

Original article

Time-series nasal epithelial transcriptomics during natural pollen exposure in healthy subjects and allergic patients

Background: The role of epithelium has recently awakened interest in the studies of type I hypersensitivity.

Objective: We analysed the nasal transcriptomics epithelial response to natural birch pollen exposure in a time series manner.

Methods: Human nasal epithelial cell swabs were collected from birch pollen allergic patients and healthy controls in winter season. In addition, four specimens at weekly intervals were collected from the same subjects during natural birch pollen exposure in spring and transcriptomic analyses were performed.

Results: The nasal epithelium of healthy subjects responded vigorously to allergen exposure. The immune response was a dominating category of this response. Notably, the healthy subjects did not display any clinical symptoms regardless of this response detected by transcriptomic analysis. Concomitantly, the epithelium of allergic subjects responded also, but with a different set of responders. In allergic patients the regulation of dyneins, the molecular motors of intracellular transport dominated. This further supports our previous hypothesis that the birch pollen exposure results in an active uptake of allergen into the epithelium only in allergic subjects but not in healthy controls.

Conclusion: We showed that birch pollen allergen causes a defence response in healthy subjects, but not in allergic subjects. Instead, allergic patients actively transport pollen allergen through the epithelium to tissue mast cells. Our study showed that new hypotheses can arise from the application of discovery driven methodologies. To understand complex multifactorial diseases, such as type I hypersensitivity, this kind of hypotheses might be worth further analyses.

**P. Mattila¹, J. Renkonen^{2,3},
S. Toppila-Salmi⁴, V. Parviainen²,
S. Joenväärä^{1,2}, S. Alff-Tuomala¹,
D. Nicorici¹, R. Renkonen^{2,3}**

¹Medicel Ltd, Helsinki, Finland; ²Transplantation Laboratory & Infection Biology Research Program, Haartman Institute, University of Helsinki, Helsinki, Finland; ³HUSLAB, Helsinki University Central Hospital, Helsinki, Finland; ⁴Department of Clinical Medicine, University of Tampere and Department of Eye, Ear and Oral Diseases, Tampere University Hospital, Tampere, Finland

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Risto Renkonen
Transplantation Laboratory
Haartman Institute
University of Helsinki
PO Box 21
Haartmaninkatu 3
FI 00014
Finland

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The prevalence of allergic diseases has grown rapidly in Western countries (1, 2). This causes significant morbidity and indirect losses of productivity in the society. The proportion of children with allergic symptoms is even higher today, and is the most common cause of chronic diseases among children. It is hypothesized that living in urban built environments deprives people from environmental microorganisms and other factors, which prevents natural tolerance and results in hypersensitivity (3, 4). Yet, the origin of allergy is unresolved.

Seasonal acute allergic reaction, i.e. type I hypersensitivity is caused by allergens cross-linking with specific anti-IgE molecules bound on mast cells in immunologically sensitized patients (5–7). A large body of studies on pollen allergies has focused on aberrant immunoresponses, especially on T cell responses (5, 8, 9). The role of epithelium as the first line of defence against allergens has recently been emphasized (10–13). However, the role of epithelial defence mechanisms against allergens is far from clear. We have focused on the systems level

analysis of allergic reactions in human subjects, and have already shown the *in vivo* leucocyte inflammatory response after Bet v 1 pollen allergen challenge in human patients (14, 15). Recently, we put out a hypothesis of a caveolar-dependent uptake and transport of birch pollen allergen in the conjunctival and nasal epithelium of allergic patients, but not in their healthy controls (10, 11).

The aim of this study was to analyse the role of respiratory epithelium during the natural antigen uptake in a time series manner. To minimize *in vitro* artifacts of cultured cells, we collected a time series of nasal epithelial cell swabs from allergic and healthy subjects during the nonsymptomatic winter season, and during the symptomatic spring season with progressively increased exposure of birch pollen. The second aim was to analyse a human disease at systems level. Thus, the progressively developed allergic response during natural pollen exposure works as a model for a disease with inducible and progressive symptoms.

Materials and methods

Subjects

This study was performed according to the Declaration of Helsinki, and the study protocol was reviewed and accepted by the Committee on Ethics of both Helsinki University Eye and Ear Hospital and Tampere University Hospital. Informed consent was obtained from all subjects.

The diagnosis of birch allergic rhinitis was based on a positive history of intermittent allergic rhinitis, a clinical examination and a skin prick test. The selection criteria for patients included in this study were the following: having a positive history of moderate seasonal birch pollen allergic rhinitis without asthma or other allergic symptoms, showing a positive response to birch pollen allergen in a routine skin prick test, showing the symptoms of early-phase allergic response after *in vivo* nasal challenge with birch pollen extract and suffering from no other allergies nor other diseases. All persons included in this study were nonsmokers. No subjects had respiratory or other infections 4 weeks before and during the course of sample collection. The healthy nonsmoking control subjects were negative for all tested antigens and had no history of seasonal allergies. Neither group used any medication during the analyses. Transcriptomics was performed with four allergic subjects (4 females, mean age: 24 years, range: 21–27 years) and four healthy controls (3 female and 1 male, mean age: 23.8 years, range: 22–25 years).

Nasal epithelial specimens

The cell swabs were taken without any anaesthesia from the middle meatus of nasal cavity after blowing the nose. The cell swabs were put directly into RNAlate (Ambion Inc, <http://www.ambion.com/>)

to prevent mRNA degradation. The nasal cell swabs were immediately used for analysis (see later). Morphological data indicate that 97% of the cells in a cytocentrifuge preparation were epithelial cells and the rest mostly leucocytes (data not shown).

Nasal epithelial cell swab specimens both from birch pollen allergic patients and healthy control subjects were collected first during nonsymptomatic winter season and then four more samples were obtained from the same subjects at weekly intervals starting at the onset of the spring allergy season in 2007. The collection of time series specimens was launched at the beginning of the birch pollen season when the patients, but not the healthy subjects, began to generate clinical symptoms in Southern Finland, i.e. from late April to mid May. Before performing the specimens collection, all subjects filled weekly a visual analogue scale questionnaire for symptoms: nasal obstruction, runny nose, sneezing, itchy nose/eyes, loss of smell, post nasal discharge, facial pain/pressure). A pooled sum (graded semi quantitatively as –, +, ++, +++) was used to characterize the clinical symptoms at each time point.

Transcriptomics and pathway analysis

Nasal epithelial cells from four healthy and four allergic subjects were collected during winter and periodically during spring. For RNA isolations Rneasy Mini Kit (Qiagen, <http://www1.qiagen.com/>) was used according to the instructions of the Manufacturer. RNA integrity and concentration were measured using RNA 6000 Nano kit for Agilent Bioanalyser (Agilent, <http://www.agilent.com/>). The processed RNA samples were hybridized on Illumina human WG-6 v2 chips and conducted in Finnish DNA Microarray Centre (Turku Centre for Biotechnology, Turku, Finland).

The *in silico* analysis of the time series transcriptomics was carried out in a Medice workflow environment (<http://www.medice.com>, Fig. 1). Quantile normalization was used for preprocessing of the all

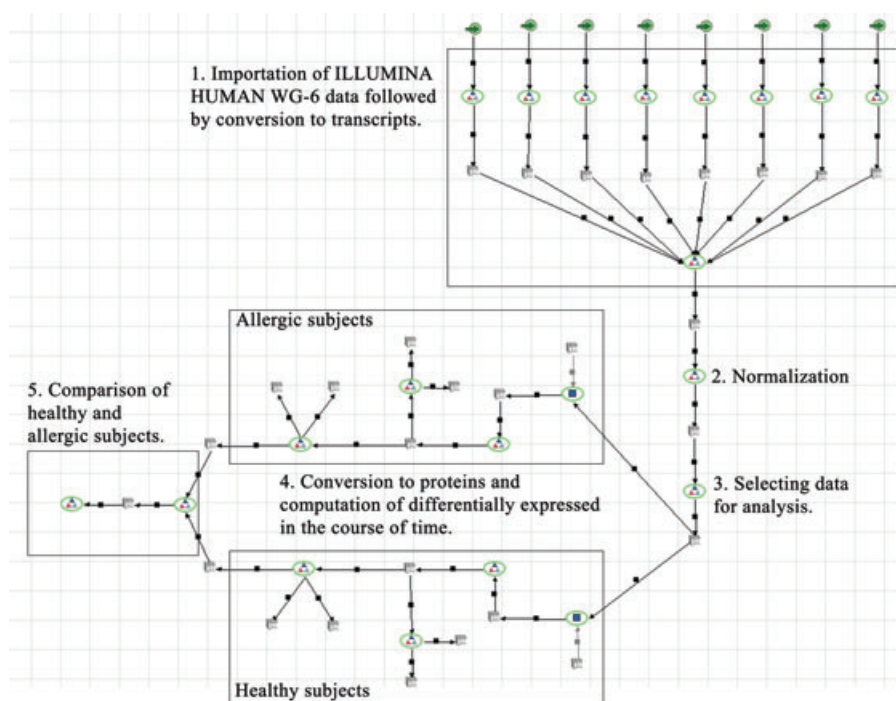


Figure 1. The *in silico* workflow analysis of the transcriptome data. All the analyses were carried out in a graphical workflow (Medice Ltd, Helsinki, Finland) which allowed maintaining all the metadata with the *in silico* analysis results.

genes on Illumina BeadArrays. This is the most commonly used approach for normalization of signal intensities detected on microarrays. The assumption behind this method is that most of the genes are equally expressed in the compared samples the proportion of the differentially expressed genes being low. Quantile normalization enforces the chips to have identical intensity distribution and performs well in this kind of analyses (16, 17). Then median values for each individual probe converting to a unique protein were calculated from each time point. The subset of the most differentially expressed proteins based on the deviation over the time course was chosen (deviation > 0.7). Heat maps in Fig. 2 depict graphically the median values of the significantly regulated proteins over the time series found in pooled specimens from healthy and allergic subjects, respectively. For the numerical values for all the transcripts/proteins see Table S1 in the Online Repository.

Instead of using probe annotations of the Manufacturer, the probe sequences were blasted (Blastall, NCBI) against the whole integrated transcriptome of Ensembl. For normalization the measurement values were converted to logarithmic scale before using quantile normalization method. The values corresponding to probes known to represent only one transcript were selected for analysis and the row labels were converted from transcripts to UNIPROT proteins as well as to Medcel reference proteins, the nonredundant objects in the Reference Pathway Model (<http://www.medcel.com>, Fig. S1 in the Online Repository).

Proteins with the highest discrimination power were selected based on the ratio of between-group to within-group sums of squares (BSS/WSS) for the two groups (18). We chose the subset of the most differentially expressed proteins based on *P* values. On the other hand, median value for each protein of the samples repre-

sented each timepoint was computed both for the allergic and for the healthy group followed by computation of distribution statistics over the five time points. The hierarchical clustering with average linkage and correlation coefficient as metric is used for presenting the results as a heat map. The overlapping proteins among the most differentiating groups of allergic and healthy patients were visualized with a Venn chart.

The nonredundant objects in the Reference Pathway Model depict data for genes, their transcripts and corresponding proteins integrated from various biological databases to a data warehouse, i.e. the Integrator (Medicel Ltd). The data analysis was performed using the Medicel Integrator (<http://www.medicel.com>). For microarray data analysis the following integrated databases (version number in parentheses) were used for probe annotations and conversions from transcripts to proteins: Illumina human WG-6 v2, NCBI/RefSeq/RNA (24/5/2007), Ensembl (45-a), SwissProt (53.2-a) and Medicel Reference Model. In data analysis and in finding protein-protein interactions for the differentially expressed genes the following databases were used: GeneOntology (30/7/2007), KEGG (1/12/2006 to 15/12/2006), Intact (27/7/2007), PID (5/3/2008) and Reactome (5/3/2008). The microarray data were submitted to ArrayExpress, accession (numbers not available yet).

Results

The study set-up is summarized in Fig. 3. We collected nasal epithelial cell swab specimens from both birch pollen allergic patients and healthy control subjects

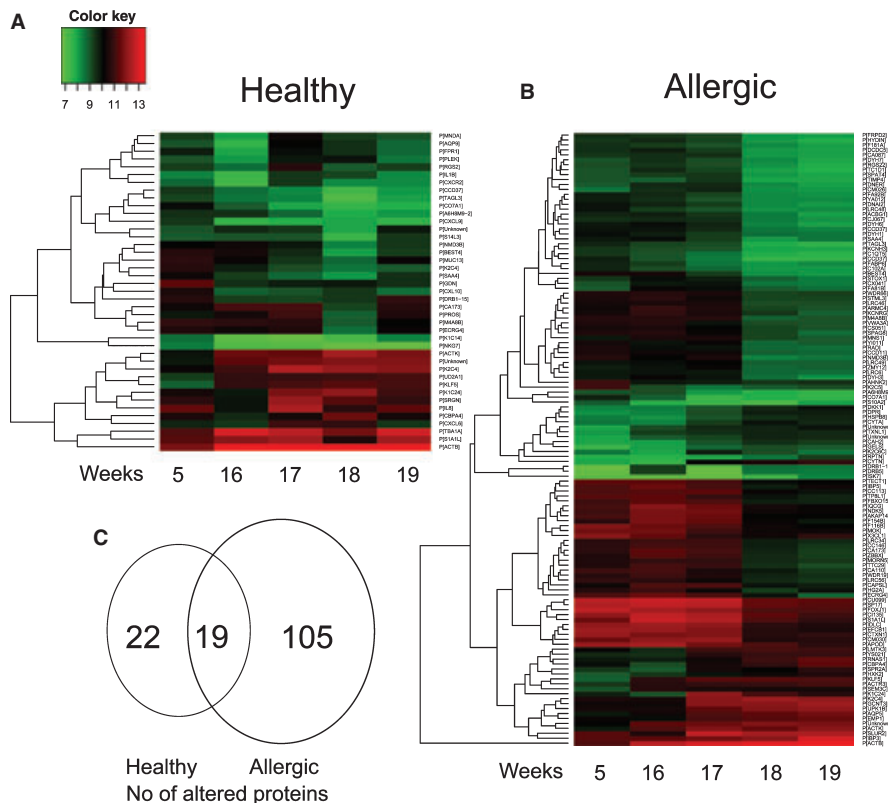


Figure 2. The most regulated proteins are displayed in the heat map and also clustered hierarchically. Panel (A) shows the median values of the most regulated proteins at each time point in the healthy and panel (B) in the allergic subjects. Panel (C) displays a Venn diagram of the altered proteins and shows that 19 of them are common.

	Winter	Spring			
Week	5	16	17	18	19
Allergen in air	-	+	++	+++	+++
Symptoms:					
Healthy subjects	-	-	-	-	-
Allergic subjects	-	+	++	+++	+++

Figure 3. The schema of the experiment. Five nasal epithelial cell swabs were taken from each subject in a time series manner. One specimen during winter, when no allergens were in the air, and four other specimens at weekly intervals during the spring pollen allergen season. The degree of symptoms increased in the allergic patients while the healthy controls remained symptom-free throughout the experiment.

during nonsymptomatic winter season, and during symptomatic spring season at weekly intervals starting at the onset of birch pollen season. At the beginning of the birch pollen season in southern Finland, i.e. in the late April, the patients but not the healthy subjects began to have clinical symptoms. While the healthy subjects remained asymptomatic throughout the study, the allergic subjects gradually generated more severe symptoms during the course of the sample collection (Fig. 3).

The healthy subjects displayed 41 significantly altered transcripts during the pollen season, which were for further analyses converted to proteins as Gene Ontologies use protein nomenclatures (Fig. 2A). Of these 19 were also regulated in the allergic patients and thus 22 were found only in healthy subjects. A significantly high proportion of the responding proteins were found uniquely in the time series specimens from healthy subjects. Of the 22 proteins 9 (40.9%) belonged to the Gene Ontology category Immune response (GO: 0006955). The protein members of the immune response modified only in healthy subjects were: CXCL10, CXCL6, CXCL9, CXCR2, interleukin (IL)1 and IL8, natural killer cell protein 7 (NKG7), Serglycin (PGSG) and fMet-Leu-Phe receptor (FPR1). The category Immune response has altogether 550 genes of the 12420 genes (i.e. 4.4%) annotated in the Amigo browser (<http://amigo.geneontology.org/cgi-bin/amigo/go.cgi>). Thus the pollen allergen induced a strong epithelial immune response in the clinically nonreactive healthy subjects and this response was not detected in the allergic subjects.

Among the allergic subjects, 124 regulated proteins were observed in epithelial specimens during the progression of clinical symptoms (Fig. 2B). One-hundred and five of these proteins were uniquely modified. The detailed analysis indicated that dyneins are among the most enriched gene ontology categories. The whole human proteome has less than 20 dynein proteins

(0.1%) and we observed six of them among the 105 modified epithelial proteins (5.7%) in the specimens obtained from allergic subjects. Axonemal dyneins, heavy chains 1, 3, 6, 7 (DYH1, DYH 3, DYH6, DYH7), intermediate 2 DNAI2 and light intermediate polypeptide 1 were mostly down regulated during the allergy season (Fig. 2B and Table S1 in the Online Repository). Dyneins are cellular molecular motors responsible for the epithelial ciliary movements as well as intracellular cargo traffic along the microtubuli. Also the expression of LRC49 (Leucine-rich repeat-containing protein 49), a regulator of dynein interactions (19), was shown to be regulated in allergic subjects.

To understand this data more we employed the Medcel Integrator data warehouse and the reference pathway model. Here, the integrated data concerning genes and transcripts were integrated from Ensembl and NCBI/RefSeq and protein data from UniProt, Ensembl and NCBI/RefSeq. In addition to the previously mentioned databases, the Medcel Reference Pathway takes advantage of the interactions of Intact, PID, Reactome and KEGG databases. All data is under a common information model, which allowed us to build cellular networks from genes–transcripts–proteins, from protein–protein interactions and from enzymatic reactions as a data (<http://www.medicel.com>).

Figure 4A shows first the whole overlay of the 124 proteins found to be regulated during allergen exposure in allergic patients. Sixty-nine proteins were not connected to anything other than their own transcripts and genes and are displayed at the bottom of the figure. On the contrary, 53 proteins showed interactions also with other objects within the data warehouse, when only one hop from the original protein was allowed in the network. There are a few major hubs, i.e. proteins that have a large set of known interacting proteins, such as beta actin (ACTB), heat shock protein beta-8 (HSPB8) and serine protease inhibitor kazal-type 7 (ISK7). These proteins are all upregulated in allergic patients, ACTB expression was very strong, while HSPB8 and esophagus cancer-related gene 2 protein (ECG2) increased only to medium levels by the end on the allergy season.

Smaller pathways (Fig. 4B and C) can also provide further perspective to the allergic reactions. As an example, we focused on one such pathway depicted by the Medcel Integrator. This small pathway/network of interacting proteins participates in saliva and tear production and is located within lipid rafts (Fig. 4C). Lipid raft-associated aquaporin 5 (AQP5) is upregulated during the allergy season in allergic patients and it is linked to a hub protein MyoD family inhibitor (MDFI). This, in turn, regulates essential *c-Jun* N-terminal kinase and wingless and Int i.e. WNT signalling pathways, and among others interacts with Nectin2, a herpes virus receptor as well as with Dickkopf-1 (DKK1). Dickkopf-1 was also upregulated in allergic patients during birch pollen season and it participates with its binding partners

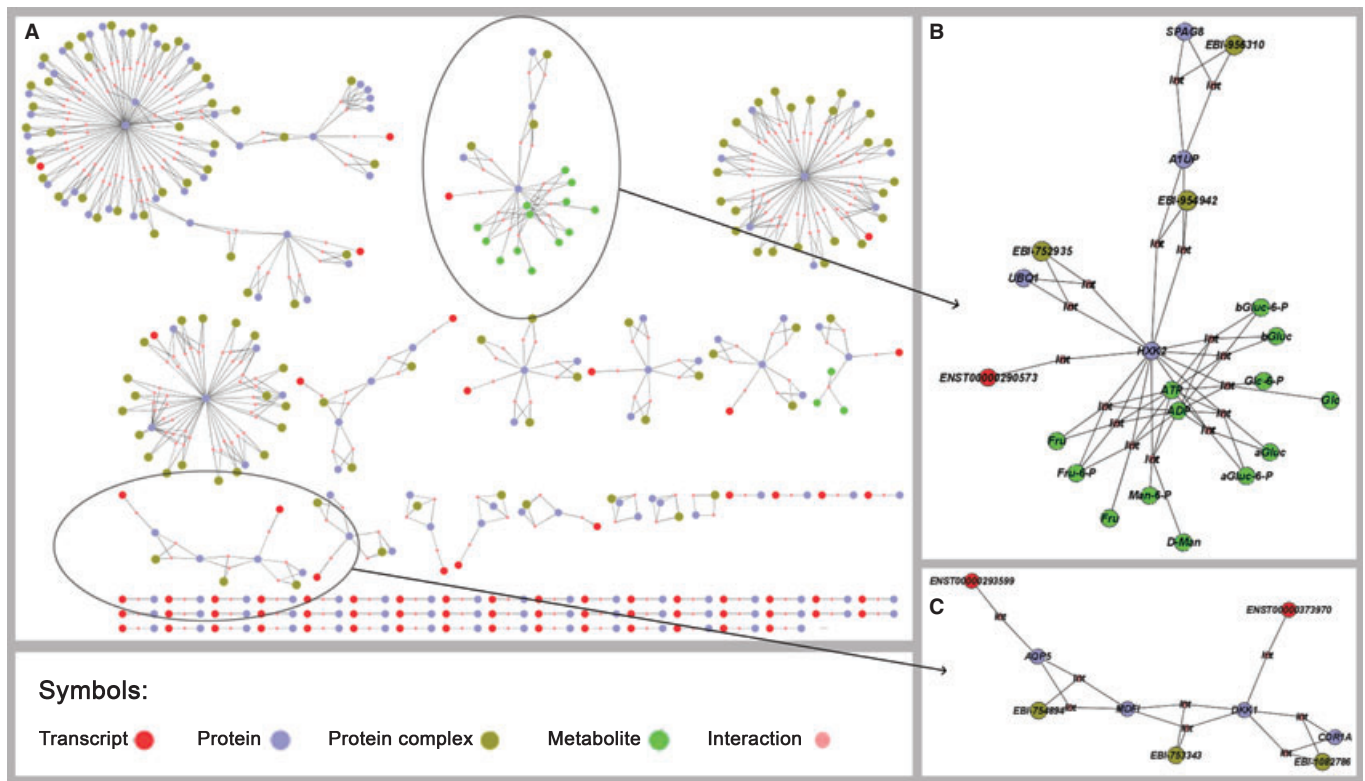


Figure 4. The network of proteins found to be regulated within allergic epithelium during the spring season. The red dots represent transcripts, which were actually measured, their corresponding proteins are shown with blue dots, protein–protein complexes are yellow-green, metabolites green and interactions between objects pink dots. (Panel A) A network of 124 proteins from the allergic patients (see Fig. 2B and Table S1 in the Online Repository). Over 50% of the proteins are not connected to other proteins; a few proteins are major hubs with very many binding partners. A number of smaller pieces of this network are composed of only a few proteins. A few smaller networks are shown in higher magnification, panel B shows a pathway starting with hexokinase (HKK2) and its corresponding transcript, and interactions with a number of glyco-metabolites. Another sub pathway showing the connection between aquaporin 5 (AQP5) and Dickkopf-1 (DKK1) is shown in panel C.

low-density lipoprotein receptor-related protein 6 (LRP6), Kremen1 and Coronin-1A (Cor1a) in inducing and regulating endocytosis. Cor1a is an actin-binding cytoskeleton protein functioning in the invagination of large pieces of plasma membrane. Thus, we can depict novel networks putatively having a role in the development of allergic reactions in the epithelium by integrating the data from our transcriptomics analysis to the data integrated from the external databases.

Discussion

For reasons of acute nature and accessibility of specimens from patients generating symptoms ‘online’ type 1 hypersensitivity is an ideal target to analyse a relevant human inflammatory disease with a systems biology approach. Here, we first designed a study setup where we could collect nasal epithelial specimens and perform a gene expression profiling from the samples in a time series manner before, at the onset of, and during the pollen

allergy season with concomitant acceleration of symptoms within the patients.

The first major observation made during this study was that not only previously sensitized, i.e. allergic, but also healthy subjects clearly respond to birch pollen exposure. Although the healthy subjects do not generate clinical symptoms, their epithelial cells strongly respond to allergen exposure. Most importantly, this response was characterized by a strong induction of transcripts coding proteins that participate in immune responses. Uniquely in the specimens from healthy subjects, a significantly high proportion, i.e. 9 of the 22, of responding transcripts and their corresponding proteins belonged to Gene Ontology category immune responses. Of crucial importance is the observation that similar defensive immune responses lacked from the epithelium of allergic patients. Our previous and the present studies show that concomitantly with the inability of generating immune responses after the exposure, the epithelium of allergic subjects failed to prevent Bet v 1 pollen allergen invasion (10, 11, 13). Thus our results suggest that at least partially the

development of allergy and its symptoms could be because of failures in certain effector mechanisms of immune responses preventing the allergen uptake by epithelium. This is clearly a novel finding adding to the current concept that allergic diseases are only caused by overactive immunological responses. It must be noted that while or epithelial cell specimens are >97 pure, even a small contaminant could alter these results, while we did not observe any differences of the contamination between these groups. Also the nonresponsiveness within the allergic patients with relation to the genes/proteins annotated to immunology was not because of upregulated expression already in the nonsymptomatic winter period.

The ability of the healthy subjects to respond so vigorously to the pollen challenge is of further interest. Thus, we compared these observations with the findings of the susceptibility analysis of various allergic diseases. A great majority of the structural genetic variations (single nucleotide polymorphisms, SNP) shown to link susceptibility to allergic diseases have been found in genes coding for proteins involved in immune responses (20, 21). Thus, it could be that the SNPs associated with the susceptibility of allergic diseases could result either from the lack of this first line of immune response against allergens making contact with the epithelium and/or because of the aberrantly regulated immunological responses leading to Th2- and IgE-dominated allergic responses.

Several of the epithelial genes regulated uniquely in allergic subjects have previously been shown to carry SNPs putatively relevant in various immunological diseases such as allergy, atopy, asthma or other autoimmune disorders. Foxj1 transcription factor was strongly upregulated during spring in allergic patients. It has been shown to have three dominant SNPs significantly associated with allergic rhinitis (22), systemic lupus erythematosus and rheumatoid arthritis (23). Foxj1 is also predominantly expressed in embryonic stem cells and early embryos (24). There are also three other genes showing regulated expression only in allergic subjects that have been associated to autoimmune diseases. Secreted mammalian Ly-6/urokinase plasminogen activator receptor-related protein-2 and Cystatin A have been proposed to have structural variation, which is associated with psoriasis, an inflammatory disease of the skin (25). Cystatin A is an inhibitor against the proteolytic activity of the major mite allergen Der p 1, and it blocks the upregulation of IL8 release from human keratinocytes stimulated with this allergen (26). Secreted mammalian Ly-6/urokinase plasminogen activator receptor-related protein-2 binds to alpha3 nicotinic acetylcholine receptors and prevents apoptosis (27). In our study small proline-rich protein 2A (SPR2A) was also regulated only in our allergic patients during spring pollen season. Small proline-rich protein 2A has also been found among allergen-induced genes in a mouse model of experimental asthma (28) and a SNP variation within this gene has been associated with Sjogren's syndrome patients. The

overexpression of SPR2A showed that it functions as on SH3 domain ligand that increases resistance to oxidative injury and promotes wound healing and response to stress (29).

The second other major observation of this study was the marked response seen in allergic subjects along the development of clinical symptoms. While the human proteome has some 20 dynein proteins (<0.1%), we observed six of them within the 105 modified epithelial proteins (5.7%) in the specimens obtained from allergic subjects. A markedly decreased expression was seen in most of the dyneins together with the progression of the disease symptoms. Dyneins are microtubule-binding proteins known to execute cargo transport within the cells (30). Our previous work showed that the pollen allergen is taken up by the epithelial cells and tissue by a caveolar mechanism. This protein transport through the epithelium to the mast cells, situated usually under the basal lamina, requires controlled intracellular machinery. Caveolae are transported within the cells via the cytoskeleton.

Microtubules serve as structural components of the cytoskeleton within cells and also part of the cilia and flagella (31). They are involved in several cellular processes including mitosis, cytokinesis and caveolar transport. Dyneins are motor proteins, which convert the chemical energy contained in adenosine triphosphate into the mechanical energy of movement. Dyneins transport cellular cargo such as caveolae along the cytoskeleton by sliding one of its arms along the microtubule (32, 33). While the arms are used for 'walking' the dyneins carry their cargo bound to the light chain (30). Among other cargos, dyneins can carry human immunodeficiency (HI) virus to the nuclei of infected cells (34). Thus, we can put out a hypothesis that dyneins could be consumed during the allergen transport in allergic subjects as this was not seen within the healthy subjects.

Instead of concentrating our analysis on just one or a few altered genes or their corresponding proteins, we have tried to generate larger protein-protein interaction networks from our data. No single database alone can provide such a connected network. To understand the new systems levels of diseases we decided to build the integrated data warehouse, by which the information was integrated from a number of databases. Thus, we can show that of the 124 proteins that were regulated in allergic patients during the pollen exposure, more than half were only connected to their own transcripts and genes based on the prior legacy knowledge. Of the 53 proteins that were connected to other proteins, a few were major hubs, i.e. proteins that have a large set of known interacting proteins. Proteins known to have many interacting proteins are ACTB, heat shock protein, HSPB8, and serine protease, ISK7. Among its other tasks, ACTB is involved together with dyneins in the organization of nuclear interchromosomal interactions (35). HSPB8 is a temperature-dependent chaperone. Activity defects in HSPB8 cause an autosomal dominant

disorder of lower motor neurons characterized by distal muscle weakness (distal spinal muscular atrophies and Charcot-Marie-Tooth disease) (36, 37).

There are several other smaller networks of interactions generated from the input of 124 proteins regulated within allergic subjects. Hexokinase (HXK2) phosphorylates several hexoses, like glucose, mannose and fructose. It also has a direct binding interaction with ubiquitin-4 (i.e. A1UP), which binds the signal sequences of proteins, which are targeted to the endoplasmic reticulum for degradation. A1UP interacts with sperm-associated antigen 8, which in turn participates in the microtubule formation. In addition, ubiquilins form aggresomes, which are mediated by the dynein and microtubule-based transport of misfolded proteins to the centrosome and involve several regulators, including ubiquitin (38). Aggresome formation is recognized as a cytoprotective response serving to sequester potentially toxic misfolded proteins and facilitate their clearance by autophagy (39).

Hence, not only the entry of the pollen allergen Bet v 1 resembles virus entry into cells but also the later phases of virus infection and allergen fate in the epithelial cells resemble each other. Thus also the ubiquitin pathway is activated in viral infections (40).

Smaller pathways can also provide further perspectives to allergic reactions. As an example, we depicted a smaller pathway linked around two proteins (AQP5) and DKK1 shown to display different expression in the allergic subjects. Aquaporin 5 is localized in lipid rafts and it was found to be increased during the allergy season in allergic patients, while it was relatively highly expressed throughout the season in healthy controls (41). Aquaporin 5 is a multipass membrane protein, which forms a water-specific channel and is involved in the generation of saliva, tears and pulmonary secretions. The ectopic or overexpression of AQP5 has been reported to be frequently associated with various cancers, such as chronic myeloid leukaemia, nonsmall cell lung cancer and colon cancer (42–44).

Aquaporin 5 is linked to a hub protein called MDFI. MyoD family inhibitor is a hub protein binding to 30 other proteins. One of them is poliovirus receptor-related protein 2 (PVRL2) (Nectin 2), which is a Herpes virus entry receptor. MyoD family inhibitor also interacts with DKK1, which, on the other hand, participates with its binding partners LRP6, Kremen2 and Cor1A in inducing and regulating endocytosis (45, 46). Cor1A (Coronin) binds DKK1 and is an actin-binding cytoskeleton protein functioning in the invagination of large pieces such as mycobacteria. Dickkopf-1 also binds the ubiquitin ligases

G2, UB2G2, which links this pathway to ubiquitinylation. This is also seen in another small pathway where HXK2, strongly upregulated in allergic patients during pollen season, also directly linked to the Ubiquitin pathway. Finally, Cor 1A also interacts with the Arp2/3 complex and actin and thus regulates traffic via actin cytoskeleton (47). We can now depict novel functionally active networks putatively having a role in the transport of the pollen allergen through the epithelium, which participate in the development of allergic reactions.

Taken together, two novel observations were made in this work. First, the epithelium of healthy subjects showed a strong immune response to the birch pollen challenge, a phenomenon not observed in allergic subjects. Second, the set of regulated transcripts found to be altered during the development of allergic symptoms in allergic subjects was found to contain a significant amount of dyneins, important in the caveolar transport of the pollen antigen through the epithelium. Furthermore, several previously found genes, displaying structural variations associated with susceptibility to allergic diseases were observed to display also modified expression levels.

Acknowledgments

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Supporting Information

Additional Supporting information may be found online of this article:

Figure S1. Medcel reference proteins, the nonredundant objects in the Reference Pathway Model, <http://www.medcel.com>.

Table S1. The numerical values for all the altered transcripts/proteins in the time series analyses.

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Online Repository

Time-series nasal epithelial transcriptomics during
natural pollen exposure in healthy subjects and allergic
patients

Pirkko Mattila PhD ^a, Jutta Renkonen DDS ^{b,c}, Sanna Toppila-Salmi MD ^d, Ville
Parviainen BS ^b, Sakari Joenväärä BS ^{a,b}, Susanne Alff-Tuomala MTech ^a, Daniel
Nicorici PhD ^a, Risto Renkonen MD ^{b, c}

^a MediceL Ltd, ^b Transplantation Laboratory & Infection Biology Research Program,
Haartman Institute, University of Helsinki, and ^c HUSLAB, Helsinki University
Central Hospital, Helsinki, Finland; ^d Department of Clinical Medicine, University of
Tampere and Department of Eye, Ear and Oral diseases, Tampere University
Hospital, Tampere, Finland

Correspondence to
Risto Renkonen
Transplantation Laboratory
Haartman Institute
P.O.BOX 21 (Haartmaninkatu 3)
FI- 00014 University of Helsinki, Finland.
tel +358-9-1912 5111
fax +358-9-2411227
E-mail risto.renkonen@helsinki.fi

Online Repository

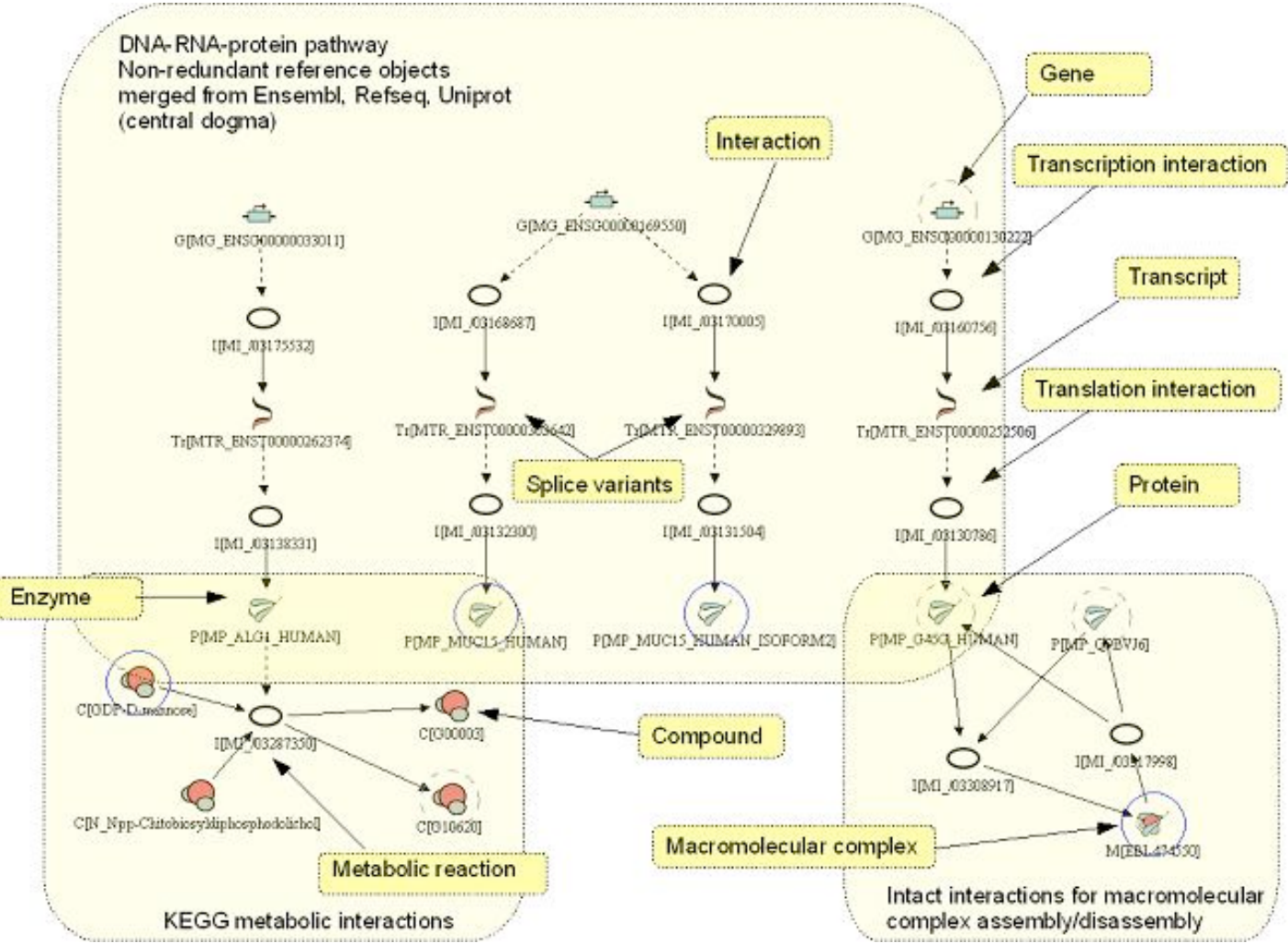
Figure 1.

Medicel non-redundant reference pathway, where genes, transcripts, proteins, metabolites and various macromolecular complexes are integrated from various individual databases to a common data warehouse under a common information model. This allows a novel approach to analyse the results obtained in high throughput analyses and begin to perform systems level interpretations.

Table 1.

Mean logarithmic values for all modified transcripts during the time series analysis, displayed in Fig 3.

Medicel non-redundant reference model



Taulukko1

Mean transcript values [log]
Data in Fig 3

Allergic patients

Corresponding protein

-	week 5	week 16	week 17	week 18	week 19
ACBG1	9,68	9,80	9,34	8,40	7,91
ACTB	11,40	12,85	13,29	13,48	13,90
ACTR3	9,40	10,79	11,01	11,11	11,24
AKAP14	11,29	11,99	11,76	10,15	10,12
APOD	12,36	12,51	11,87	11,35	10,82
AQP5	9,89	10,41	11,37	11,53	11,84
ARMC4	10,35	11,05	10,68	9,20	8,94
BEST4	10,46	10,43	9,29	8,03	8,02
CJ067	9,47	9,61	9,50	8,36	8,07
CM026	8,50	9,48	9,67	8,09	8,02
CM030	11,79	12,25	12,36	10,69	10,78
F181A	9,50	9,30	8,95	8,15	7,84
AHNK2	11,13	9,92	9,75	9,38	9,47
CS051	10,45	10,54	10,18	9,13	8,98
CA110	11,16	11,22	10,60	9,83	9,38
CA173	10,83	11,52	11,24	9,69	9,29
CA087	9,41	9,52	9,49	7,93	7,90
C1QT5	9,42	8,93	8,50	7,66	7,53
CU099	12,91	13,06	13,00	11,67	11,59
ECRG4	11,40	11,75	11,34	9,95	8,71
CI135	12,25	12,99	12,64	11,55	11,12
MORN5	10,66	10,98	11,01	9,58	9,34
CAH2	7,73	7,92	8,95	9,42	9,29
CAPSL	10,35	11,36	11,32	10,00	9,75
C102A	9,67	9,04	8,72	7,94	8,00
CCD11	10,53	10,35	10,34	8,76	8,63
CC113	11,41	11,42	11,59	10,24	9,94
CCD37	9,78	9,00	8,37	7,42	7,34
HG2A	10,80	11,16	10,41	9,24	9,94
CO7A1	9,72	8,78	7,30	7,54	7,34
CBPA4	10,03	9,08	10,50	10,93	11,53
CYTN	7,79	7,40	10,20	10,53	10,93
CYTA	8,51	8,27	9,26	9,97	9,61
CTXN1	12,40	12,00	12,04	10,82	10,97
X3CL1	12,13	11,67	10,86	10,33	10,93
CX041	9,02	9,90	9,97	8,43	8,36
DCDC5	9,51	9,79	9,23	7,94	7,89
F154B	10,89	11,92	11,65	10,27	9,89
DKK1	8,31	8,40	8,99	9,55	9,96
DYH1	10,12	9,76	9,26	8,56	8,47
DYH3	9,83	10,28	9,97	8,60	8,13
DYH7	8,96	9,52	9,08	8,19	7,79
DNAI2	9,93	9,66	9,24	8,17	7,96
IDLC	12,29	12,41	12,25	10,87	10,71
DNER	8,89	9,62	9,57	8,20	7,92
EFCB1	12,41	12,31	12,08	10,77	10,61

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2			Taulukko1			
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4	EMP1	10,29	10,11	11,31	11,49	11,71
5	FABP6	9,66	9,22	8,47	8,01	8,19
6	F116B	12,11	11,79	11,38	10,39	10,20
7	FA81B	8,84	10,23	10,08	8,90	8,53
8	FA92B	9,79	9,53	9,17	8,17	8,08
9	FBXO15	11,15	11,52	11,40	9,73	9,68
10	ACTK	9,90	11,40	12,05	11,64	12,18
11	CCD37	9,98	9,97	9,56	8,52	8,26
12	ZBBX	10,71	11,47	11,14	9,56	9,30
13	S1A1L	11,76	13,10	12,80	11,34	10,99
14	YI011	10,45	9,96	10,26	8,67	8,90
15	FOXJ1	12,91	12,59	12,30	11,34	11,35
16	FRPD2	9,42	9,36	9,21	7,98	7,90
17	GCNT3	10,41	10,41	12,11	12,17	12,46
18	NMD3B	10,73	10,19	10,55	8,28	8,46
19	GELS	7,27	7,92	8,65	9,09	8,87
20	HXK2	8,92	9,37	9,98	10,42	10,68
21	DRB1-15	6,50	9,29	6,62	8,53	8,49
22	DRB5	6,48	9,25	6,43	8,45	8,36
23	DYH6	9,83	10,04	9,62	8,25	7,90
24	HSPB8	8,33	8,04	8,96	9,48	9,70
25	HYDIN	9,37	9,55	9,13	8,02	7,68
26	IBP3	11,44	11,57	11,85	12,11	13,23
27	IBP5	11,38	11,77	11,28	9,76	10,33
28	IQCG	11,22	12,21	11,74	10,48	10,30
29	KCNH3	9,39	9,10	8,96	7,76	7,73
30	KCNRG	10,48	10,96	10,57	9,35	8,81
31	CC146	10,83	11,28	11,13	9,76	9,34
32	KLF5	8,78	10,88	11,50	11,51	11,32
33	K1C24	9,74	8,93	12,30	11,29	10,78
34	K2C4	10,58	10,49	12,18	12,42	12,26
35	K2C5	11,12	9,07	8,46	8,84	9,08
36	K2C6C	8,47	7,76	9,78	9,04	8,49
37	LMTK3	10,27	9,61	11,12	11,24	11,18
38	VWA3A	10,68	10,72	9,98	9,09	8,97
39	YS021	9,59	9,51	10,75	11,07	11,02
40	A6H8M9-2	9,02	9,06	8,00	7,54	7,42
41	Unknown	7,79	9,11	9,60	9,66	9,95
42	Unknown	10,15	11,58	11,74	12,07	12,00
43	Unknown	7,75	8,91	9,21	9,64	9,60
44	LRC34	10,86	11,18	11,14	9,78	9,53
45	LRC46	10,47	10,55	10,58	9,09	9,10
46	LRC48	9,98	9,28	8,97	8,05	8,16
47	LRC49	9,99	10,05	10,20	8,72	8,81
48	LRC56	10,97	10,89	10,36	9,60	9,38
49	LRC6	9,87	10,02	9,84	8,68	8,59
50	SLUR2	11,49	10,70	12,96	12,54	12,71
51	YA012	9,76	9,49	9,00	8,22	8,15
52	MNS1	9,83	10,55	10,56	9,22	8,87
53	M4A8B	10,72	10,80	10,74	8,73	8,97
54	NDK5	11,16	11,98	11,82	10,39	10,28
55	MOK	11,82	11,85	11,16	10,41	10,36
56	RGS22	9,04	9,35	9,10	8,12	7,70
57	RNAS1	9,86	9,75	10,36	11,20	11,65
58	RPTN	7,53	7,45	7,74	8,44	9,23
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Taulukko1

RAD	10,53	10,04	9,93	8,70	8,58
S10A2	8,85	7,46	7,33	7,03	7,90
SAA4	9,90	10,10	9,23	8,02	8,45
DPR	8,07	8,29	9,11	9,72	9,81
SEM3C	8,98	10,74	10,58	10,51	10,50
SP17	12,80	12,96	12,89	11,62	11,50
SPAG8	10,65	10,79	10,22	9,14	8,57
SPAT4	8,92	9,30	9,36	7,93	7,81
ISK7	6,67	6,56	6,52	7,15	8,28
SPR2A	9,33	8,91	10,44	10,16	10,87
STML3	10,54	10,86	10,36	9,14	9,21
STOX1	9,38	10,05	9,82	8,77	8,34
TAGL3	9,71	9,15	9,03	7,68	7,38
TC1D1	8,98	9,56	9,17	7,92	7,56
TECT1	11,57	11,58	11,26	10,08	10,20
TIMP4	8,98	8,82	9,34	7,82	7,80
TP8L1	11,51	11,60	11,07	10,02	9,71
TTC29	10,67	11,30	11,07	9,43	9,05
TXNL1	7,96	9,30	9,51	9,70	9,62
UPK1B	10,43	10,42	11,60	12,02	12,31
WDR19	10,81	11,17	10,68	9,67	9,49
WDR66	10,65	10,78	10,54	9,20	9,24
ZMY12	9,78	9,96	10,26	8,67	8,80

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Taulukko1

Healthy subjects

ACTB	11,79	13,38	13,39	13,5125	13,55
AQP9	9,04	7,60	10,11	9,51	8,71
BEST4	10,67	10,42	9,57	8,00	9,27
CA173	10,30	11,09	11,11	9,20	10,64
Unknown	9,04	9,58	9,42	7,89	9,88
ECRG4	10,58	10,30	10,59	8,63	10,24
CCD37	9,41	8,70	8,33	7,42	8,11
CO7A1	10,01	8,96	7,92	7,56	7,74
CBPA4	10,53	9,55	10,36	11,55	10,38
CXL10	10,54	8,83	9,07	9,40	8,71
CXCL6	9,66	9,37	11,07	10,85	9,38
CXCL9	9,39	7,53	7,78	7,96	7,57
ACTK	9,93	11,57	11,52	12,31	11,80
S1A1L	11,28	11,96	12,26	10,55	12,25
FPR1	9,79	7,90	10,00	9,51	8,79
NMD3B	10,09	10,30	9,83	8,41	9,39
DRB1-15	10,71	9,28	9,20	9,40	10,85
IL1B	8,35	7,46	9,37	9,19	8,32
IL8	11,47	9,90	12,37	10,78	11,45
CXCR2	8,77	7,43	9,36	8,27	8,30
KLF5	8,83	10,83	11,12	11,27	11,17
K1C14	8,70	6,97	6,71	7,28	8,43
K1C24	10,38	9,89	11,81	12,03	11,03
K2C4	10,25	11,07	12,47	12,16	11,99
K2C4	10,66	9,77	9,10	8,62	8,95
A6H8M9-2	9,53	9,53	8,74	7,87	8,13
Unknown	9,83	11,45	11,66	11,84	11,89
MNDA	9,27	8,02	10,34	9,31	9,00
M4A8B	10,88	10,73	10,64	8,97	10,13
MUC13	10,79	10,23	9,40	8,72	10,25
NKG7	8,48	7,12	7,03	6,77	6,78
PLEK	9,04	8,08	9,83	9,91	8,55
PROS	9,89	10,43	11,06	8,84	10,60
RGS2	9,31	8,64	10,64	8,96	9,28
SAA4	10,29	9,33	8,80	8,08	9,45
S14L3	8,95	9,02	8,99	7,51	9,40
GDN	11,43	9,47	9,80	9,85	9,44
SRGN	10,72	9,93	12,12	11,30	10,88
TAGL3	9,20	8,57	8,42	7,14	8,00
TBA1A	11,21	13,00	12,63	11,63	12,90
UD2A1	9,34	10,86	10,96	11,01	11,16