that are either secreted or remain in the cytoplasm or nucleus. Some authors describe CLU as a pro- and some as an antiapoptotic factor in various cancer cell lines like prostate or breast cancer (90). In its secreted form it protects from apoptosis and cytolysis (90-92).

Out of season, following proteins were significantly more abundant in allergic rhinitis patients (Table 4): Glutathione S-transferase P/GSTP1, neutrophil elastase/ELANE, histone H2B type 1-K/HIST1H2BK, protein S100-A8/S100A8, protein S100-A12/S100A12 and rho GDP-dissociation inhibitor 2/ARHGDIB. GSTP1 negatively regulates cyclin dependent kinase-5/CDK5 directly by dislodging p25/p35 and indirectly by reducing oxidative stress (93). Thus it prevents neurodegeneration which could prominently influence Alzheimer's disease. Apart from that it negatively regulates acute inflammatory responses and apoptosis by catalyzing the conjugation of reduced gluthathione to toxic substances which leads to detoxification (93). Moreover, it enhances neutrophilic inflammation in asthmatics by modulating NF-kappaB (94).

ELANE, S100A8 and S100A12 contrarily have a proinflammatory function promoting neutrophil chemotaxis and cytokine production. ELANE furthermore destroys bacteria through its serine protease activity (95-97). Likewise HIST1H2BK has antimicrobial humoral and proinflammatory properties apart from its function in regulation of gene transcription (98-100). These proteins underline the inflammatory response of the nasal mucus on a proteome level contrary to clinical presentation in a symptom-free interval. ARHGDIB influences immune response and besides negatively regulates cell adhesion and cytoskeleton organization. Due to epithelial remodeling through ongoing inflammatory processes out of pollen season this protein could be secreted in nasal mucus after epithelial cell damage. Its role in cancer metastasis has been studied more extensively where it promotes cell invasion, tumor cell motility and neovascularization via regulation of Rho GTPases (101,102).

Contrary to that, healthy controls showed nine significantly more abundant proteins in season (Table 4): Polyubiquitin-C/UBC, tubulin alpha-1B/TUBA1B, hemoglobin beta/HBB, FA-binding protein epidermal/FABP5, Ig kappa chain V-I region DEE/ N/A, clusterin/CLU, thioredoxin/TXN, beta-microseminoprotein/MSMB and Ig heavy chain V-III region BRO with the first four exclusively present in season. UBC is responsible for ubiquitination of proteins everywhere in the body, where covalently bound to target proteins, it leads to degradation of the latter via lysosomal degradation, proteasome degradation or endocytic trafficking (103,104). Different covalent modifications of the target proteins have been shown to affect chromosome structure and regulation of transcription (103). Extracellular proteins can also be modified and - in any case - target proteins are modified to different fates like endocytosis and lysosomal degradation. UBC and CLU interact in a way that ubiquitinated proteins are internalized into cells by CLU though membrane receptor binding. Hence these target proteins become degraded. Secreted CLU protects from apoptosis and cytolysis by complement (90-92). Their high abundance in healthy controls in season could reflect degradation of allergens preventing them from sensitizing the immune system upon presentation to MHC II cells (103-105). CLU was the only protein that was significantly more abundant in season in allergic rhinitis patients as well as in healthy controls. In allergic rhinitis patients, where

UBC is not present to interact with CLU as described above, CLU could be responsible for clearing cellular debris as a result of epithelial damage during allergen exposure.

TUBA1B as a major constituent of microtubules could be responsible for cell organelle positioning and intracellular trafficking in healthy controls. Microtubules are also responsible for cell polarization anchoring tight junction proteins and giving epithelial cells and apico-basal orientation which could reflect a higher integrity of nasal mucosa in healthy controls in season as a reaction against epithelial damage and transmigration of allergens through the epithelium as postulated by Renkonen *et al.* on the one hand, and/or increased epithelial remodeling on the other hand. This could be explained by allergen damage on the healthy epithelium which reacts by a higher turnover of cells to maintain epithelial integrity (106,107).

HBB is involved in oxygen transport from the lungs to the periphery. Jordakieva *et al.* found that erythrocytes as well as hemoglobin in peripheral blood dropped upon allergen challenge in sensitized mice and pollen-allergic human subjects. They concluded that the recruitment of erythrocytes to the lungs could be a compensation for hypoxia in the inflamed mucosa. However they did not show hemoglobin levels in the mucosa (108). In our study hemoglobin beta was detected in healthy controls only in season in the mucus. Perhaps this is a defense mechanism to allergen challenge trough enhancing the oxygenation of the mucosa in healthy controls. Despite the lack of sensitization, harmful pollen contents like proteases still affect healthy mucosa (62,109,110). In allergic rhinitis patients HBB was 3-fold higher in season compared to out of season not reaching significance. On the one hand this underlines the theory that hemoglobin could be a reaction to hypoxia also in allergic subjects, on the other hand the presence out of season reflects the perennial inflammatory state of the mucus and mucosa.

FABP5 is important for the development of keratinocytes and epidermal appendices like sebaceous glands. Albeit it is responsible for the integrity of the cutaneous barrier it could also share the same function for the mucosal barrier and epithelial cells, which should be subject to further investigation. Another explanation could be its origin from the nasal vestibule despite the fact that we meticulously obtained mucus from the nasal cavity proper and the middle meatus (111). TXN regulates reactive oxidative metabolism

playing an important role in scavenging reactive oxygen species. It was shown to suppress inflammation in an animal asthma model and eosinophil recruitment as well as goblet cell hyperplasia. Since it was significantly more abundant in season in healthy controls it could also decrease oxidative stress caused upon allergen exposure (112-115).

MSMB is known for its presence in prostate and involvement in prostate cancer. Furthermore it is also expressed in other tissues like pancreas, esophagus, stomach, duodenum, colon, trachea, lung, salivary glands and fallopian tube (116). Van den Kiebboom *et al.* (117) recently described MSMB and its involvement in respiratory syncytial virus infection of the nasopharynx where its expression was down-regulated upon infection. Bockkov *et al.* (118) found that MSMB was down-regulated in asthmatic patients. Its higher abundance in healthy controls' mucus in season could be another defense response of that group during allergen exposure. Its function there needs to be further elucidated since as of today it has been attributed as tumor suppressor gene.

Mucin-5B/MUC5B, antileukoproteinase/SLPI and protein S100-P/S100P were significantly more abundant out of season in healthy controls. MUC5B is a major constituent of nasal mucus influencing its viscosity. It reflects a normal state of the mucus (26,119). SLPI has antiprotease activity for trypsin, chymotrypsin, elastase, and cathepsin G. Its function is to prevent protease damage to the mucosa (120-124) thus we would have expected a higher abundance of SLPI in season in healthy controls.

Although described in malignant cells as growth promoter, S100P is involved in the formation of microvilli. It is thus responsible for normal mucosal cell function and ciliary beat necessary for mucociliary clearance (125,126).

Summarizing the seasonal proteomic differences in allergic rhinitis patients and healthy controls it seems that allergic rhinitis patients show an enhanced immune response even out of the pollen season as well as epithelial remodeling. Healthy controls, however, seem to react to pollen exposure by expressing proteins that could degrade allergenic proteins (CLU, UBC), improve oxygenation of the mucosa (HBB), react to reactive oxygen species (TXN) and modulate immune response through immunoglobulin expression in season.

In allergic rhinitis patients we found 10 proteins that were significantly more abundant than in healthy controls during pollen season. These were complement C4-B/C4B, alpha-1-acid glycoprotein 2/ORM2, phospholipid transfer protein/PLTP, which were exclusively present in allergic rhinitis; as well as alpha-2-macroglobulin/A2M, apolipoprotein A-II/APOA2, vitamin D-binding protein/GC, complement C3/C3, apolipoprotein A-I/APOA1, BPI fold-containing family B member 2/BPIFB2, and clusterin/CLU (Table 5). Of these C3, A2M, APOA1 and APOA2 were also found to be significantly more abundant in allergic rhinitis patients in chapter 1 of our study (Table 3). This underlines the reproducibility of proteomic results obtained by mass spectrometry. Apolipoproteins could act as anti-inflammatory agents apart from their involvement in lipid metabolism (127,128) and their high abundance could be regulated by a local mechanisms since plasma levels of APOs did not differ between allergic rhinitis patients and healthy controls as previously shown (85). However, further studies are needed to prove the production of APOs in the epithelium and to exclude that their high abundance is due to increased plasma exudation. Contrary, other authors suggest that in chronic inflammation they could also act pro-inflammatory through posttranslational modifications and complexing with other molecules (129). PLTP is responsible for phospholipid transfer between lipoproteins. Interestingly, it binds to apolipoprotein A-I, apolipoprotein E and clusterin and forms complexes responsible for immune responses. PLTP is elevated in plasma during acute inflammation (130).

C3 - like C4B - is a pro-inflammatory molecule activating the complement system and is also a marker for plasma exudation (131,132), which underlines the disintegration of the epithelial barrier in allergy. On one hand, A2M also serves as a marker for increased plasma exudation; on the other hand, it is an anti-protease with the potential of inhibiting all classes of pollen proteases released upon contact with the nasal mucus (133-135).

ORM2 is an acute phase protein which seems to be locally up-regulated as pro-inflammatory molecule in the reproductive system which could be similar for the nasal mucosa (136). As ORM2 was newly identified in the nasal mucus in the present study, its role in the upper airway tract needs to be elucidated. BPIFB2 is a PLUNC protein family member, expressed in upper airway epithelial cells and mediates innate immune responses and antimicrobial activity (64,137,138). GC and vitamin D seem to be

responsible for the late phase inflammatory reaction after allergen challenge in asthma. Here, they act through activation of monocyte/macrophage system and activation of complement C5 (139). Taken together, differences between allergic rhinitis patients and healthy controls in season also show a preponderance of pro-inflammatory and immune reactions in allergy.

On the basis of the nasal mucus proteome, allergic rhinitis patients show a perennial inflammatory response combined with an augmentation during the season, despite reduced defense mechanisms of the immune system and reduced integrity of the epithelial barrier. Moreover, fewer proteins are expressed in season, which reflect a reduced plasticity of the allergic proteome that can also be seen in the reduced number of enriched biological processes as compared to the total human proteome. In season, allergic rhinitis patients do not show processes like cytoskeleton organization, cell proliferation or cellular homeostasis. Healthy controls express all these important biological processes in season whereas out of season several biological processes are not present in their proteome.

5. Conclusion

Contrary to their symptom pattern, allergic rhinitis patients show an increased inflammatory response in their nasal mucus proteome even out of pollen season. In combination with reduced defense mechanisms and an increase in inflammation in season, the nasal mucus proteome of allergic subjects reflects a decreased plasticity and thus inadequate reaction to allergen stress as compared to healthy controls. Our results also show that healthy controls express proteins in their mucus that obviously protect them against harmful pollen content and subsequent sensitization to allergens. Some of our identified proteins could serve as novel biomarkers for allergic rhinitis or even serve as new therapies if supplemented to the allergic mucosa. Therefore, functional analyses of distinct, significantly altered proteins will further increase our

understanding of the pathophysiology of nasal mucus as defense barrier, which may be the basis of novel therapeutic strategies. .

6. Bibliography

(1) Neskey D, Eloy JA, Casiano RR. Nasal, septal, and turbinate anatomy and embryology. Otolaryngol Clin North Am.2009;42:193-205.

(2) Junqueira L, Carneiro J. Histologie. 5th ed. Berlin, Heidelberg, New York: Springer Verlag; 2002.

(3) Bloom W, Fawcett D. A Textbook of Histology. 9th ed. Philadelphia, London, Toronto: WB Saunders Company; 1969.

(4) Hoover KC. Evolution of olfactory receptors. Methods Mol Biol 2013;1003:241-249.

(5) Donald P, Gluckman J, Rice D. The Sinuses. 1st ed. New York: Raven Press; 1995.

(6) Bousquet J, Schünemann HJ, Samolinski B et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy 2008;63 Suppl 86:8-160.

(7) Bousquet J, Schunemann HJ, FAU - Zuberbier T, Zuberbier T, FAU - Bachert C, Bachert C, et al. Development and implementation of guidelines in allergic rhinitis - an ARIA-GA2LEN paper.; Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines: 2010 revision. Allergy 2010;65:1212-1221.

(8) Mygind N, Dahl R, Pedersen S, Thestrup-Pedersen K. Essential Allergy. 2nd ed. Oxford: Blackwell Science; 1996.

(9) Heppt W, Renz H, Röcken M. Allergologie. 1st ed. Berlin, Heidelberg, New York: Springer Verlag; 1998.

(10) Kapp A, Klimek L, Werfel T. Allergische Entzündungen. 1st ed. Stuttgart, New York: Georg Thieme Verlag; 2002.

(11) Sturm GJ, Kranzelbinder B, Sturm EM, Heinemann A, Groselj-Strele A, Aberer W. The basophil activation test in the diagnosis of allergy: technical issues and critical factors. Allergy 2009;64:1319-1326.

(12) Sturm GJ, Jin C, Kranzelbinder B, Hemmer W, Sturm EM, Griesbacher A, et al. Inconsistent results of diagnostic tools hamper the differentiation between bee and vespid venom allergy. PLoS One 2011;6:e20842.

(13) Broide DH. Immunomodulation of allergic disease. Annu Rev Med 2009;60:279-291.

(14) Gevaert P, Van Bruaene N, Cattaert T, Van Steen K, et al. Mepolizumab, a humanized anti-IL-5 mAb, as a treatment option for severe nasal polyposis. J Allergy Clin Immunol 2011;128:989-995.

(15) Rogers DF. Physiology of airway mucus secretion and pathophysiology of hypersecretion. Respir Care 2007;52:1134-1146.

(16) Evans CM, Koo JS. Airway mucus: the good, the bad, the sticky. Pharmacol Ther 2009;121:332-348.

(17) Williams OW, Sharafkhaneh A, Kim V, Dickey BF, Evans CM. Airway mucus: From production to secretion. Am J Respir Cell Mol Biol 2006;34:527-536.

(18) Raphael GD, Jeney EV, Baraniuk JN, Kim I, Meredith SD, Kaliner MA. Pathophysiology of rhinitis. Lactoferrin and lysozyme in nasal secretions. J Clin Invest 1989;84:1528-1535.

(19) Raphael GD, Baraniuk JN, Kaliner MA. How and why the nose runs. J Allergy Clin Immunol 1991;87:457-467.

(20) Casado B, Pannell LK, Iadarola P, Baraniuk JN. Identification of human nasal mucous proteins using proteomics. Proteomics 2005;5:2949-2959.

(21) Persson CG, Erjefalt I, Alkner U, Baumgarten C, Greiff L, Gustafsson B, et al. Plasma exudation as a first line respiratory mucosal defence. Clin Exp Allergy 1991;21:17-24.

(22) Stammberger H. Functional Endoscopic Sinus Surgery. 1st ed. Philadelphia, Pennsylvania: B.C. Decker; 1991.

(23) Ren J, Deng Y, Xiao B, Wang G, Tao Z. Protective effects of exogenous surfactant protein A in allergic rhinitis: a mouse model. Ann Otol Rhinol Laryngol 2013;122:240-246.

(24) Nogaki T, Asano K, Furuta A, Kanai K, Suzaki I, Kanei A, et al. Enhancement of clara cell 10-kD protein (CC10) production from nasal epithelial cells by fexofenadine hydrochloride. Asian Pac J Allergy Immunol 2012;30:139-145.

(25) Voynow JA, Selby DM, Rose MC. Mucin gene expression (MUC1, MUC2, and MUC5/5AC) in nasal epithelial cells of cystic fibrosis, allergic rhinitis, and normal individuals. Lung 1998;176:345-354.

(26) Thornton DJ, Sheehan JK. From mucins to mucus: toward a more coherent understanding of this essential barrier. Proc Am Thorac Soc 2004;1:54-61.

(27) Lin L, Zhao X, Yan W, Guo Y, Liang S. Amelioration of Muc5b mucin hypersecretion is enhanced by IL-33 after 2-APB administration in a murine model of allergic rhinitis. Biotech Histochem 2014;89:273-286.

(28) Small P, Barrett D, Frenkiel S, Rochon L, Cohen C, Black M. Measurement of antigen-specific IgE in nasal secretions of patients with perennial rhinitis. Ann Allergy 1985;55:68-71.

(29) Platts-Mills TA, von Maur RK, Ishizaka K, Norman PS, Lichtenstein LM. IgA and IgG anti-ragweed antibodies in nasal secretions. Quantitative measurements of antibodies and correlation with inhibition of histamine release. J Clin Invest 1976;57:1041-1050.

(30) Johansson SG, Deuschl H. Immunoglobulins in nasal secretion with special reference to IgE. I. Methodological studies. Int Arch Allergy Appl Immunol 1976;52:364-375.

(31) Deuschl H. Immunoglobulins in nasal secretion with special reference to IgE. II. Seasonal studies of Timothy-specific IgE-antibody in patients with allergic rhinitis. Int Arch Allergy Appl Immunol 1976;52:376-383.

(32) Huggins KG, Brostoff J. Local production of specific IgE antibodies in allergic-rhinitis patients with negative skin tests. Lancet 1975;2:148-150.

(33) Mygind N, Weeke B, Ullman S. Quantitative determination of immunoglobulins in nasal secretion. Int Arch Allergy Appl Immunol 1975;49:99-107.

(34) Yunginger JW, Gleich GJ. Seasonal changes in serum and nassal IgE concentrations. J Allergy Clin Immunol 1973;51:174-186.

(35) Tse KS, Wicher K, Arbesman CE. IgE antibodies in nasal secretions of ragweed-allergic subjects. J Allergy 1970;46:352-357.

(36) Woof JM, Mestecky J. Mucosal immunoglobulins. Immunol Rev 2005;206:64-82.

(37) Hamaguchi Y, Taya M, Suzumura H, Sakakura Y. Lysosomal proteases and protease inhibitors in nasal allergy and non-atopic sinusitis. Am J Otolaryngol 1990;11:37-43.

(38) Baumgarten CR, Nichols RC, Naclerio RM, Lichtenstein LM, Norman PS, Proud D. Plasma kallikrein during experimentally induced allergic rhinitis: role in kinin formation and contribution to TAME-esterase activity in nasal secretions. J Immunol 1986;137:977-982.

(39) Baumgarten CR, Nichols RC, Naclerio RM, Proud D. Concentrations of glandular kallikrein in human nasal secretions increase during experimentally induced allergic rhinitis. J Immunol 1986;137:1323-1328.

(40) Baraniuk JN, Silver PB, Kaliner MA, Barnes PJ. Effects of ipratropium bromide on bradykinin nasal provocation in chronic allergic rhinitis. Clin Exp Allergy 1994;24:724-729.

(41) Schaper C, Gustavus B, Koch B, Ewert R, Hanf G, Kunkel G, et al. Effects of fexofenadine on inflammatory mediators in nasal lavage fluid in intermittent allergic rhinitis. J Investig Allergol Clin Immunol 2009;19:459-464.

(42) Linder A, Venge P, Deuschl H. Eosinophil cationic protein and myeloperoxidase in nasal secretion as markers of inflammation in allergic rhinitis. Allergy 1987;42:583-590.

(43) Beppu T, Ohta N, Gon S, Sakata K, Inamura K, Fukase S, et al. Eosinophil and eosinophil cationic protein in allergic rhinitis. Acta Otolaryngol Suppl 1994;511:221-223.

(44) Wang DY, Clement P, Smitz J, De Waele M, Derde MP. Quantification of eosinophil cationic protein and eosinophils in nasal secretions of allergen-induced nasal inflammation. Allergol Immunopathol (Madr) 1994;22:179-183.

(45) Rasp G, Thomas PA, Bujia J. Eosinophil inflammation of the nasal mucosa in allergic and non-allergic rhinitis measured by eosinophil cationic protein levels in native nasal fluid and serum. Clin Exp Allergy 1994;24:1151-1156.

(46) Groger M, Bernt A, Wolf M, Mack B, Pfrogner E, Becker S, et al. Eosinophils and mast cells: a comparison of nasal mucosa histology and cytology to markers in nasal discharge in patients with chronic sino-nasal diseases. Eur Arch Otorhinolaryngol 2013;270:2667-2676.

(47) Kramer MF, Ostertag P, Pfrogner E, Rasp G. Nasal interleukin-5, immunoglobulin E, eosinophilic cationic protein, and soluble intercellular adhesion molecule-1 in chronic sinusitis, allergic rhinitis, and nasal polyposis. Laryngoscope 2000;110:1056-1062.

(48) Menzella F, Lusuardi M, Galeone C, Taddei S, Zucchi L. Profile of anti-IL-5 mAb mepolizumab in the treatment of severe refractory asthma and hypereosinophilic diseases. J Asthma Allergy 2015;8:105-114.

(49) Nishioka K, Ogawa T, Saito C, Nishioka S, Nakagawa F, Ohomichi T, et al. Major basic protein, eosinophil cationic protein, and arylsulfatase in nasal secretions of patients with Japanese cedar pollinosis. Acta Med Okayama 1995;49:29-33.

(50) Tonnesen P, Hindberg I, Schaffalitzky de Muckadell OB, Mygind N. Effect of nasal allergen challenge on serotonin, substance P and vasoactive intestinal peptide in plasma and nasal secretions. Allergy 1988;43:310-317.

(51) Raphael GD, Igarashi Y, White MV, Kaliner MA. The pathophysiology of rhinitis. V. Sources of protein in allergen-induced nasal secretions. J Allergy Clin Immunol 1991;88:33-42.

(52) Ichikawa K, Okuda M, Naka F, Yago H. The sources of chemical substances in allergic nasal fluid. Rhinology 1991;29:143-149.

(53) Raphael GD, Druce HM, Baraniuk JN, Kaliner MA. Pathophysiology of rhinitis. 1. Assessment of the sources of protein in methacholine-induced nasal secretions. Am Rev Respir Dis 1988;138:413-420.

(54) Raphael GD, Meredith SD, Baraniuk JN, Druce HM, Banks SM, Kaliner MA. The pathophysiology of rhinitis. II. Assessment of the sources of protein in histamine-induced nasal secretions. Am Rev Respir Dis 1989;139:791-800.

(55) Wang D, Clement P, Smitz J, Derde MP. Concentrations of chemical mediators in nasal secretions of patients with hay fever during natural allergen exposure. Acta Otolaryngol 1994;114:552-555.

(56) Peters-Golden M, Henderson WR,Jr. The role of leukotrienes in allergic rhinitis. Ann Allergy Asthma Immunol 2005;94:609-618.

(57) Laumonnier Y, Schmudde I, Kohl J. The role of complement in the diagnosis and management of allergic rhinitis and allergic asthma. Curr Allergy Asthma Rep 2011;11:122-130.

(58) Erin EM, Zacharasiewicz AS, Nicholson GC, Tan AJ, Higgins LA, Williams TJ, et al. Topical corticosteroid inhibits interleukin-4, -5 and -13 in nasal secretions following allergen challenge. Clin Exp Allergy 2005;35:1608-1614.

(59) Hsin CH, Shun CT, Liu CM. Immunoglobulins in nasal secretions of patients with allergic rhinitis and chronic rhinosinusitis. Eur Arch Otorhinolaryngol 2008;265:539-542.

(60) Svensson C, Andersson M, Persson CG, Venge P, Alkner U, Pipkorn U. Albumin, bradykinins, and eosinophil cationic protein on the nasal mucosal surface in patients with hay fever during natural allergen exposure. J Allergy Clin Immunol 1990;85:828-833.

(61) Sanico AM, Atsuta S, Proud D, Togias A. Plasma extravasation through neuronal stimulation in human nasal mucosa in the setting of allergic rhinitis. J Appl Physiol) 1998;84:537-543.

(62) Runswick S, Mitchell T, Davies P, Robinson C, Garrod DR. Pollen proteolytic enzymes degrade tight junctions. Respirology 2007;12:834-842.

(63) Belkowski SM, Boot JD, Mascelli MA, Diamant Z, de Garavilla L, Hertzog B, et al. Cleaved secretory leucocyte protease inhibitor as a biomarker of chymase activity in allergic airway disease. Clin Exp Allergy 2009;39:1179-1186.

(64) Wiesner J, Vilcinskas A. Antimicrobial peptides: the ancient arm of the human immune system. Virulence 2010;1:440-464.

(65) Pham CT. Neutrophil serine proteases: specific regulators of inflammation. Nat Rev Immunol 2006l;6:541-550.

(66) Bryborn M, Adner M, Cardell LO. Psoriasin, one of several new proteins identified in nasal lavage fluid from allergic and non-allergic individuals using 2-dimensional gel electrophoresis and mass spectrometry. Respir Res 200519;6:118.

(67) Kvarnhammar AM, Rydberg C, Jarnkrants M, Eriksson M, Uddman R, Benson M, et al. Diminished levels of nasal S100A7 (psoriasin) in seasonal allergic rhinitis: an effect mediated by Th2 cytokines. Respir Res 2012;13:2-9921-13-2.

(68) Iguchi Y, Yao K, Okamoto M. A characteristic protein in nasal discharge differentiating non-allergic chronic rhinosinusitis from allergic rhinitis. Rhinology 2002;40:13-17.

(69) Tosun F, Sezen I, Gerek M, Ozkaptan Y, Yapar M, Caliskaner Z, et al. Electrophoretic evaluation of nasal discharge in patients with allergic rhinitis and vasomotor rhinitis. Am J Rhinol 2002;16:141-144.

(70) Park YB, Mo EK, Lee JY, Kim JH, Kim CH, Hyun IG, et al. Association between pet ownership and the sensitization to pet allergens in adults with various allergic diseases. Allergy Asthma Immunol Res 2013;5:295-300.

(71) Choi IJ, Rhee CS, Lee CH, Kim DY. Effect of allergic rhinitis on the expression of human beta-defensin 2 in tonsils. Ann Allergy Asthma Immunol 2013;110:178-183.

(72) Musa M, Wilson K, Sun L, Mulay A, Bingle L, Marriott HM, et al. Differential localisation of BPIFA1 (SPLUNC1) and BPIFB1 (LPLUNC1) in the nasal and oral cavities of mice. Cell Tissue Res 2012;350:455-464.

(73) Bingle L, Bingle CD. Distribution of human PLUNC/BPI fold-containing (BPIF) proteins. Biochem Soc Trans 2011;39:1023-1027.

(74) Ghafouri B, Kihlstrom E, Stahlbom B, Tagesson C, Lindahl M. PLUNC (palate, lung and nasal epithelial clone) proteins in human nasal lavage fluid. Biochem Soc Trans 2003;31:810-814.

(75) Graves PR, Haystead TA. Molecular biologist's guide to proteomics. - Microbiol Mol Biol Rev.2002;66:39-63.

(76) Verrills NM. Clinical proteomics: present and future prospects. Clin Biochem Rev.2006;27:99-116.

(77) Zhu W, Smith JW, Huang CM. Mass spectrometry-based label-free quantitative proteomics. J Biomed Biotechnol.2010;840518.

(78) Neilson KA, Ali NA, Muralidharan S, Mirzaei M, Mariani M, Assadourian G, et al. Less label, more free: approaches in label-free quantitative mass spectrometry. Proteomics 201111:535-553.

(79) Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, Cohen N, Cervin A, Douglas R, Gevaert P, Georgalas C, Goossens H, Harvey R, Hellings P, Hopkins C, Jones N, Joos G, Kalogjera L, Kern B, Kowalski M, Price D, Riechelmann H, Schlosser R, Senior B, Thomas M, Toskala E, Voegels R, Wang de Y, Wormald PJ. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. Rhinol Suppl.2012;1-298.

(80) Martinez-Bartolome S, Deutsch EW, Binz PA, Jones AR, Eisenacher M, Mayer G, et al. Guidelines for reporting quantitative mass spectrometry based experiments in proteomics. J Proteomics 201314;1874-3919.

(81) Lundgren DH, Hwang SI, Wu L, Han DK. Role of spectral counting in quantitative proteomics. Expert Rev Proteomics 2010;7:39-53.

(82) Old WM, Meyer-Arendt K, Aveline-Wolf L, Pierce KG, Mendoza A, Sevinsky JR, et al. Comparison of label-free methods for quantifying human proteins by shotgun proteomics. Mol Cell Proteomics 2005;4:1487-1502.

(83) Maere S, Heymans K, Kuiper M. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. - Bioinformatics 2005;21):3448-3449.

(84) Tomazic PV, Birner-Gruenberger R, Leitner A, Obrist B, Spoerk S, Lang-Loidolt D. Nasal mucus proteomic changes reflect altered immune responses and epithelial permeability in patients with allergic rhinitis. J Allergy Clin Immunol 2014;133:741-750.

(85) Tomazic PV, Birner-Gruenberger R, Leitner A, Darnhofer B, et al. Apolipoproteins have a potential role in nasal mucus of allergic rhinitis patients: A proteomic study.Laryngoscope 2015;125:E91-E96.

(86) Tomazic PV, Birner-Gruenberger R, Leitner A, Spoerk S, Lang-Loidolt D. Seasonal proteome changes of nasal mucus reflect perennial inflammatory response and reduced defence mechanisms and plasticity in allergic rhinitis. J Proteomics 20165;133:153-160.

(87) Voynow JA, Rubin BK. Mucins, mucus, and sputum. Chest 2009;135:505-512.

(88) Zuhdi Alimam M, Piazza FM, Selby DM, Letwin N, Huang L, Rose MC. Muc-5/5ac mucin messenger RNA and protein expression is a marker of goblet cell metaplasia in murine airways. Am J Respir Cell Mol Biol 2000;22:253-260.

(89) Mortstedt H, Karedal MH, Jonsson BA, Lindh CH. Screening method using selected reaction monitoring for targeted proteomics studies of nasal lavage fluid. J Proteome Res 2013;12:234-247.

(90) Zhang H, Kim JK, Edwards CA, Xu Z, et al. Clusterin inhibits apoptosis by interacting with activated Bax. Nat Cell Biol 2005;7:909-915.

(91) Jones SE, Jomary C. Clusterin. Int J Biochem Cell Biol.2002;34:427-431.

(92) Leskov KS, Klokov DY, Li J, Kinsella TJ, et al. Synthesis and functional analyses of nuclear clusterin, a cell death protein. J Biol Chem.2003;278):11590-1600.

(93) Sun KH, Chang KH, Clawson S, Ghosh S, et al. Glutathione-S-transferase P1 is a critical regulator of Cdk5 kinase activity. J Neurochem.2011;118:902-914.

(94) Polosukhin VV, Polosukhin IV, Hoskins A, Han W, Abdolrasulnia R, Blackwell TS, et al. Glutathione S-transferase M1 modulates allergen-induced NF-kappaB activation in asthmatic airway epithelium. Allergy 2014;69:1666-1672.

(95) Tateosian NL, Reiteri RM, Amiano NO, Costa MJ, Villalonga X, Guerrieri D, et al. Neutrophil elastase treated dendritic cells promote the generation of CD4(+)FOXP3(+) regulatory T cells in vitro. Cell Immunol 2011;269:128-134.

(96) Ryckman C, Vandal K, Rouleau P, Talbot M, et al. Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. J Immunol 2003;170:3233-3242.

(97) Yang Z, Yan WX, Cai H, Tedla N, et al. S100A12 provokes mast cell activation: a potential amplification pathway in asthma and innate immunity. J Allergy Clin Immunol 2007;119:106-114.

(98) Drab T, Kracmerova J, Hanzlikova E, Cerna T, Litvakova R, Pohlova A, et al. The antimicrobial action of histones in the reproductive tract of cow. Biochem Biophys Res Commun 2014;443:987-990.

(99) Valapour M, Guo J, Schroeder JT, Keen J, Cianferoni A, Casolaro V, et al. Histone deacetylation inhibits IL4 gene expression in T cells. J Allergy Clin Immunol 2002;109:238-245.

(100) Ekaney ML, Otto GP, Sossdorf M, Sponholz C, et al. Impact of plasma histones in human sepsis and their contribution to cellular injury and inflammation. Crit Care.2014;18:543.

(101) Griner EM, Theodorescu D, Cho HJ, Baek KE, Yoo J. The faces and friends of RhoGDI2. Cancer Metastasis Rev 2012;31:519-528.

(102) Cho HJ, Baek KE, Yoo J. RhoGDI2 as a therapeutic target in cancer. Expert Opin Ther Targets.2010;14:67-75.

(103) Komander D. The emerging complexity of protein ubiquitination. Biochem Soc Trans.2009;37:937-953.

(104) Huang F, Kirkpatrick D, Jiang X, Gygi S, et al. Differential regulation of EGF receptor internalization and degradation by multiubiquitination within the kinase domain. Mol Cell 200617;21:737-748.

(105) Wilkinson KD. Regulation of proteolysis by human deubiquitinating enzymes. Biochim Biophys Acta 2014;1843:114-128.

(106) Renkonen R, Renkonen J, Joenvaara S, Mattila P, Parviainen V, Toppila-Salmi S. Allergens are transported through the respiratory epithelium. Expert Rev Clin Immunol 2010;6:55-59.

(107) de Forges H, Bouissou A, Perez F. Interplay between microtubule dynamics and intracellular organization. Int J Biochem Cell Biol 2012;44:266-274.

(108) Jordakieva G, Wallmann J, Schmutz R, Lemell P, Wegmann M, Nittke T, et al. Peripheral erythrocytes decrease upon specific respiratory challenge with grass pollen allergen in sensitized mice and in human subjects. PLoS One 201422;9:e86701.

(109) Vinhas R, Cortes L, Cardoso I, Mendes VM, Manadas B, Todo-Bom A, et al. Pollen proteases compromise the airway epithelial barrier through degradation of transmembrane adhesion proteins and lung bioactive peptides. Allergy 2011;66:1088-1098.

(110) Baraniuk JN, Bolick M, Esch R, Buckley CE 3rd. Quantification of pollen solute release using pollen grain column chromatography. Allergy 1992;47:411-417.

(111) Lin MH, Khnykin D. Fatty acid transporters in skin development, function and disease. Biochim Biophys Acta 2014;1841:362-368.

(112) Imaoka H, Hoshino T, Okamoto M, Sakazaki Y, Sawada M, Takei S, et al. Endogenous and exogenous thioredoxin 1 prevents goblet cell hyperplasia in a chronic antigen exposure asthma model. Allergol Int 2009;58:403-410.

(113) Kobayashi N, Yamada Y, Ito W, Ueki S, Kayaba H, Nakamura H, et al. Thioredoxin reduces C-C chemokine-induced chemotaxis of human eosinophils. Allergy 2009;64:1130-1135.

(114) Ito W, Kobayashi N, Takeda M, Ueki S, et al. Thioredoxin in allergic inflammation. Int Arch Allergy Immunol 2011;155 Suppl 1:142-146.

(115) Torii M, Wang L, Ma N, Saito K, et al. Thioredoxin suppresses airway inflammation independently of systemic Th1/Th2 immune modulation. Eur J Immunol 2010;40:787-796.

(116) Whitaker HC, Warren AY, Eeles R, Kote-Jarai Z, et al. The potential value of microseminoprotein-beta as a prostate cancer biomarker and therapeutic target. Prostate 201015;70:333-340.

(117) van den Kieboom CH, Ahout IM, Zomer A, Brand KH, de Groot R, Ferwerda G, et al. Nasopharyngeal gene expression, a novel approach to study the course of respiratory syncytial virus infection. Eur Respir J 2015;45:718-725.

(118) Bochkov YA, Hanson KM, Keles S, Brockman-Schneider RA, et al. Rhinovirus-induced modulation of gene expression in bronchial epithelial cells from subjects with asthma. Mucosal Immunol 2010;3:69-80.

(119) Thornton DJ, Rousseau K, McGuckin MA. Structure and function of the polymeric mucins in airways mucus. Annu Rev Physiol 2008;70:459-486.

(120) Garcia-Verdugo I, Descamps D, Chignard M, Touqui L, Sallenave JM. Lung protease/anti-protease network and modulation of mucus production and surfactant activity. Biochimie 2010;92:1608-1617.

(121) Scott A, Weldon S, Taggart CC. SLPI and elafin: multifunctional antiproteases of the WFDC family. Biochem Soc Trans 2011;39:1437-1440.

(122) Sallenave JM, Ryle AP. Purification and characterization of elastase-specific inhibitor. Sequence homology with mucus proteinase inhibitor. Biol Chem Hoppe Seyler 1991;372:13-21.

(123) Heinzel R, Appelhans H, Gassen G, Seemuller U, Machleidt W, Fritz H, et al. Molecular cloning and expression of cDNA for human antileukoprotease from cervix uterus. Eur J Biochem 1986;160:61-67.

(124) Tewfik MA, Latterich M, DiFalco MR, Samaha M. Proteomics of nasal mucus in chronic rhinosinusitis. Am J Rhinol 2007;21:680-685.

(125) Jiang H, Hu H, Tong X, Jiang Q, Zhu H, Zhang S. Calcium-binding protein S100P and cancer: mechanisms and clinical relevance. J Cancer Res Clin Oncol 2012;138:1-9.

(126) Parkkila S, Pan PW, Ward A, Gibadulinova A, Oveckova I, Pastorekova S, et al. The calcium-binding protein S100P in normal and malignant human tissues. BMC Clin Pathol 2008;8:2.

(127) Furlaneto CJ, Ribeiro FP, Hatanaka E, Souza GM, Cassatella MA, Campa A. Apolipoproteins A-I and A-II downregulate neutrophil functions. Lipids.2002;37:925-928.

(128) Park SW, Lee EH, Lee EJ, Kim HJ, Bae DJ, Han S, et al. Apolipoprotein A1 potentiates lipoxin A4 synthesis and recovery of allergen-induced disrupted tight junctions in the airway epithelium.; Overexpression of apolipoprotein A1 in the lung abrogates fibrosis in experimental silicosis.; Apolipoproteins and apolipoprotein mimetic peptides modulate phagocyte trafficking through chemotactic activity. Clin Exp Allergy 2013;43:914-927.

(129) Vuilleumier N, Dayer JM, von Eckardstein A, Roux-Lombard P. Pro- or anti-inflammatory role of apolipoprotein A-1 in high-density lipoproteins? Swiss Med Wkly.2013;143:w13781.

(130) Cheung MC, Vaisar T, Han X, Heinecke JW, et al. Phospholipid transfer protein in human plasma associates with proteins linked to immunity and inflammation. - Biochemistry 2010;49:7314-7322.

(131) Hugli TE. Human anaphylatoxin (C3a) from the third component of complement. Primary structure. J Biol Chem 1975;250:8293-8301.

(132) Jun SW, Kim TH, Lee HM, Lee SH, Kim WJ, Park SJ, et al. Overexpression of the anaphylatoxin receptors, complement anaphylatoxin 3a receptor and complement anaphylatoxin 5a receptor, in the nasal mucosa of patients with mild and severe persistent allergic rhinitis. J Allergy Clin Immunol 2008;122:119-125.

(133) Craig-Barnes HA, Doumouras BS, Palaniyar N. Surfactant protein D interacts with alpha2-macroglobulin and increases its innate immune potential. J Biol Chem 2010;285:13461-13470.

(134) Ho AS, Cheng CC, Lee SC, Liu ML, Lee JY, Wang WM, et al. Novel biomarkers predict liver fibrosis in hepatitis C patients: alpha 2 macroglobulin, vitamin D binding protein and apolipoprotein AI. J Biomed Sci 2010;17:58.

(135) Svensson C, Gronneberg R, Andersson M, Alkner U, Andersson O, Billing B, et al. Allergen challenge-induced entry of alpha 2-macroglobulin and tryptase into human nasal and bronchial airways. J Allergy Clin Immunol 1995 ;96:239-246.

(136) Kowsar R, Hambruch N, Marey MA, Liu J, et al. Evidence for a novel, local acute-phase response in the bovine oviduct: progesterone and lipopolysaccharide up-regulate alpha 1-acid-glycoprotein expression in epithelial cells in vitro. Mol Reprod Dev 2014;81:861-870.

(137) Gakhar L, Bartlett JA, Penterman J, Mizrachi D, Singh PK, Mallampalli RK, et al. PLUNC is a novel airway surfactant protein with anti-biofilm activity. PLoS One 2010;5:e9098.

(138) Bingle CD, Craven CJ. PLUNC: a novel family of candidate host defence proteins expressed in the upper airways and nasopharynx. Hum Mol Genet 2002;11:937-943.

(139) Bratke K, Wendt A, Garbe K, Kuepper M, et al. Vitamin D binding protein and vitamin D in human allergen-induced endobronchial inflammation. Clin Exp Immunol 2014;177:366-372.

7. Appendix

Table E1: Table for Chapter 1 with epidemiologic data for each individual with gender, age, group (HC=healthy controls, AR=allergic rhinitis), skin prick test (SPT) for clinically relevant allergens and total IgE whenever available

ID	Gender	Age	Group	Alder Hasel Birch	Grasspollen	Ragweedpollen	Total-IgE kU/l
1	female	23	HC	neg	neg	neg	17.6
2	male	31	AR	pos	pos	neg	78.5
3	male	29	AR	neg	pos	pos	158.0
4	male	38	HC	neg	neg	neg	2.0
5	male	58	HC	neg	neg	neg	87.9
6	male	26	AR	pos	pos	pos	47.1
7	female	39	HC	neg	neg	neg	39.0
8	female	47	HC	neg	neg	neg	9.6
9	male	24	HC	neg	neg	neg	39.1
10	male	45	AR	neg	pos	neg	22.4
11	male	31	HC	neg	neg	neg	54.5
12	female	22	HC	neg	neg	neg	26.5
13	female	46	HC	neg	neg	neg	8.8
14	female	37	HC	neg	neg	neg	2.7
15	male	41	HC	neg	neg	neg	27.3
16	male	38	AR	pos	pos	neg	146.0
17	male	38	HC	neg	neg	neg	6.6
18	female	52	HC	neg	neg	neg	
19	male	37	HC	neg	neg	neg	573.0
20	female	41	HC	neg	neg	neg	10.0
21	female	34	HC	neg	neg	neg	8.4
22	female	29	AR	pos	pos	neg	433.0
23	male	29	AR	pos	pos	neg	144.0
24	female	26	AR	pos	pos	neg	200.0
25	male	48	AR	pos	pos	neg	81.7
26	male	27	AR	pos	pos	neg	49.6
27	male	43	HC	neg	neg	neg	12.9

28	male	28	HC	neg	neg	neg	91.9
29	male	46	HC	neg	neg	neg	21.9
30	male	20	AR	neg	pos	neg	81.2
31	female	32	AR	pos	pos	neg	308.0
32	male	38	AR	pos	pos	neg	
33	female	38	AR	pos	pos	pos	85.4
34	female	30	AR	pos	pos	neg	
35	male	26	AR	pos	pos	neg	104.0
36	male	44	HC	neg	neg	neg	21.9
37	male	29	HC	neg	neg	neg	75.6
38	female	48	AR	neg	pos	pos	24.2
39	male	26	AR	pos	pos	neg	127.0
40	male	26	AR	pos	pos	neg	
41	female	38	AR	pos	pos	pos	87.6
42	female	33	AR	pos	neg	neg	30.7
43	male	25	AR	pos	pos	neg	63.5
44	male	35	AR	pos	pos	pos	184.0
45	female	48	AR	neg	pos	pos	33.1
46	male	21	AR	neg	pos	neg	47.5
47	male	36	AR	pos	pos	pos	131.0
48	female	36	HC	neg	neg	neg	16.5
49	male	27	AR	pos	pos	neg	114.0
50	male	29	AR	pos	pos	neg	53.8
51	female	28	HC	neg	neg	neg	106.0
52	female	26	AR	pos	pos	neg	117.0
53	female	21	HC	neg	neg	neg	43.0
54	female	36	HC	neg	neg	neg	4.7
55	female	35	HC	neg	neg	neg	33.0
56	female	33	HC	neg	neg	neg	66.0
57	female	52	HC	neg	neg	neg	6.0
58	female	37	HC	neg	neg	neg	4.7

Table E2: Table for Chapter 2 with epidemiologic data for each individual with gender, age, group (HC=healthy controls, AR=allergic rhinitis), symptoms, skin prick test (SPT) for clinically relevant allergens and total IgE whenever available

ID	Gen-der	Age	Group	Symp-toms during pollen season	Birch alder hasel	Grass-pollen	Ragweed -pollen	Total-IgE kU/l
1	female	23	HC	no	neg	neg	neg	17.6
2	male	58	HC	no	neg	neg	neg	87.9
3	female	39	HC	no	neg	neg	neg	39.0
4	female	34	HC	no	neg	neg	neg	8.4
5	female	29	AR	yes	pos	pos	neg	433.0
6	male	27	AR	yes	pos	pos	neg	49.6
7	male	28	HC	no	neg	neg	neg	91.9
8	male	20	AR	yes	neg	pos	neg	81.2
9	female	38	AR	yes	pos	pos	pos	85.4
10	male	26	AR	yes	pos	pos	neg	104.0
11	female	48	AR	yes	neg	pos	pos	24.2
12	male	26	AR	yes	pos	pos	neg	127.0
13	female	33	AR	yes	pos	neg	neg	30.7
14	male	35	AR	yes	pos	pos	pos	184.0
15	female	36	HC	no	neg	neg	neg	16.5
16	female	28	HC	no	neg	neg	neg	106.0
17	female	26	AR	yes	pos	pos	neg	117.0
18	female	21	HC	no	neg	neg	neg	43.0
19	female	36	HC	no	neg	neg	neg	4.7
20	female	35	HC	no	neg	neg	neg	33.0
21	female	33	HC	no	neg	neg	neg	66.0
22	female	52	HC	no	neg	neg	neg	6.0

Table E3: Table for Chapter 1 of all proteins (N=247) detected in healthy controls (n=29) including SwissProt accession number, NCBI Gene symbols, sequence coverage (%), number of unique peptides and Spectrum Mill score, mean spectral count (SC) and standard error of mean (SEM). Proteins with grey background have been newly identified in nasal mucus

SwissProt acc. no.	NCBI Gene Symbols	sequence coverage (%)	unique peptides	score	Group	mean SC "H"	SEM SC "H"
P02768	ALB	86	74	1406.03	H & A	208.59	38.27
P02788	LTF	84	85	1596.16	H & A	88.48	12.93
P01876	IGHA1	76	23	435.07	H & A	77.34	7.06
P01833	PIGR	50	38	690.85	H & A	57.55	5.21
P12273	PIP	76	15	285.21	H & A	41.69	5.42
P01834	IGKC	89	10	193.46	H & A	38.93	3.64
Q8TDL5	BPIFB1	76	27	524.57	H & A	32.24	4.77
P61626	LYZ	81	22	369.66	H & A	30.07	2.76
P0CG05	IGLC2	84	7	124.32	H & A	29.97	2.35
P01857	IGHG1	63	16	283.12	H & A	19.48	3.50
P06702	S100A9	85	12	223.3	H & A	19.31	6.27
Q9NP55	BPIFA1	59	14	289.87	H & A	19.00	2.55
P63261	ACTG1	68	19	365.75	H & A	17.03	2.11
Q9HC84	MUC5B	20	70	1308.84	H & A	16.17	3.10
Q96DA0	ZG16B	55	14	261.56	H & A	16.03	1.83
P02787	TF	64	42	759.91	H & A	15.86	3.25
P31025	LCN1	75	18	327.88	H & A	15.62	5.06
P03973	SLPI	72	14	246.03	H & A	12.34	1.96
P01591	IGJ	66	9	158.29	H & A	11.07	1.19
P00738	HP	61	25	445.46	H & A	9.83	1.26
P04264	KRT1	47	38	669.22	H & A	9.76	2.30
P25311	AZGP1	55	17	291.9	H & A	7.90	1.58
P05109	S100A8	81	13	215.63	H & A	7.10	2.10
P80188	LCN2	58	9	183.81	H & A	6.76	1.05
P01871	IGHM	34	11	188.72	H & A	5.90	0.86
P02647	APOA1	55	22	396.78	H & A	5.90	1.27
P13645	KRT10	62	36	642.75	H & A	5.45	1.26
P06733	ENO1	52	16	304.27	H & A	5.41	0.86
Q9GZZ8	LACRT	39	10	169.1	H & A	5.21	1.50

Q6P5S2	C6orf58	32	8	151.77	H & A	5.21	1.25
P04406	GAPDH	71	15	283.89	H & A	5.17	1.05
O75556	SCGB2A1	73	8	150.4	H & A	5.14	1.34
P20061	TCN1	30	11	211.05	H & A	5.10	0.66
P04083	ANXA1	62	17	298.87	H & A	5.00	0.90
P02675	FGB	56	21	392.69	H & A	4.79	1.14
P63104	YWHAZ	43	9	170.45	H & A	4.59	1.05
P07737	PFN1	65	8	141.55	H & A	4.48	0.92
Q16378	PRR4	32	5	77.64	H & A	4.34	2.59
Q14508	WFDC2	45	5	88.4	H & A	4.31	0.53
P09211	GSTP1	80	10	199.32	H & A	4.14	0.77
P01024	C3	45	53	921.8	H & A	4.07	0.85
P01766	N/A	24	2	40.89	H & A	4.03	0.46
P00352	ALDH1A1	66	21	411.28	H & A	3.90	1.17
P61769	B2M	72	7	121.64	H & A	3.59	0.47
Q8N4F0	BPIFB2	31	9	175.2	H & A	3.55	0.65
P59666	DEFA3	26	5	66.41	H & A	3.48	0.91
P05164	MPO	44	24	393.12	H & A	3.34	1.40
Q06830	PRDX1	74	13	233.72	H & A	3.24	0.95
P13796	LCP1	68	28	498.35	H & A	3.10	1.05
P68871	HBB	89	14	262.68	H & A	3.03	1.26
P30838	ALDH3A1	60	16	275.77	H & A	3.00	0.91
P01036	CST4	67	10	173.38	H & A	2.93	0.82
P04206	N/A	30	2	38.74	H & A	2.79	0.36
P08107	HSPA1A	34	17	283.68	H & A	2.79	0.84
P06744	GPI	26	9	163.83	H & A	2.76	0.68
P62805	HIST1H4A H4/A H4FA	58	7	122.49	H & A	2.66	1.16
P31949	S100A11	73	6	112.55	H & A	2.59	0.43
P14618	PKM	62	24	435.04	H & A	2.24	0.95
P02679	FGG	42	13	228.77	H & A	2.21	0.59
P68371	TUBB4B	66	18	332.84	H & A	2.21	1.11
Q99879	HIST1H2BM	35	5	74.76	H & A	2.17	0.58
P29508	SERPINB3	61	22	392.21	H & A	2.14	0.78
P01593	N/A	30	2	41.37	H & A	2.10	0.40
P01034	CST3	44	5	91.91	H & A	2.07	0.37
P69905	HBA1	83	9	181.14	H & A	2.03	0.99
P68363	TUBA1B	54	16	287.56	H & A	1.93	0.94
P29401	TKT	30	11	189.98	H & A	1.83	0.50
P01779	N/A	25	2	39.72	H & A	1.83	0.25
P01009	SERPINA1	50	16	295.71	H & A	1.79	0.49
P02671	FGA	22	13	229.43	H & A	1.79	0.51
P16050	ALOX15	49	22	376.8	H & A	1.76	0.77
P02774	GC	20	7	125.38	H & A	1.72	0.41

P06396	GSN	26	12	209.9	H & A	1.72	0.42
P00558	PGK1	49	14	238.15	H & A	1.69	0.53
P10599	TXN	65	6	93.53	H & A	1.69	0.29
P62937	PPIA	37	7	125.13	H & A	1.66	0.42
P02763	ORM1	40	7	119.96	H & A	1.66	0.36
P35527	KRT9	44	19	284.7	H & A	1.59	0.64
Q9Y6R7	FCGBP	13	36	669.15	H & A	1.59	0.90
O95994	AGR2	42	5	95.64	H & A	1.55	0.54
P07900	HSP90AA1	25	14	230.55	H & A	1.52	0.65
P02790	HPX	45	13	234.56	H & A	1.52	0.43
P10909	CLU	17	5	97.77	H & A	1.45	0.34
P23528	CFL1	42	6	119.81	H & A	1.45	0.33
O95969	SCGB1D2	33	4	66.13	H & A	1.45	0.55
P30044	PRDX5	55	10	177.71	H & A	1.41	0.68
P25815	S100P	70	4	74.53	H & A	1.41	0.26
P01023	A2M	31	28	487.78	H & A	1.38	0.49
Q9UGM3	DMBT1	13	23	379.68	H & A	1.38	0.75
P31146	CORO1A	31	7	124.45	H & A	1.38	0.40
P52566	ARHGDIB	52	7	122.78	H & A	1.34	0.36
P07339	CTSD	24	6	109.96	H & A	1.34	0.45
P02808	STATH	54	3	60.49	H & A	1.31	0.30
P04792	HSPB1	60	6	111.08	H & A	1.24	0.36
P07195	LDHB	35	8	141.04	H & A	1.17	0.37
P08670	VIM	25	11	178.05	H & A	1.17	0.37
P18669	PGAM1	36	6	98.78	H & A	1.14	0.25
P20671	HIST1H2AD	38	5	74.75	H & A	1.14	0.32
Q5VTE0	EEF1A1P5	33	9	177.47	H & A	1.10	0.51
P08246	ELANE	36	7	115.18	H & A	1.10	0.26
P24158	PRTN3	43	6	119.38	H & A	1.10	0.32
Q01469	FABP5	40	5	73.1	H & A	1.03	0.37
P02812	PRB2	31	3	53.44	H only	0.97	0.58
Q01518	CAP1	23	6	98.81	H & A	0.97	0.28
P02652	APOA2	69	6	110.86	H & A	0.97	0.28
P52209	PGD	31	10	185.22	H & A	0.97	0.34
P04075	ALDOA	34	8	141.99	H & A	0.93	0.34
Q99935	PROL1	31	6	101.25	H & A	0.86	0.41
O43707	ACTN4	17	10	174.55	H & A	0.86	0.30
P54108	CRISP3	33	4	71.93	H & A	0.76	0.20
P01011	SERPINA3	32	9	157.55	H & A	0.76	0.27
P07355	ANXA2	45	10	172.09	H & A	0.69	0.29
Q8WZ42	TTN	0	2	23.32	H & A	0.66	0.19
P31944	CASP14	28	7	111.63	H & A	0.66	0.31
P80723	BASP1	57	6	100.59	H & A	0.66	0.19

P60174	TPI1	46	8	134.53	H & A	0.62	0.19
P22079	LPO	26	9	154.29	H & A	0.62	0.22
P06703	S100A6	47	5	79.62	H & A	0.59	0.14
P30740	SERPINB1	24	7	118.09	H & A	0.59	0.22
P09210	GSTA2	33	6	102.94	H & A	0.59	0.34
P14780	MMP9	14	8	124.88	H & A	0.59	0.30
P59827	BPIFB4	27	10	190.82	H & A	0.55	0.33
Q07654	TFF3	36	2	33	H & A	0.55	0.20
P05783	KRT18	30	9	154.97	H & A	0.52	0.29
P02765	AHSG	26	5	87.13	H & A	0.52	0.19
P00326	ADH1C	30	8	149.11	H & A	0.52	0.29
P62328	TMSB4X	45	3	48.68	H & A	0.48	0.18
P37837	TALDO1	16	5	69.75	H & A	0.48	0.17
Q13938	CAPS	25	4	68.59	H & A	0.48	0.29
P01625	N/A	35	3	53.92	H & A	0.48	0.18
P00441	SOD1	53	3	63.44	H & A	0.48	0.24
Q08380	LGALS3BP	17	6	113.39	H & A	0.45	0.18
P06576	ATP5B	19	7	111.22	H & A	0.45	0.28
Q71DI3	HIST2H3A	43	5	68.9	H & A	0.41	0.18
Q6UWW0	LCN15	40	6	100.37	H & A	0.41	0.20
P62158	CALM1	61	4	73.17	H & A	0.41	0.22
P12429	ANXA3	30	7	125.24	H & A	0.41	0.21
P23381	WARS	39	12	214.3	H & A	0.38	0.26
P00751	CFB	19	9	137.75	H & A	0.38	0.17
P15311	EZR	10	5	87.22	H & A	0.38	0.17
P50395	GDI2	17	6	104.05	H & A	0.38	0.13
O95968	SCGB1D1	24	4	58.67	H only	0.38	0.17
P11684	SCGB1A1	41	3	56.6	H & A	0.38	0.13
Q9BW30	TPPP3	29	4	82.02	H & A	0.34	0.24
P22528	SPRR1B	28	2	31.01	H & A	0.34	0.17
Q00610	CLTC	4	5	92.25	H & A	0.34	0.19
P00450	CP	22	12	212.56	H & A	0.34	0.22
P01617	N/A	34	3	45.68	H & A	0.34	0.10
P20930	FLG	3	8	130.4	H & A	0.34	0.31
P30041	PRDX6	37	6	99.52	H & A	0.31	0.19
P30086	PEBP1	34	4	79.4	H & A	0.31	0.19
O00299	CLIC1	31	4	64.64	H & A	0.31	0.14
Q8TAX7	MUC7	9	4	56.74	H & A	0.31	0.20
P13639	EEF2	15	8	150.73	H & A	0.31	0.20
P02766	TTR	68	7	127.21	H & A	0.28	0.11
P40394	ADH7	32	8	141.97	H & A	0.28	0.24
P80303	NUCB2	17	4	64.55	H & A	0.28	0.15
P61978	HNRNPK	17	5	83.6	H only	0.28	0.16

P59998	ARPC4	18	2	39.1	H & A	0.28	0.11
P01781	N/A	17	2	35.78	H & A	0.28	0.14
Q00796	SORD	25	5	98.11	H & A	0.24	0.17
P35321	SPRR1A	44	3	41.58	H & A	0.24	0.13
P19957	PI3	18	2	36.09	H & A	0.24	0.09
P35325	SPRR2B	48	3	42.04	H & A	0.24	0.13
P16402	HIST1H1D	10	2	32.27	H & A	0.24	0.13
Q13228	SELENBP1	28	7	122.16	H & A	0.24	0.18
P09960	LTA4H	5	2	39.53	H & A	0.24	0.12
Q29RF7	PDS5A	1	3	24.21	H & A	0.21	0.09
P22626	HNRNPA2B1	19	5	85.11	H & A	0.21	0.13
P16152	CBR1	24	5	87.54	H & A	0.21	0.09
P40925	MDH1	23	5	83.37	H & A	0.21	0.21
P0C0L5	C4B	25	21	379.44	H & A	0.21	0.13
Q06323	PSME1	18	3	54.75	H & A	0.21	0.14
P31151	S100A7	57	6	95.87	H & A	0.21	0.13
P40926	MDH2	13	3	52.88	H & A	0.21	0.13
P81605	DCD	22	2	24.47	H & A	0.21	0.12
Q99497	PARK7	34	4	62.94	H & A	0.21	0.17
P07858	CTSB	25	6	107.93	H & A	0.21	0.13
P55072	VCP	10	5	77.28	H & A	0.21	0.14
P08311	CTSG	35	7	108.88	H & A	0.21	0.17
P61158	ACTR3	22	5	81.43	H & A	0.17	0.14
P28799	GRN	13	4	69.16	H only	0.17	0.10
P22894	MMP8	17	6	105.18	H & A	0.17	0.17
Q9NZT1	CALML5	26	4	63.6	H & A	0.17	0.11
P14555	PLA2G2A	48	6	88.78	H only	0.17	0.17
Q9UBC9	SPRR3	24	3	42.14	H & A	0.14	0.08
Q04828	AKR1C1	30	5	96.99	H & A	0.14	0.10
P80511	S100A12	29	3	41.64	H & A	0.14	0.08
P20618	PSMB1	13	2	27.85	H only	0.14	0.11
P14550	AKR1A1	32	8	139.72	H & A	0.14	0.14
P28838	LAP3	11	4	73	H & A	0.14	0.14
P84077	ARF1	22	3	54.33	H & A	0.14	0.10
P17213	BPI	14	4	79.75	H & A	0.14	0.11
P26447	S100A4	18	2	23.38	H only	0.14	0.08
P35579	MYH9	4	5	86.63	H & A	0.14	0.08
P61160	ACTR2	12	4	76.44	H only	0.14	0.14
P04040	CAT	9	3	51.11	H & A	0.14	0.11
P01033	TIMP1	32	4	63.56	H & A	0.14	0.08
P01714	N/A	24	2	25.21	H only	0.10	0.06
Q6UXB2	CXCL17	17	2	30.06	H & A	0.10	0.06
P15559	NQO1	22	3	64.61	H & A	0.10	0.10

Q9Y265	RUVBL1	10	3	52.54	H only	0.10	0.10
P12724	RNASE3	34	4	67.47	H & A	0.10	0.08
P43490	NAMPT	9	2	41.03	H & A	0.10	0.06
P02545	LMNA	4	2	34.21	H only	0.10	0.10
P05387	RPLP2	24	2	32.57	H only	0.10	0.08
Q96KP4	CNDP2	21	5	68.49	H & A	0.10	0.10
P41218	MNDA	15	4	65.08	H & A	0.10	0.10
Q9Y3Z3	SAMHD1	6	3	57.89	H & A	0.10	0.10
O00391	QSOX1	8	4	71.08	H & A	0.10	0.08
P07998	RNASE1	21	2	21.91	H only	0.07	0.05
P80748	N/A	21	2	30.28	H only	0.07	0.07
P11216	PYGB	2	2	27.79	H & A	0.07	0.05
P06753	TPM3	8	2	30.26	H only	0.07	0.05
P55058	PLTP	8	2	34.18	H & A	0.07	0.05
P04217	A1BG	8	2	35.58	H & A	0.07	0.05
P12277	CKB	11	2	31.72	H only	0.07	0.07
P22314	UBA1	5	3	50.04	H & A	0.07	0.07
P07602	PSAP	8	3	45.55	H & A	0.07	0.07
P07237	P4HB	5	2	30.2	H & A	0.07	0.05
Q14103	HNRNPD	6	2	32.89	H & A	0.07	0.07
Q16881	TXNRD1	5	2	33.61	H only	0.07	0.07
Q9UL46	PSME2	33	5	90.21	H & A	0.07	0.07
P37802	TAGLN2	35	4	75.81	H & A	0.07	0.07
P04080	CSTB	45	3	57.31	H & A	0.07	0.07
P07910	HNRNPC	7	2	30.54	H only	0.07	0.07
P08758	ANXA5	12	3	48.82	H only	0.07	0.07
P52565	ARHGDIA	15	2	32.32	H only	0.07	0.07
P04196	HRG	6	2	32.54	H only	0.07	0.05
P07108	DBI	41	2	28.01	H only	0.07	0.05
P11413	G6PD	5	2	31.26	H only	0.07	0.07
P30101	PDIA3	7	3	36.21	H & A	0.07	0.05
P08118	MSMB	44	2	25.64	H & A	0.07	0.07
O75874	IDH1	9	3	53.32	H & A	0.07	0.05
P02749	APOH	26	5	85.8	H & A	0.07	0.07
P32320	CDA	26	2	28.69	H only	0.07	0.07
O76013	KRT36	3	2	23.59	H & A	0.07	0.05
P22392	NME2	28	3	54.39	H & A	0.03	0.03
P52272	HNRNPM	4	2	29.08	H & A	0.03	0.03
P27169	PON1	10	2	43.39	H & A	0.03	0.03
P63241	EIF5A	27	2	36.59	H & A	0.03	0.03
P61916	NPC2	25	2	37.44	H & A	0.03	0.03
P19971	TYMP	12	3	52.29	H only	0.03	0.03
P01042	KNG1	8	4	52.62	H & A	0.03	0.03

P00734	F2	6	2	34.53	H & A	0.03	0.03
P01008	SERPINC1	10	3	44.7	H & A	0.03	0.03
Q9HD89	RETN	24	2	40.06	H & A	0.03	0.03

Table E4: Table for Chapter 2 of all proteins (N=430) detected including SwissProt accession number, NCBI Gene symbols entry name, sequence coverage (%), number of unique peptides and Mascot score, group/seasonal specificity represented by mean normalized areas under the curve AUC [%] and standard error of mean (SEM). Proteins with grey background have been newly identified in nasal mucus

					allergic rhinitis				healthy controls			
					IN season		OUT season		IN season		OUT season	
Acc. no.	NCBI Gene symbols	seq. cov.	No. Pept.	score	mean AUC [%]	SEM	mean AUC [%]	SEM	mean AUC [%]	SEM	mean AUC [%]	SEM
P31946	YWHAB	48.37	7	202	.0	.0	.0	.0	.0	.0	.0	.0
P62258	YWHAE	34.90	7	166	.0	.0	.1	.0	.0	.0	.0	.0
P61981	YWHAG	19.43	2	22	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P31947	SFN	57.66	8	243	.0	.0	.1	.0	.0	.0	0.0	0.0
P27348	YWHAQ	25.71	3	77	.0	.0	.0	.0	.0	.0	.0	.0
P63104	YWHAZ	46.53	7	441	.1	.0	.2	.0	.1	.0	.1	.0
P16885	PLCG2	1.11	1	27	.3	.3	0.0	0.0	0.0	0.0	0.0	0.0
P05386	RPLP1	14.04	1	4	0.0	0.0	.0	.0	.0	.0	.0	.0
P05387	RPLP2	69.57	4	8	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P52209	PGD	31.47	10	133	.0	.0	.1	.0	.0	.0	.0	.0
Q7Z5M8	ABHD12B	1.66	1	59	.1	.1	0.0	0.0	.1	.0	.1	.1
Q13510	ASAH1	10.89	2	8	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P68032	ACTC1	36.34	1	1493	.3	.1	.6	.1	.9	.2	.6	.2
P60709	ACTB	78.93	10	3139	1.2	.2	1.5	.1	1.4	.3	1.3	.3
P59998	ARPC4	18.45	2	25	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
O15511	ARPC5	12.58	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P61158	ACTR3	25.60	6	12	0.0	0.0	.0	.0	.0	.0	0.0	0.0
O95433	AHSA1	6.51	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P07108	DBI	41.38	2	5	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P00568	AK1	7.22	1	3	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
Q01518	CAP1	23.79	6	79	.0	.0	.1	.0	.0	.0	.1	.1
P61204	ARF3	22.10	3	14	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q5JQC9	AKAP4	1.64	1	20	.1	.1	.0	.0	.1	.1	0.0	0.0

P49588	AARS	2.79	1	1	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P14550	AKR1A1	40.00	9	24	0.0	0.0	.0	.0	.0	.0	.0	.0
P00326	ADH1C	34.40	9	104	.0	.0	.0	.0	.0	.0	.0	.0
P40394	ADH7	36.01	9	52	.0	.0	.0	.0	.0	.0	.0	.0
P30838	ALDH3A1	60.26	17	492	.1	.0	.1	.0	.2	.1	.2	.1
Q04828	AKR1C1	35.91	1	26	.0	.0	.0	.0	0.0	0.0	.0	.0
P52895	AKR1C2	26.01	1	24	.0	.0	.0	.0	0.0	0.0	.0	.0
P42330	AKR1C3	14.55	1	13	.0	.0	.0	.0	0.0	0.0	0.0	0.0
P02763	ORM1	40.80	5	257	.2	.1	.2	.0	.1	.0	.1	.1
P19652	ORM2	30.35	2	49	.1	.0	.1	.0	0.0	0.0	.0	.0
P01011	SERPINA3	32.62	9	116	.1	.0	.1	.0	.1	.0	.1	.0
P01009	SERPINA1	55.26	21	501	.2	.1	.1	.0	.1	.0	.1	.0
P04217	A1BG	8.48	2	27	.0	.0	.0	.0	0.0	0.0	.0	.0
P02765	AHSG	26.43	5	84	.2	.1	.2	.1	.0	.0	0.0	0.0
P01023	A2M	36.30	33	479	.2	.1	.1	.0	.0	.0	.1	.0
P12814	ACTN1	17.15	5	48	.0	.0	.0	.0	.0	.0	.1	.0
O43707	ACTN4	22.06	7	56	0.0	0.0	.0	.0	.0	.0	.0	.0
P06733	ENO1	53.69	17	659	.1	.0	.3	.0	.2	.0	.1	.0
Q9C0B1	FTO	1.58	1	4	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
Q9NP70	AMBN	1.12	1	55	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P01019	AGT	30.93	6	52	.0	.0	.0	.0	0.0	0.0	.0	.0
Q9NU02	ANKEF1	0.64	1	2	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P04083	ANXA1	62.43	18	740	.2	.0	.2	.0	.3	.1	.1	.1
P27216	ANXA13	3.80	1	2	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P07355	ANXA2	46.02	11	82	.0	.0	.0	.0	.0	.0	.0	.0
P12429	ANXA3	34.67	8	26	0.0	0.0	.0	.0	.0	.0	.0	.0
P08758	ANXA5	24.06	6	10	0.0	0.0	0.0	0.0	.0	.0	.0	.0
O95994	AGR2	42.29	6	198	.1	.0	.1	.0	.1	.0	.0	.0
P03973	SLPI	72.73	14	1992	.6	.2	1.4	.3	.6	.1	2.4	.6
P01008	SERPINC1	14.66	5	7	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P02647	APOA1	58.43	21	1899	1.5	.5	.5	.1	.4	.2	.5	.2
P02652	APOA2	69.00	6	1008	.5	.2	.4	.1	.1	.0	.1	.1
P06727	APOA4	6.82	2	7	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P04114	APOB	1.47	3	3	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P16050	ALOX15	50.30	23	295	.0	.0	.1	.0	.0	.0	.0	.0
P25705	ATP5A1	7.78	3	39	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P06576	ATP5B	25.33	8	33	.0	.0	.0	.0	.0	.0	0.0	0.0
Q8IVW6	ARID3B	1.25	1	1	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P20160	AZU1	7.97	2	39	0.0	0.0	0.0	0.0	.1	.1	0.0	0.0
P17213	BPI	14.58	4	10	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P98160	HSPG2	0.61	2	11	.0	.0	0.0	0.0	.0	.0	.2	.2

P02812	PRB2	4.81	1	72	0.0	0.0	0.0	0.0	0.0	0.0	.1	.1
P02749	APOH	26.67	5	25	.0	.0	.0	.0	0.0	0.0	0.0	0.0
P61769	B2M	65.55	6	686	.7	.2	1.2	.2	1.0	.3	1.0	.2
Q562R1	ACTBL2	28.46	2	638	.2	.1	.4	.2	.8	.3	.6	.3
P08118	MSMB	8.77	2	58	.1	.0	.0	.0	.2	.0	.1	.1
P07738	BPGM	5.02	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q9NP55	BPIFA1	61.33	15	4973	3.2	.7	2.8	.6	2.6	.7	2.4	.4
Q8TDL5	BPIFB1	80.58	32	8321	2.4	.5	1.9	.3	1.7	.5	1.8	.3
Q8N4F0	BPIFB2	40.61	10	860	.4	.1	.5	.1	.1	.0	.3	.1
P59826	BPIFB3	6.51	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P59827	BPIFB4	27.52	10	116	.0	.0	.0	.0	.0	.0	0.0	0.0
P80723	BASP1	44.93	5	66	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
Q13938	CAPS	48.15	8	75	.0	.0	.1	.0	.1	.0	.0	.0
P62158	CALM1	61.74	4	76	.0	.0	.1	.0	.1	.0	.0	.0
P04632	CAPNS1	8.58	1	9	0.0	0.0	0.0	0.0	.0	.0	.0	.0
P07384	CAPN1	4.06	1	3	.0	.0	.0	.0	0.0	0.0	0.0	0.0
Q99439	CNN2	5.50	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P00918	CA2	6.15	1	1	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P16152	CBR1	24.91	5	15	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P04040	CAT	9.30	3	7	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P49913	CAMP	20.59	3	16	0.0	0.0	0.0	0.0	.0	.0	.0	.0
P07339	CTSD	26.46	7	146	.0	.0	.1	.0	.1	.0	.0	.0
P08311	CTSG	31.37	6	45	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P25774	CTSS	4.83	1	2	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
A5YKK6	CNOT1	0.46	1	3	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P00450	CP	26.67	15	78	.0	.0	.1	.0	.0	.0	.0	.0
Q15782	CHI3L2	2.82	1	6	.0	.0	.0	.0	.0	.0	0.0	0.0
Q0VF96	CGNL1	0.61	1	3	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
O00299	CLIC1	31.12	6	38	.0	.0	.0	.0	.0	.0	0.0	0.0
Q00610	CLTC	5.85	6	23	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P10909	CLU	17.59	5	254	.2	.0	.1	.0	.1	.0	.0	.0
P23528	CFL1	42.17	6	213	.1	.1	.1	.0	.1	.0	.1	.0
Q68D86	CCDC102B	2.14	1	27	.3	.3	.5	.5	.6	.4	1.2	.9
Q6TFL3	CCDC171	1.06	1	9	0.0	0.0	0.0	0.0	.1	.0	0.0	0.0
Q2UY09	COL28A1	1.24	2	3	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P25940	COL5A3	0.40	1	14	0.0	0.0	.0	.0	0.0	0.0	.0	.0
P01024	C3	51.05	58	808	.3	.1	.3	.1	.1	.0	.2	.1
P0C0L5	C4B	22.88	19	114	.1	.0	.0	.0	0.0	0.0	.0	.0
P01031	C5	2.45	2	15	.0	.0	.0	.0	0.0	0.0	.0	.0
P00751	CFB	23.30	10	71	.0	.0	.1	.0	.0	.0	.0	.0
P08603	CFH	6.09	5	8	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0

Q02246	CNTN2	1.73	1	4	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P22528	SPRR1B	37.08	1	16	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P31146	CORO1A	28.20	7	106	.0	.0	.1	.0	.1	.0	.1	.0
P12277	CKB	16.27	3	3	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q13616	CUL1	0.77	1	2	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q00532	CDKL1	3.36	1	3	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P35520	CBS	2.18	1	1	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P04080	CSTB	45.92	3	25	.0	.0	.0	.0	.0	.0	.0	.0
P01034	CST3	44.52	5	228	.0	.0	.1	.0	.1	.0	.1	.0
P01036	CST4	67.38	5	439	.3	.3	.3	.2	.3	.2	.3	.2
P09228	CST2	23.40	1	16	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P01037	CST1	45.39	3	197	.1	.1	.2	.1	.0	.0	.2	.2
P54108	CRISP3	37.14	5	74	.1	.1	.0	.0	.1	.0	.1	.0
P32320	CDA	26.03	2	2	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q14008	CKAP5	0.69	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P28838	LAP3	17.15	6	15	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
Q96KP4	CNDP2	26.11	6	11	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q9UKG1	APPL1	1.13	1	13	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
Q9UGM3	DMBT1	38.17	26	3186	1.5	.4	1.2	.4	1.7	.2	2.0	.5
Q13011	ECH1	9.15	2	2	.1	.1	0.0	0.0	0.0	0.0	0.0	0.0
P81605	DCD	32.73	3	22	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P60981	DSTN	23.64	3	6	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P56282	POLE2	1.90	1	6	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P19957	PI3	18.80	2	16	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P68104	EEF1A1	35.28	10	153	.1	.1	.0	.0	.1	.1	.1	.0
P13639	EEF2	18.76	9	48	.0	.0	0.0	0.0	.0	.0	.0	.0
P12724	RNASE3	16.88	2	58	.3	.3	.0	.0	.1	.0	0.0	0.0
Q05315	CLC	19.01	2	21	.1	.1	0.0	0.0	0.0	0.0	0.0	0.0
P11678	EPX	9.79	5	25	.0	.0	.0	.0	0.0	0.0	0.0	0.0
P61916	NPC2	25.83	2	8	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P58107	EPPK1	1.96	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P60842	EIF4A1	3.20	1	2	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P63241	EIF5A	42.86	3	8	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P55060	CSE1L	2.37	2	87	0.0	0.0	0.0	0.0	.4	.3	.0	.0
Q9GZZ8	LACRT	39.86	10	911	.2	.1	.2	.1	.4	.2	.6	.3
P15311	EZR	17.06	7	53	.0	.0	.0	.0	.0	.0	.0	.0
Q01469	FABP5	52.59	6	116	.0	.0	.0	.0	.1	.0	0.0	0.0
P52907	CAPZA1	6.99	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P47756	CAPZB	9.03	1	6	.0	.0	.0	.0	.0	.0	0.0	0.0
Q9UK61	FAM208A	0.48	1	7	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
Q8NCA5	FAM98A	1.93	1	4	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0

Q5FWF7	FBXO48	3.23	1	15	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P02671	FGA	22.86	13	227	.2	.1	.1	.0	.2	.1	.0	.0
P02675	FGB	56.82	21	628	.3	.1	.2	.1	.2	.1	.1	.0
P02679	FGG	45.92	15	369	.3	.1	.2	.1	.1	.0	.1	.0
Q96M85	N/A	2.82	1	24	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P09467	FBP1	8.28	2	17	.0	.0	0.0	0.0	.0	.0	.0	.0
P04075	ALDOA	45.33	11	119	.1	.0	.0	.0	.1	.0	.0	.0
P09972	ALDOC	6.32	1	10	.0	.0	.0	.0	.0	.0	0.0	0.0
P17931	LGALS3	6.00	1	10	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
Q08380	LGALS3BP	21.37	7	81	.1	.0	.2	.0	.0	.0	.1	.0
P06396	GSN	25.19	11	194	.1	.0	.1	.0	.1	.0	.1	.0
P06744	GPI	29.39	10	321	.1	.0	.1	.0	.1	.0	.1	.0
P35754	GLRX	27.36	1	4	.0	.0	.0	.0	0.0	0.0	0.0	0.0
P00390	GSR	3.64	1	10	.0	.0	.0	.0	0.0	0.0	0.0	0.0
P09211	GSTP1	85.71	11	614	.2	.1	.4	.1	.3	.1	.4	.2
P04406	GAPDH	71.94	15	910	.2	.1	.4	.1	.3	.1	.3	.1
P28799	GRN	13.32	4	11	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P08263	GSTA1	58.56	12	185	.1	.0	.2	.1	.0	.0	.1	.1
P00738	HP	61.58	25	1475	.6	.2	.4	.1	.5	.1	.4	.1
P11142	HSPA8	38.54	16	114	.0	.0	.0	.0	.0	.0	.0	.0
Q96RW7	HMCN1	0.16	1	4	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P69905	HBA1	85.21	10	1117	.9	.6	.3	.2	.0	.0	0.0	0.0
P68871	HBB	95.24	10	1069	2.3	1.5	.8	.6	.1	.1	0.0	0.0
P02042	HBD	63.95	3	191	.3	.3	0.0	0.0	.1	.0	0.0	0.0
P69891	HBG1	19.73	1	118	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P02790	HPX	45.67	14	280	.2	.1	.1	.0	.1	.0	.1	.0
P09429	HMGB1	14.42	2	5	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P26583	HMGB2	12.92	1	4	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P04196	HRG	9.52	3	31	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P07305	H1F0	6.70	1	3	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P10412	HIST1H1E	17.81	3	26	0.0	0.0	0.0	0.0	.1	.0	0.0	0.0
Q96KK5	HIST1H2AH	58.59	1	229	.1	.1	.1	.0	.2	.1	.2	.1
Q16777	HIST2H2AC	58.14	1	270	.1	.1	.2	.1	.2	.1	.2	.1
P0C0S5	H2AFZ	35.16	1	78	.0	.0	.1	.0	.0	.0	.0	.0
P06899	HIST1H2BJ	35.71	1	550	.2	.1	.4	.1	.5	.2	.2	.1
O60814	HIST1H2BK	35.71	2	598	.2	.1	.7	.2	.5	.2	.3	.1
P68431	HIST1H3A	38.24	1	62	.1	.0	.1	.0	.0	.0	.0	.0
Q71DI3	HIST2H3A	38.24	1	68	.1	.0	.0	.0	.1	.0	.1	.0
P84243	H3F3A	38.24	1	43	.0	.0	.0	.0	.0	.0	.0	.0
P62805	HIST1H4A	59.22	8	516	.0	.0	.1	.0	.2	.1	.1	.0
P08107	HSPA1A	34.79	15	216	.1	.0	.1	.0	.1	.0	.0	.0

P07900	HSP90AA1	23.91	7	133	.0	.0	.1	.0	.0	.0	.0	.0
P08238	HSP90AB1	21.69	5	68	.0	.0	.0	.0	.0	.0	0.0	0.0
P04792	HSPB1	65.85	8	236	.1	.1	.1	.0	.1	.0	.1	.0
P01876	IGHA1	84.14	12	17560	6.5	.6	6.2	.6	6.8	.9	6.8	.8
P01877	IGHA2	71.18	4	11543	5.9	.6	5.2	.5	5.3	.9	5.0	.8
P01857	IGHG1	63.03	8	3323	1.8	.5	1.7	.3	.9	.2	1.2	.4
P01859	IGHG2	46.01	5	1292	.7	.3	.7	.1	.3	.1	.4	.2
P01860	IGHG3	52.25	6	1121	.6	.3	.2	.1	.2	.1	.4	.2
P01861	IGHG4	49.54	4	577	.2	.1	.2	.1	.1	.1	.6	.2
P23083	N/A	10.26	1	2	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P06331	N/A	17.12	2	24	.0	.0	.0	.0	.0	.0	0.0	0.0
P01766	N/A	25.00	2	378	.6	.2	.3	.0	.6	.1	.2	.1
P01767	N/A	9.57	1	11	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P01768	N/A	22.13	2	12	0.0	0.0	0.0	0.0	.2	.2	0.0	0.0
P01769	N/A	15.57	1	8	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P01781	N/A	17.24	2	28	.0	.0	.0	.0	.0	.0	.0	.0
P01765	N/A	26.09	1	158	.2	.1	.2	.1	.1	.0	.1	.1
P01779	N/A	26.72	1	184	.2	.1	.2	.0	.2	.0	.1	.1
P01834	IGKC	90.57	9	9721	4.4	.8	2.1	.3	3.1	.6	3.1	.3
P04430	N/A	16.67	1	5	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P01596	N/A	16.82	1	116	.1	.1	.1	.1	.1	.0	.0	.0
P01597	N/A	16.67	1	318	.3	.3	.2	.1	.4	.1	.1	.1
P01602	IGKV1-5	13.68	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P01605	N/A	25.00	2	8	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P01612	N/A	16.51	1	78	.0	.0	.0	.0	.0	.0	0.0	0.0
P01613	N/A	30.36	2	41	.1	.1	0.0	0.0	.1	.0	.0	.0
P01609	N/A	27.78	1	252	.4	.3	.2	.1	.5	.1	.2	.1
P01611	N/A	16.67	1	114	.0	.0	.0	.0	.1	.0	.0	.0
P01617	N/A	32.74	2	123	.1	.1	.1	.1	.0	.0	.2	.1
P01619	N/A	16.67	2	46	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P18135	N/A	29.46	2	43	.1	.1	.0	.0	.1	.0	.1	.1
P01621	N/A	25.00	1	4	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P04433	N/A	7.83	1	6	0.0	0.0	0.0	0.0	.0	.0	.0	.0
P01625	N/A	36.84	3	118	.1	.0	.1	.0	.1	.0	.0	.0
P04208	N/A	11.93	1	36	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P80748	N/A	21.62	2	66	.0	.0	.0	.0	.0	.0	.1	.1
P01714	N/A	25.00	2	10	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P01717	N/A	17.76	1	23	.1	.1	.0	.0	.0	.0	0.0	0.0
P0CG05	IGLC2	96.23	1	6496	3.0	.4	2.8	.4	2.2	.3	3.6	.6
P0CG06	IGLC3	89.62	1	6443	3.0	.4	2.8	.4	2.2	.3	3.6	.6
A0M8Q6	IGLC7	50.94	1	745	.3	.3	0.0	0.0	.2	.2	.2	.2

P01871	IGHM	32.52	7	714	.2	.1	.1	.0	.2	.0	.2	.1
P04220	N/A	29.92	2	645	.2	.0	.2	.0	.1	.0	.2	.0
Q9Y6R7	FCGBP	32.27	58	267	.1	.0	.1	.1	.1	.0	.0	.0
P01591	IGJ	66.04	9	1733	.7	.1	.5	.1	.8	.1	1.7	.6
B9A064	IGLL5	48.13	3	3273	2.0	.5	2.5	.4	1.9	.2	2.7	.8
Q6UXS9	CASP12	6.16	1	2	0.0	0.0	0.0	0.0	.1	.1	0.0	0.0
Q8NI35	INADL	0.33	1	6	.0	.0	0.0	0.0	.0	.0	0.0	0.0
Q14571	ITPR2	0.26	1	7	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P19827	ITIH1	2.09	1	4	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P78413	IRX4	3.28	1	5	0.0	0.0	0.0	0.0	.1	.1	0.0	0.0
O75874	IDH1	16.18	5	11	0.0	0.0	0.0	0.0	0.0	0.0	.1	.1
O43526	KCNQ2	1.26	1	4	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
Q8N239	KLHL34	1.86	1	13	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P04264	KRT1	47.52	32	1190	.1	.1	.0	.0	.1	.1	.0	.0
P13645	KRT10	62.67	31	853	.0	.0	.0	.0	.0	.0	.1	.1
P13646	KRT13	20.09	3	47	0.0	0.0	0.0	0.0	.0	.0	.0	.0
P02533	KRT14	33.69	7	115	0.0	0.0	0.0	0.0	0.0	0.0	.1	.1
P08779	KRT16	17.97	2	32	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P05783	KRT18	28.60	8	73	0.0	0.0	.0	.0	.0	.0	.0	.0
P08727	KRT19	66.50	20	639	.1	.0	.1	.0	.1	.0	.1	.1
P35908	KRT2	38.97	16	266	.0	.0	0.0	0.0	.0	.0	.0	.0
P19013	KRT4	6.37	1	10	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P13647	KRT5	21.02	5	95	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P02538	KRT6A	26.77	2	125	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P04259	KRT6B	26.77	1	164	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P08729	KRT7	18.55	6	35	.0	.0	0.0	0.0	.0	.0	.0	.0
P05787	KRT8	55.07	22	465	.0	.0	.1	.1	.1	.1	.2	.1
P35527	KRT9	43.66	17	237	.1	.1	0.0	0.0	.1	.0	0.0	0.0
Q86T90	KIAA1328	1.21	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q9NZS2	KLRF1	8.62	2	21	0.0	0.0	.0	.0	.0	.0	.0	.0
P01042	KNG1	11.65	6	10	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P22079	LPO	26.54	9	58	.1	.0	.1	.0	.0	.0	.0	.0
P02788	LTF	90.14	90	18768	4.1	1.1	3.2	.4	3.7	.9	4.2	.6
Q9NZR2	LRP1B	0.15	1	1	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
P30740	SERPINB1	24.27	7	33	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P31025	LCN1	75.57	18	2709	.7	.4	1.8	1.1	1.1	.6	2.0	1.2
Q6UWW0	LCN15	35.87	5	59	.1	.0	.1	.0	.0	.0	.0	.0
P23141	CES1	4.94	2	2	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P00338	LDHA	43.37	9	83	.0	.0	.0	.0	.0	.0	.0	.0
P07195	LDHB	38.02	8	50	.0	.0	.0	.0	.0	.0	.0	.0
P33241	LSP1	7.96	1	5	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0

P29375	KDM5A	0.59	2	6	.0	.0	0.0	0.0	0.0	0.0	.0	.0
P61626	LYZ	81.08	23	7973	7.0	1.2	9.9	1.6	9.1	1.4	7.2	1.8
P14174	MIF	17.39	2	6	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P40121	CAPG	7.18	2	2	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P40925	MDH1	26.35	5	35	0.0	0.0	0.0	0.0	.0	.0	.0	.0
P40926	MDH2	13.61	3	38	.0	.0	0.0	0.0	.0	.0	0.0	0.0
O75556	SCGB2A1	70.53	7	802	.6	.4	.5	.2	.4	.3	.8	.4
P14780	MMP9	14.14	8	46	0.0	0.0	.0	.0	.0	.0	.0	.0
Q8IWI9	MGA	0.56	2	13	0.0	0.0	.0	.0	.0	.0	.0	.0
P42679	MATK	3.75	1	4	.0	.0	0.0	0.0	.1	.1	0.0	0.0
Q13421	MSLN	14.13	5	21	.0	.0	.0	.0	.0	.0	0.0	0.0
P01033	TIMP1	32.37	4	19	.0	.0	.0	.0	.0	.0	.0	.0
P26038	MSN	6.76	2	4	0.0	0.0	0.0	0.0	.0	.0	.0	.0
O15403	SLC16A6	2.10	1	24	0.0	0.0	.0	.0	.0	.0	.3	.2
P98088	MUC5AC	21.25	44	667	.2	.1	.2	.1	.2	.1	.1	.0
Q9HC84	MUC5B	22.37	73	4942	1.0	.2	1.0	.2	.5	.1	1.0	.2
Q8TAX7	MUC7	9.81	4	57	.0	.0	.0	.0	0.0	0.0	.0	.0
P24158	PRTN3	43.36	6	129	.1	.1	.1	.0	.1	.0	.1	.1
P41218	MNDA	19.41	5	7	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P05164	MPO	42.01	23	439	.1	.0	.2	.1	.2	.1	.1	.1
P60660	MYL6	19.21	2	3	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P35579	MYH9	4.59	5	10	.0	.0	.0	.0	.0	.0	0.0	0.0
Q9NPC6	MYOZ2	1.89	1	9	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P29966	MARCKS	5.42	1	1	.2	.2	0.0	0.0	0.0	0.0	0.0	0.0
P15559	NQO1	22.63	4	20	0.0	0.0	.0	.0	.0	.0	0.0	0.0
Q92859	NEO1	0.48	1	31	.0	.0	0.0	0.0	.1	.1	0.0	0.0
P22894	MMP8	21.20	7	14	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P59665	DEFA1	26.60	5	680	.3	.1	.6	.2	.9	.4	1.3	.6
P08246	ELANE	47.19	8	174	.1	.0	.2	.0	.3	.1	.1	.1
P80188	LCN2	58.08	9	946	.4	.1	.7	.3	.5	.1	.4	.1
P43490	NAMPT	9.98	2	11	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P10153	RNASE2	19.88	3	31	.1	.1	0.0	0.0	0.0	0.0	0.0	0.0
P80303	NUCB2	21.19	5	13	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P06748	NPM1	7.14	1	6	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
Q8NGR2	OR1L6	1.73	1	15	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q9NWT1	PAK1IP1	2.30	1	1	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
O00151	PDLIM1	13.07	2	5	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
O75594	PGLYRP1	7.65	1	2	0.0	0.0	.0	.0	.0	.0	0.0	0.0
P62937	PPIA	48.48	8	290	.1	.0	.1	.0	.1	.0	.1	.0
P62942	FKBP1A	12.96	1	4	0.0	0.0	.0	.0	0.0	0.0	.0	.0
Q06830	PRDX1	78.89	13	524	.0	.0	.1	.0	.2	.1	.1	.1

P32119	PRDX2	14.65	2	13	.0	.0	0.0	0.0	.0	.0	.0	.0
P30044	PRDX5	55.61	10	200	.0	.0	.0	.0	.1	.1	.1	.0
P30041	PRDX6	52.23	8	26	0.0	0.0	0.0	0.0	.0	.0	.0	.0
Q8IWS0	PHF6	2.47	1	2	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P30086	PEBP1	51.87	5	37	.0	.0	.0	.0	.0	.0	.0	.0
P00558	PGK1	59.47	17	163	.0	.0	.0	.0	.1	.0	.0	.0
P18669	PGAM1	45.28	8	146	.1	.0	.1	.0	.1	.0	.1	.0
P14555	PLA2G2A	45.83	5	32	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P55058	PLTP	14.60	3	22	.1	.1	.0	.0	0.0	0.0	0.0	0.0
Q93100	PHKB	1.46	1	1	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P36955	SERPINF1	15.31	3	16	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
Q8TC59	PIWIL2	1.23	1	2	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P00747	PLG	5.56	2	5	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P13796	LCP1	71.29	30	345	.0	.0	.1	.0	.1	.0	.1	.0
Q96QH2	PRAM1	2.51	1	1	0.0	0.0	0.0	0.0	.1	.1	0.0	0.0
Q9UKK3	PARP4	0.52	1	32	.0	.0	.0	.0	.0	.0	.0	.0
P0CB38	PABPC4L	4.05	1	2	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P01833	PIGR	54.32	37	11447	3.0	.6	3.5	.6	2.9	.4	3.1	.5
P26599	PTBP1	3.95	1	10	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P0CG48	UBC	21.02	1	34	.0	.0	.1	.0	.1	.0	0.0	0.0
P07602	PSAP	8.78	3	15	.0	.0	.0	.0	0.0	0.0	.0	.0
P07737	PFN1	55.71	6	531	.2	.1	.4	.1	.3	.1	.3	.1
P12273	PIP	77.40	17	12982	4.1	1.0	2.6	.5	4.6	.9	4.5	.9
Q99935	PROL1	34.27	8	81	.0	.0	.0	.0	.0	.0	.0	.0
Q16378	PRR4	37.31	6	666	1.7	1.3	.6	.3	1.1	.7	.4	.2
Q99497	PARK7	34.39	4	10	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P28066	PSMA5	7.88	1	5	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P31949	S100A11	73.33	6	507	.2	.1	.3	.0	.2	.1	.2	.1
P80511	S100A12	31.52	5	167	.0	.0	.2	.1	.2	.1	.2	.1
P06703	S100A6	47.78	5	98	.1	.0	.3	.1	.0	.0	.1	.0
P31151	S100A7	44.55	5	28	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P06702	S100A9	85.09	12	3328	.5	.1	1.3	.5	1.5	.7	1.3	.5
P25815	S100P	71.58	4	238	.0	.0	.1	.0	.0	.0	.1	.0
Q68CR1	SEL1L3	0.44	1	2	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q01105	SET	14.48	2	22	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P00734	F2	6.11	2	9	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
P00540	MOS	2.60	1	60	0.0	0.0	0.0	0.0	.1	.1	0.0	0.0
P98179	RBM3	20.38	1	13	.0	.0	.0	.0	0.0	0.0	.0	.0
P14618	PKM	64.22	25	324	.0	.0	.0	.0	.1	.0	.1	.0
P31150	GDI1	11.41	1	9	0.0	0.0	.2	.2	0.0	0.0	0.0	0.0
P50395	GDI2	20.90	4	46	.0	.0	.0	.0	.0	.0	.0	.0

P46940	IQGAP1	1.75	1	4	.0	.0	.0	.0	0.0	0.0	0.0	0.0
P15153	RAC2	7.29	1	2	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q9HD89	RETN	24.07	2	6	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P00352	ALDH1A1	69.66	23	754	.1	.1	.2	.1	.2	.1	.2	.1
P52565	ARHGDIA	22.55	3	6	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P52566	ARHGDIB	52.74	7	106	.0	.0	.2	.0	.1	.1	.1	.1
P07998	RNASE1	35.26	3	6	0.0	0.0	0.0	0.0	.0	.0	.0	.0
Q32P51	HNRNPA1L2	8.44	2	7	.0	.0	.0	.0	0.0	0.0	.0	.0
P22626	HNRNPA2B1	14.45	4	30	0.0	0.0	.0	.0	.0	.0	.0	.0
P51991	HNRNPA3	4.23	1	4	0.0	0.0	0.0	0.0	.0	.0	.0	.0
P07910	HNRNPC	7.52	2	3	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q14103	HNRNPD	11.27	3	7	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P61978	HNRNPK	17.71	5	23	0.0	0.0	.0	.0	.0	.0	.0	.0
Q13950	RUNX2	1.15	1	1	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q9Y265	RUVBL1	10.31	3	3	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P05109	S100A8	82.80	15	1125	.1	.0	.4	.2	.6	.3	.6	.4
P02810	PRH1	26.51	1	10	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0
Q9Y3Z3	SAMHD1	6.55	3	10	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q8TD33	SCGB1C1	12.63	1	1	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
O95968	SCGB1D1	24.44	6	62	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
O95969	SCGB1D2	33.33	4	204	.1	.1	.1	.0	.1	.0	.1	.1
Q13228	SELENBP1	30.51	8	39	.0	.0	.0	.0	.0	.0	.0	.0
P02787	TF	63.18	40	2733	.5	.1	.4	.1	.3	.1	.3	.1
P29508	SERPINB3	63.33	20	683	.1	.1	.2	.1	.1	.0	.1	.1
P48594	SERPINB4	25.38	6	78	.0	.0	.0	.0	0.0	0.0	.0	.0
P02768	ALB	85.55	75	50081	8.3	2.0	8.6	.8	9.6	2.5	8.6	1.5
P27169	PON1	10.99	3	42	.0	.0	.0	.0	0.0	0.0	0.0	0.0
Q9H299	SH3BGRL3	66.67	3	3	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
O43295	SRGAP3	0.73	1	5	.1	.1	0.0	0.0	0.0	0.0	0.0	0.0
P62314	SNRPD1	16.81	1	10	.0	.0	.0	.0	.0	.0	0.0	0.0
Q9Y6X4	FAM169A	0.90	1	2	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P01241	GH1	8.76	1	5	0.0	0.0	.0	.0	.0	.0	0.0	0.0
Q00796	SORD	25.21	5	22	.0	.0	.0	.0	0.0	0.0	0.0	0.0
P02808	STATH	54.84	3	246	.7	.4	3.3	1.7	3.7	1.2	1.0	.5
O00391	QSOX1	8.03	4	10	0.0	0.0	0.0	0.0	.1	.1	.1	.1
P00441	SOD1	60.39	4	43	0.0	0.0	.0	.0	.1	.1	.0	.0
O95425	SVIL	0.36	1	1	0.0	0.0	0.0	0.0	0.0	0.0	.1	.1
Q92922	SMARCC1	1.09	1	1	0.0	0.0	0.0	0.0	0.0	0.0	.1	.1
P22105	TNXB	0.40	2	8	.0	.0	.1	.1	.1	.1	.0	.0
O60635	TSPAN1	5.39	1	16	.0	.0	.1	.0	.0	.0	0.0	0.0
P10599	TXN	65.71	7	193	.1	.0	.0	.0	.1	.0	.0	.0

Q16881	TXNRD1	5.86	2	2	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P19971	TYMP	14.73	4	15	.0	.0	0.0	0.0	.0	.0	0.0	0.0
P62328	TMSB4X	56.82	4	34	0.0	0.0	0.0	0.0	0.0	0.0	.0	.0
Q8WZ42	TTN	0.08	3	60	.1	.1	0.0	0.0	.1	.1	0.0	0.0
P37837	TALDO1	16.02	5	33	0.0	0.0	0.0	0.0	.0	.0	.0	.0
P20061	TCN1	30.95	11	781	.4	.1	.5	.1	.3	.0	.4	.1
P37802	TAGLN2	37.19	5	34	.0	.0	.0	.0	.0	.0	0.0	0.0
P55072	VCP	10.17	6	23	.0	.0	0.0	0.0	0.0	0.0	.0	.0
P29401	TKT	32.10	11	215	.1	.1	.1	.0	.1	.0	.1	.0
A6NKL6	TMEM200C	1.13	1	50	.1	.1	0.0	0.0	.1	.0	0.0	0.0
Q66K66	TMEM198	2.50	1	8	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P02766	TTR	52.38	6	47	.1	.1	.0	.0	.0	.0	.0	.0
Q07654	TFF3	36.25	2	308	1.1	.4	1.3	.3	.4	.1	.6	.2
P60174	TPI1	54.20	10	102	.1	.0	.1	.0	.1	.0	.1	.0
P06753	TPM3	8.10	2	7	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P23381	WARS	44.59	13	56	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
Q71U36	TUBA1A	56.32	2	199	.0	.0	.1	.0	.2	.1	.1	.1
P68363	TUBA1B	56.32	2	183	0.0	0.0	.1	.0	.2	.1	0.0	0.0
P07437	TUBB	54.73	1	194	0.0	0.0	.0	.0	.1	.1	.1	.1
P04350	TUBB4A	59.01	2	386	.0	.0	.1	.0	.2	.1	.1	.1
P68371	TUBB4B	70.11	3	434	.0	.0	.1	.0	.2	.1	.1	.1
Q9BW30	TPPP3	29.55	4	42	.0	.0	.0	.0	.0	.0	.0	.0
Q9Y2Z9	COQ6	1.92	1	22	0.0	0.0	0.0	0.0	.3	.3	0.0	0.0
P22314	UBA1	7.37	4	10	0.0	0.0	0.0	0.0	.0	.0	.0	.0
O60701	UGDH	2.63	1	2	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
Q6P5S2	C6orf58	53.03	14	1632	.7	.3	.9	.2	.4	.1	.7	.2
P11684	SCGB1A1	50.55	4	192	.1	.1	.4	.2	.2	.1	.5	.2
P08670	VIM	30.69	11	98	0.0	0.0	0.0	0.0	.1	.0	.1	.0
P02774	GC	23.00	7	353	.2	.1	.4	.1	.1	.0	.1	.0
Q7Z5L0	VMO1	21.29	2	3	0.0	0.0	.0	.0	0.0	0.0	0.0	0.0
P04004	VTN	9.00	3	5	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
Q14508	WFDC2	45.16	5	584	.4	.1	.6	.2	.4	.2	.5	.1
P13010	XRCC5	9.56	3	7	.0	.0	0.0	0.0	0.0	0.0	0.0	0.0
Q9H2Y7	ZNF106	0.74	1	2	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P49910	ZNF165	1.03	1	10	0.0	0.0	0.0	0.0	.0	.0	.0	.0
O14978	ZNF263	1.02	1	12	0.0	0.0	0.0	0.0	.0	.0	0.0	0.0
P25311	AZGP1	55.37	18	2120	.9	.2	1.5	.2	.9	.2	1.3	.1
Q96DA0	ZG16B	55.29	16	5352	2.9	.9	1.9	.4	2.3	.4	1.5	.3