

Original article

Birch pollen allergen Bet v 1 binds to and is transported through conjunctival epithelium in allergic patients

Background: Previous work in type-I pollen allergies has mainly focused on lymphocytes and immune responses. Here, we begin to analyse with a systems biology view the differences in conjunctival epithelium obtained from healthy and allergic subjects.

Methods: Transcriptomics analysis combined with light and electron microscopic analysis of birch pollen allergen Bet v 1 located within conjunctival epithelial cells and tissues from birch allergic subjects and healthy controls was carried out.

Results: Bet v 1 pollen allergen bound to conjunctival epithelial cells within minutes after the exposure even during the nonsymptomatic winter season only in allergic, but not in healthy individuals. Light- and electron microscopy showed that Bet v 1 was transported through the epithelium within lipid rafts/caveolae and reached mast cells only in allergic patients, but not in healthy individuals. Transcriptomics yielded 22 putative receptors expressed at higher levels in allergic epithelium compared with healthy specimens. A literature search indicated that out of these receptors, eight (i.e. 37%) were associated with lipid rafts/caveolae, which suggested again that Bet v 1 transport is lipid raft/caveola-dependent.

Conclusions: We show a clear difference in the binding and uptake of Bet v 1 allergen by conjunctival epithelial cells in allergic vs healthy subjects and several putative lipid raft/caveolar receptors were identified, which could mediate or be co-transported with this entry. The application of discovery driven methodologies on human conjunctival epithelial cells and tissues can provide new hypotheses worth a further analysis to the molecular mechanisms of a complex multifactorial disease such as type-I birch pollen allergy.

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Allergic conjunctivitis is a type-I hypersensitivity reaction caused by antigens, such as pollen allergens, cross-linking specific anti-IgE molecules on the previously presensitized mast cells. Most of the previous work on the pathogenesis of the pollen allergy has focused on the mechanisms of the immune responses elicited by the allergen (1–5). We have begun to analyse the molecular mechanisms facilitating the pollen entry into the epithelium and thus allowing its interaction with tissue-bound mast cells Joenvaara S et al., unpublished data. We have previously introduced a noninvasive application of the confocal reflected light microscopy enabling the direct and repeatable quantitative analysis of the very early phases of conjunctival allergic inflammation in human patients (6, 7). The challenge with the birch pollen allergen Bet v 1 led within minutes to the leucocyte extravasation into the conjunctival sites of inflammation only in allergic, but not in healthy individuals (8).

The role of epithelial cells as the first line of defence has recently been emphasized (8, 5–11). The aim of this

system level analysis is to begin to dissect the role of conjunctival epithelial cells in the early pathogenesis of type-I allergic reactions. We focused on this disease as it is common, i.e. up to 15–20% of the population in Europe suffer from pollen allergies (4, 11–13). Allergic symptoms cause a lot of morbidity and thus are a major indirect reason for the losses of productivity in the society (14). Type-I allergy is an example of an acute disease where its causative agents such as the birch pollen allergen Bet v 1 have been identified (1, 4, 15). Conjunctival epithelial cells and tissues can be obtained from healthy controls and allergic patients during winter (a symptom-free period for both patients and controls) before and after the *in vivo* conjunctival pollen challenge. To minimize the *in vitro* artefacts, we collected, analysed and compared only freshly isolated conjunctival epithelial cell swabs and conjunctival tissue biopsies from healthy control subjects and birch pollen allergic individuals during the nonsymptomatic winter before and after the pollen challenge.

This study is a part of a large systems biology effort to begin to understand the early events in the type-I allergy. We selected this disease as a starting point of the system level approach as it is an acute disease. These are clearly less difficult, if not easy, to study compared with chronic diseases. Here, we can challenge the subjects with a known allergen such as the birch pollen during the nonsymptomatic winter season and induce the acute disease reactions within minutes, collect cell and tissue specimens in a time series manner both from allergic patients and healthy control subjects. In this larger series of our top-down analysis of a human type-I allergy, we collected not only conjunctival but also nasal epithelial cells and tissues Joenvaara S et al., unpublished data. These specimens were analysed before and after the birch pollen challenge and the following data domains were gathered; transcriptomics with various platforms (Affymetrix, Illumina and ABI), Bet v 1-associated proteins with various HPLC methods combined with MS/MS analysis and the light and electron microscopy analysis.

In this report, we show the results obtained from conjunctival epithelial cells. Comparing the conjunctival epithelial cells and tissues from healthy and allergic subjects, we show with the light and electron microscopic analysis that the Bet v 1 pollen allergen bound to epithelial cells within minutes even during the nonsymptomatic winter season only in allergic, but not in healthy individuals. It also was transported through the morphologically intact epithelium and reached mast cells located close to or under the basal lamina during the nonsymptomatic winter season only in allergic patients. Co-localization together with caveolin 2 suggested a traffic within lipid rafts/caveolae.

Thus, the application of discovery driven methodologies on human conjunctival epithelial cells and tissues can provide a new hypothesis worth a further analysis to the molecular mechanisms of a complex multifactorial disease such as the type-I birch pollen allergy.

Material and methods

Subjects

This study was performed in accordance with the Declaration of Helsinki, and the study protocol was reviewed and accepted by the Committee on Ethics of Helsinki University Eye and Ear Hospital. All subjects consisting of five allergic subjects (mean age 29.4 ± 4.7 years, range 12 years, two females, three males) and five healthy controls (mean age 33.4 ± 8.4 years, range 19 years, two females and three males) gave their informed consent. All the microscopic and transcriptomic analysis were carried out separately for each specimen.

The status of birch and other pollen allergies was tested in the routine hospital laboratory with skin prick tests. The tested allergens were; birch (*Betula verrucosa*), timothy grass (*Phleum pratense*), meadow fescue (*Festuca pratensis*), mugwort or common wormwood (*Artemisia vulgaris*), fungal allergen (*Cladosporium*

herbarum), cat, dog, horse, cow, house dust mite, latex and histamine as a positive and saline as a negative control. The birch pollen allergic patients selected for this study showed no other pollen allergies and the healthy control subjects were negative for all tested antigens. The selected patients also reacted to the *in vivo* conjunctival challenge with the birch pollen, while the healthy controls did not. The allergic patients also had a positive history of seasonal allergy in May; the time birch pollen is in the air in Finland. All allergic subjects selected for this study fulfilled these criteria; positive in the prick test only to the birch allergen, reacted to the conjunctival pollen challenge and had a positive history of seasonal allergy in May–June (birch pollen season in southern Finland). Concomitantly, the healthy controls did not react to any allergen in the prick test, did not react to conjunctival birch pollen challenge and had no history of any allergy.

Conjunctival epithelial specimens

Biopsies from conjunctival epithelium as well as conjunctival epithelial cell swabs from both five healthy subjects and five allergic patients, verified with skin prick tests against the birch pollen extract, were obtained.

Immunohistochemistry

Bet v 1 location within the epithelial tissue was studied with polyclonal anti Bet v 1 antibodies (first a gift from R Valenta, University of Vienna, Austria and later our own polyclonal rabbit anti-Bet v 1 antibody towards recombinant Bet v 1). Rabbit IgG and the depletion of the primary antibody were used as negative controls. Secondary fluorescent anti-rabbit antibody (Alexa 488; Molecular Probes, Eugene, OR, USA) was used to detect the signal and ConA lectin coupled with Alexa 633 (Molecular Probes) was used to detect epithelial tissue.

Immuno transmission electron microscopy

Immuno transmission electron microscopy (immunoTEM) was performed to analyse the presence of the Bet v 1 allergen within the conjunctival epithelial tissue as described previously (6, 17).

The quantification of the immunohistochemistry as well as immunoTEM specimens were carried out with dedicated *in silico* workflows (Medicel Ltd, Helsinki, Finland), which allowed not only the automated counting, but also the traceability of results with all their metadata and their easy combination to other data domains such as mass spectrometry and transcriptomics (www.medical.com).

Transcriptomics

Conjunctival epithelial cells from healthy and allergic subjects were collected during winter before and after the *in vivo* birch pollen challenge. A basic analysis was performed from five healthy and five allergic subjects during winter when no one had any clinical symptoms. The RNA isolation was carried out according to the Queen's Rnaeasy Mini Handbook, the concentration measurement with RNA 6000 Nano kit for Agilent Bioanalyser, Agilent Technologies, Santa Clara, CA 95051, USA and the specimens were hybridized with Affymetrix chip platform on U133A and conducted in Finnish DNA Microarray Centre (Turku Centre for Biotechnology).

During the microarray analysis, the following integrated databases were used for probe annotations and conversions between genes, transcripts and proteins: HG-U133-PLUS-2 (2006-11-15-a),

NCBI/RefSeq/RNA (2007/05/24), Ensembl (45-a), SwissProt (53.2-a), TrEMBL (36.2), GeneOntology (2007-07-30), KeGG (2006/12/15-1) and Intact (2007-07-27).

Data analysis was performed using the Integrator (Medicel Ltd, Helsinki, Finland). The data preprocessed with the GCRMA method were normalized using quantile normalization. Only those probes known to represent one transcript were selected for the analysis. Statistically differing expressions between different groups were calculated with a workflow using ANOVA. Enrichments–depletions of gene ontology (GO) categories were essentially computed as described Joenvaara S et al., unpublished data.

Results

Study setup

This is the first part of a study of a human disease at the system level. We identified and verified a group of birch allergic subjects ($n = 5$). No other allergen(s) caused reactions to these patients in a standard skin prick test. An age-matched group of healthy controls ($n = 5$) with no symptoms and a negative skin prick test was also identified. We then collected conjunctival epithelial cell swabs and biopsies from all subjects during winter, when there was no birch pollen in the air and thus even the allergic patients did not present any symptoms (Fig. 1). During this previously nonsymptomatic period, we could also apply an *in vivo* perturbation by an application of pollen allergen to the eyes, which caused rapid symptoms in allergic subjects, but no manifestation in healthy controls. We collected a new set of cell and tissue specimens and analysed all specimens with the light and TEM before perturbations, as well as 1 and 10 min after it. We also collected mRNA specimens during the winter season both from the healthy and allergic subjects.

In this systems biology approach, we try to study the type-I allergy disease on a systems level. Here, we do not restrict only to single genes or one data domain, but instead try to begin to dissect differences within a broader content. Furthermore, we restricted only to freshly isolated human cells and tissue specimens to avoid as much as possible the *in vitro* artefacts of cell cultures. Our study is an example of a holistic top-down approach to

begin to understand the early events of an allergic reaction in patients.

Morphological alterations induced by the ocular Bet v 1 challenge to epithelial cells

During the winter season, we challenged the asymptomatic allergic patients and the healthy subjects with the conjunctival application of the birch pollen extract and collected conjunctival epithelial cell swabs before the challenge, and 1 and 10 min after it. Bet v 1 is the major allergen within the birch pollen extract used for these perturbations and thus its presence and location were analysed with the following experiments.

During the asymptomatic winter season, there were no morphological differences between conjunctival cells obtained either from healthy or allergic subjects before the birch pollen challenge (Fig. 2A,B). However, after a very short *in vivo* conjunctival perturbation with the birch pollen extract the epithelial cells from the allergic, but not from the healthy subjects, showed a gross vacuolization when analysed with the TEM (Fig. 2C,D). Thus, clearly the seemingly morphologically similar conjunctival epithelial cells during the asymptomatic period had the capacity to react differently to the birch pollen perturbation.

Bet v 1 binding to epithelium

We then analysed the presence, quantity and location of Bet v 1 in the conjunctival biopsies from these same individuals obtained during a different session during the same winter period. Already the 1-min *in vivo* ocular challenge resulted in a patchy location of the Bet v 1 binding to conjunctival epithelial surfaces only in the allergic, but not in the healthy individuals (Fig. 2E–H and Table 1). Even exposures for 10 min did not result in the Bet v 1 binding to the epithelium in healthy subjects (data not shown).

Bet v 1 entry to epithelial cells and tissues

These observations were further verified on the immunotEM, where the Bet v 1 binding to and entry in the epithelium were evident only in cells taken from the allergic, but not from the healthy individuals. During the asymptomatic phase before the pollen perturbation, epithelial cells both from the healthy and allergic subjects did not show essentially any anti-Bet v 1 immunogold staining (Table 2). Already 1 min after the perturbation, before the fulminant clinical symptoms, Bet v 1 was found both at the villae on the cell surface, as well as within the villae, cytoplasm, intracellular vesicles and also in the nuclei in epithelial cells from allergic patients. Concomitantly, there was no Bet v 1 in the conjunctival epithelial cells obtained from the healthy control subjects. At 10 min after the challenge, while the clinical symptoms

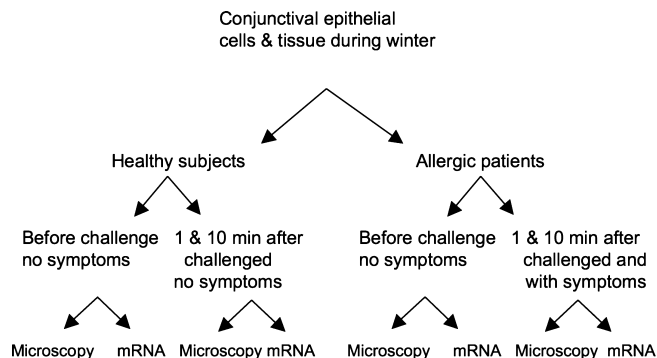


Figure 1. The study set up.

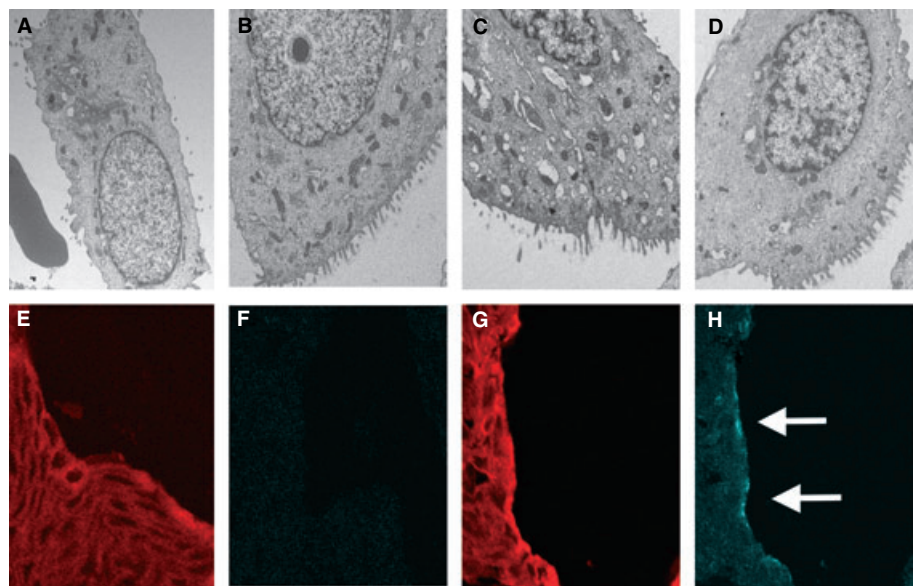


Figure 2. Bet v 1 induced a vesicular formation in the conjunctival epithelial cells obtained only from allergic, but not from healthy subjects. Conjunctival epithelial cell swabs were collected from the left eye of both (A) birch pollen allergic and (B) healthy subjects before the *in vivo* birch pollen challenge during the asymptomatic winter season. Basically, no morphological aberrations can be detected during this time. Then the right eyes were given a 10-min *in vivo* ocular birch pollen challenge, epithelial cell swabs were collected and the EM analysis showed large accumulation of cytoplasmic vesicles in the epithelial cells from allergic patients (C), but no morphological changes in the cells of the healthy subjects (D). Original magnification $\times 2500$. Bet v 1 binds to the conjunctival epithelium only in allergic patients, but not in healthy subjects. The ConA staining clearly indicated the epithelial lining in red (E and G). Healthy subject did not show any Bet v 1 binding to the epithelium (F), while on the contrary, a conjunctival epithelial biopsy taken from an allergic patient 1 min after the pollen challenge indicated a strong and patchy Bet v 1 location detected with anti-Bet v 1 antibodies (H, blue staining for Bet v 1, white arrows). Original magnification $\times 200$.

Table 1. Bet v 1 binding to conjunctival epithelial surface analysed with the immunofluorescence microscopy during the winter season after 1-min ocular pollen challenge

	Bet v 1 on the epithelial surface
Healthy subject 1	–
Healthy subject 2	–
Healthy subject 3	–
Healthy subject 4	–
Healthy subject 5	–
Allergic subject 1	++
Allergic subject 2	+++
Allergic subject 3	+++
Allergic subject 4	++
Allergic subject 5	++

–, No staining is graded; ++, medium staining is graded; +++, strong staining is graded. See also Fig. 2.

already were fulminant, most of Bet v 1 was located within the cytoplasm and not any more on the cell surface of villae (Table 2).

Conjunctival biopsies were also obtained and the immuno TEM analysis showed a rapid transport of Bet v 1 into the epithelial layers (Table 3). The stratified conjunctival epithelium was analysed in the vertical direction starting from the apical surface and entering to

Table 2. The analysis of the amount of anti-Bet v 1 gold label particles in conjunctival epithelial cells before and after the *in vivo* pollen challenge during the asymptomatic winter season

	At villus surface	Within villus	Within cytoplasm	Within vesicles	Within nucleus
0 min					
Healthy (35)	0	0.1 ± 0.1	1.0 ± 0.4	0	0.3 ± 0.2
Allergic (26)	0.9 ± 0.3	0.5 ± 0.3	2.6 ± 1.0	0.2 ± 0.2	0.5 ± 0.3
1 min					
Healthy (82)	0.5 ± 0.3	1.1 ± 0.8	0.6 ± 0.7	0.8 ± 0.7	0.5 ± 0.3
Allergic (82)	12.4 ± 7.1	8.6 ± 5.3	36.2 ± 10.4	11.6 ± 6.2	1.8 ± 1.0
10 min					
Healthy (73)	1.1 ± 1.0	0.8 ± 0.5	3.5 ± 2.2	0.6 ± 0.4	0.6 ± 0.3
Allergic (94)	0.2 ± 0.3	0.7 ± 0.4	27.3 ± 11.2	1.1 ± 0.8	2.7 ± 1.4

Bet v 1 rapidly bound to and entered into the conjunctival epithelial cells in allergic patients but not in healthy subjects. The numbers in brackets indicate the number of microscopic fields analysed with original magnification $\times 27\ 500$.

deeper epithelial layers. Although the majority of anti Bet v 1-gold label was within the most apical epithelial cells (cells no: 1–2), the staining could be detected already at this early time point all the way into the more basal epithelial cell layers (cell no 4). This indicates the extremely rapid traffic through conjunctival epithelial cells and tissues in allergic patients. Concomitantly, there was essentially no Bet v 1 binding to and traffic

Table 3. The analysis of the location of anti-Bet v 1 gold label particles within various epithelial layers of the stratified conjunctival epithelium after 1 min of the pollen perturbation

Location	Bet v 1			Background		
	Total	Desmosome	Intercellular	Total	Desmosome	Intercellular
Apical surface	1050	—	—	0	—	—
Epithelial cell 1	4600	360	80	220	10	0
Epithelial cell 2	4100	320	60	240	10	0
Epithelial cell 3	3500	290	40	270	10	0
Epithelial cell 4	2500	210	10	230	10	0

The number of anti-Bet v 1 gold particles per the whole specimen was analysed.

through the conjunctival epithelium in healthy subjects (Table 3).

In most cases, Bet v 1 was found in the small clusters of anti-Bet v 1 gold label particles (Fig. 3). The number of anti-Bet v 1 gold label particles was $25 \pm 3/\mu\text{m}^2$ in allergic patients and only on the background level, i.e. $4 \pm 0.3 \mu\text{m}^2$ (Fig. 3B) in healthy controls. These clusters were never found prior to perturbations, but already 1 min after the birch pollen challenge, the clusters were detected at the surface and also within the conjunctival epithelial cells (Fig. 3C). Likewise, most of the gold label (over 75%) was detected within such clusters in specimens from the allergic patients. As a negative control challenge, birch pollen allergic subjects were also challenged with timothy grass (*P. pratense*), but no entry of this pollen was detected (data not shown).

In most cases, Bet v 1 was present in small clusters while binding to and entering into the epithelium. We

next tried to identify further the mechanisms of this binding and entry. As a caveola was a good putative candidate for such a mechanism, we first analysed the presence of various caveolar markers in the conjunctival epithelial tissue clustering together with the Bet v 1 protein. By using the double immunoTEM, we could show that a caveolar marker, caveolin 2 but not caveolin 1 and 3, was present on the conjunctival epithelial surface in the same clusters as Bet v 1 (in the double immuno-TEM staining. Caveolin 2 showed a strong co-localization with Bet v 1, especially at the early sites of the contact (Fig. 3D–F).

Transcriptomics

We then collected epithelial cell swabs from both allergic and healthy subjects during winter, when there was no birch pollen in the air and thus not even the allergic patients had any symptoms. The conjunctival epithelial cells displayed 182 differentially expressed transcripts during the symptomatic winter period (Table S1).

The novel observation in this study is that epithelial cells and tissues obtained from allergic patients were able to uptake Bet v 1 pollen allergen while the same was not true in the healthy subjects. To begin to characterize this difference, we performed a transcriptomic analysis of the intact epithelium from both allergic and healthy subjects during the winter season when neither group had any symptoms. The aim of this analysis was to ask if the epithelium of these two groups expressed already different transcriptomic states even in the presence of no clinical symptoms of allergy. The classical study approach is to

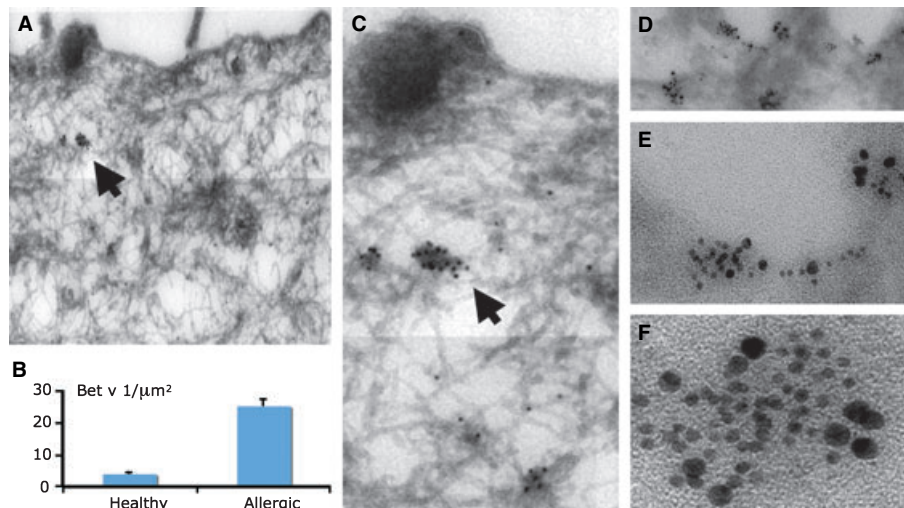


Figure 3. Bet v 1 binds to and is transported through the conjunctival epithelium only in allergic, but not in healthy subjects. The strong clustered staining of anti-Bet v 1 and secondary antibody with 10-nm gold label particles barely visible in panel A (arrow, original magnification $\times 5000$). Quantification of the Bet v 1 within upper layers of epithelial cells in panel B. A blow-up of the field in panel A is shown in panel C (arrow, original magnification $\times 27\,500$). Bet v 1 co-localized with caveolin 2. Double immunoTEM with Bet v 1 detected with 5 nm and caveolin 2 detected with 10-nm gold particles, magnification $\times 5000$ (D) $\times 27\,500$ (E) and $\times 205\,000$ (F). Bet v 1 was almost solely (75%) co-localized with a caveolar marker protein caveolin 2.

pick a few altering genes/proteins and start to work with them. Instead systems biology tries to look these differences from a broader perspective and thus we have performed a top-down analysis of the differentially expressed genes.

We next converted these differentially expressed transcripts to corresponding proteins (Table S1). In an attempt to try to get some putatively relevant knowledge from this list of proteins, we first analysed it against GO categories. They provide a controlled vocabulary to describe gene and gene product attributes in any organism, and here in human subjects. The enrichments-depletions of GO categories within our list of differentially regulated proteins between allergic and healthy subjects were essentially computed as described (6).

Gene ontology slim is an 'easy-reader' or a cut-down version of the GO containing a subset of the terms in the whole GO. Gene ontology slims give a broad overview of the ontology content without the detail of the specific fine grained terms. Gene ontology slims are particularly useful for giving a summary of the results of a GO annotation of a list of genes or proteins generated with e.g. a microarray when the broad classification of the gene product function is required (<http://www.geneontology.org/GO.slims.shtml>).

The most enriched biological process was signal transduction (Table S2). The category "Integral to plasma membrane" was the most enriched cellular compartment and transporters, cell adhesion and endopeptidase activities were the most enriched molecular functions.

Another approach to generate some information from this gene/protein list was to try to identify putative cell-surface receptors, which might be involved in the uptake of Bet v 1 into the allergic but not healthy epithelium during winter without any perturbations. From the list of upregulated genes/proteins in the specimen of allergic, but not healthy subjects, we gathered a subset of well-known cell-surface receptors. We could list 22 differentially expressed cell-surface receptors, which displayed enhanced expression levels in allergic *vs* healthy controls (Table S3). Among these receptors, (many) seven were G protein coupled receptors, three receptors involved in immune response, two linked to the extracellular matrix and one lectin known to bind venom toxin and HI viruses (Table S3). An intriguing observation is that eight of them (36%) have been found in lipid rafts/caveolae according to our manual PubMed literature search (Table S3). The frequency of caveolar/lipid raft proteins in the order of 1% within the human proteome and thus this 36% proportion is significantly higher than that expected by chance. This again could suggest that the epithelial cell surfaces of allergic and healthy epithelium differ significantly in the expression of these lipid raft/caveolar receptors. This *in silico* analysis provides a list of putative receptors, which could be involved in the uptake of Bet v 1.

Discussion

We have initiated a large systems biology effort to begin to understand the early events in the type-I allergy. We selected this disease as a starting point of a systems level approach as it is an acute disease (12, 13), which is clearly less difficult, if not easy, to study compared with chronic diseases. Here, we can challenge the subjects with a known allergen such as the birch pollen during the asymptomatic winter season and induce the acute disease reactions within minutes and collect cell and tissue specimens in a time-series manner both from allergic patients and healthy control subjects. We began to analyse with a systems biology view the differences in the nasal epithelium obtained from healthy and allergic subjects (6) and now expand these analysis to other respiratory epithelial tissues. With the light and electron microscopic analysis, we show here that the Bet v 1 pollen allergen bound to epithelial cells within minutes even during the nonsymptomatic winter season only in allergic, but not in healthy individuals. It travelled through the epithelium together with lipid rafts/caveolae and reached mast cells only in allergic patients.

The novel observation of this study is the difference between the epithelial cells and tissues obtained from allergic patients and healthy controls. We began to characterize this difference by performing the transcriptomic analysis of the intact epithelium from both allergic and healthy subjects during the winter season, when neither group had any symptoms. A classical study approach is to pick a few altering genes/proteins and start to work with them. Systems biology tries to look these differences from a broader perspective and thus we have performed a top-down analysis of the differentially expressed genes. We first try to classify them into categories (here GO categories), which could characterize the differentially expressed genes/proteins.

We found 182 statistically significantly differentially expressed transcripts (and converted them to corresponding proteins). We continued to analyse this whole set and characterized them with GO categories. An over-representation of cell receptors and proteins involved in signal transduction were/was seen.

As there were so many receptors displaying enhanced expression levels in allergic subjects compared with healthy controls, we also took a closer look at them. It could be postulated that allergic epithelium might express receptor(s) ready to putatively uptake the birch pollen or at least co-migrate along the Bet v 1 transport. A list of 22 epithelial cell-surface receptors displayed enhanced expression levels in allergic patients *vs* healthy controls. Eight of them (36%) have been found in lipid rafts/caveolae according to our manual PubMed literature search. The frequency of caveolar/lipid raft proteins in the order of 1% within the human proteome and thus this 36% proportion is significantly higher than that expected by chance. This again could suggest that the epithelial cell

surfaces of the allergic and healthy epithelium differ significantly in the expression of these lipid raft/caveolar receptors. Together with the Bet v 1 co-localization to lipid rafts/caveolae in the immuno EM analysis these observation could further strengthen our hypothesis of the caveolar/lipid raft dependent Bet v 1 transport in the allergic epithelium.

Thus, an application of discovery driven methodologies on human conjunctival epithelial cells and tissues can provide a new hypothesis worth a further analysis to the molecular mechanisms of a complex multifactorial disease such as the type-I birch pollen allergy.

The kinetics of caveolar traffic of Bet v 1 resembles in many ways other known caveolar-dependent phenomena such as the entry of viruses, bacterial toxins or even parasites (17–22). We have previously identified a small set of Bet v 1-associated proteins, where 37% were of the caveolar origin Joenvaara S et al., unpublished data. Here, we analyse the putative receptors on conjunctival epithelial cells, which could participate in this Bet v 1 uptake seen only in allergic, but not healthy subjects. Altogether 22 receptors were overexpressed in allergic conjunctiva and out of these, several could play a putative role in the Bet v 1 recognition and traffic through epithelium.

Bet v 1 protein has been shown to have an affinity for a wide section of amphiphilic molecules including sterols and fatty acids (23, 24). Bet v 1 is also able to interact and associate with lipid vesicles, which induces large conformational rearrangements to the structure of Bet v 1 (25). Thus in a separate study, we have *in silico* modelled the Bet v 1 binding to various amphiphilic ligands and lipids (Mattila K and Renkonen R, unpublished data).

Here, we show that already during winter, the conjunctival epithelium in allergic patients was able to bind and uptake the Bet v 1 allergen putatively in a caveola-dependent manner. In our immunoTEM analysis, a proportion of the gold label could be located in the nuclei of epithelial cells. While we currently do not have any direct role for this, we can put out a hypothesis that the Bet v 1 entry to the conjunctival cells modifies directly or indirectly their transcriptomic profiles, which is the subject of our next study.

The most significant aspects of this and other top-down explorative studies using systems biology approaches is that they can provide a truly new hypothesis. Before this study, few would have argued that the conjunctival

epithelium is so markedly different in healthy compared with allergic subjects already during the nonsymptomatic winter season. Now, based on these results, the mechanisms of the lipid raft/caveolar-dependent Bet v 1 pollen allergen binding to and trafficking through the epithelium can be further explored and analysed. The ability to discover aetiologically relevant disease mechanisms, which fall outside the prior medical and biological knowledge, is the major motivation for these unbiased explorative approaches.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. 182 differentially expressed transcripts in allergic vs healthy conjunctival epithelial cells during winter with no clinical symptoms.

Table S2. 182 differentially expressed transcripts were enriched using GO slim into biological process, cellular component and molecular functions. The most enriched categories are displayed. These results are calculated using the hypergeometric distribution in the GO-Term-Finder module with the Bonferroni correction. The *P*-value cut-off was 0.05. The minimum number of gene products: 3. There were 83 result(s) clearing the threshold values and 1039 not.

Table S3. 22 receptors with upregulated transcript expression levels in allergic vs healthy conjunctival specimens and their characterization in SwissProt. Lipid raft and/or caveola associated proteins are marked bold (26–33).

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1 Supplement Table 1. 182 differentially expressed transcripts in allergic versus healthy
2 conjunctival epithelial cells during winter with no clinical symptoms.
3
4

GBP2_HUMAN	CEA5_HUMAN	SYM_HUMAN
SL55_HUMAN	PLUN_HUMAN	DMWD_HUMAN
PRG4_HUMAN	Q8TCN5	MGLL_HUMAN
TPX2_HUMAN	ENC1_HUMAN	NLEX_HUMAN
DOC1_HUMAN	K0423_HUMAN	ADNP_HUMAN
MARH6_HUMAN	SARL_HUMAN	MA2B1_HUMAN
KCMB1_HUMAN	FATSO_HUMAN	Q99640
SSR4_HUMAN	PSD8_HUMAN	CNTF_HUMAN
SAA_HUMAN	CPA6_HUMAN	TPMT_HUMAN
GLNE_HUMAN	VA0E1_HUMAN	GP153_HUMAN
EMR1_HUMAN	RPB7_HUMAN	MY1A_HUMAN
IPKG_HUMAN	EPA1_HUMAN	MO15B_HUMAN
SCA5_HUMAN	MIP_HUMAN	O43529
ZNF93_HUMAN	ME18_HUMAN	ARVC_HUMAN
EFB3_HUMAN	SIX6_HUMAN	TMO1_HUMAN
PPAL_HUMAN	JAD1C_HUMAN	MGR6_HUMAN
MCAF2_HUMAN	PHLA1_HUMAN	MPP3_HUMAN
NAR1_HUMAN	AF10_HUMAN	NDK5_HUMAN
SH33_HUMAN	EDAR_HUMAN	KLK6_HUMAN
GBGU_HUMAN	SCNBA_HUMAN	SU6H_HUMAN
RHAG_HUMAN	Q9H8H3	PSD9_HUMAN
EXL2_HUMAN	Q9UNS1	Q9BWF3
TNF8_HUMAN	SP90_HUMAN	Q9BQ04
ACHO_HUMAN	CKB4_HUMAN	SLE1_HUMAN
SMB2_HUMAN	RXINP_HUMAN	CJ038_HUMAN
NCT2_HUMAN	ENSP00000236040	Q96IZ2
TMPS6_HUMAN	HEN2_HUMAN	TTC23_HUMAN
CLC1B_HUMAN	RFNG_HUMAN	CK057_HUMAN
DRP2_HUMAN	PENK_HUMAN	TUL2_HUMAN
HIS1_HUMAN	ITA10_HUMAN	REC8_HUMAN
OAH3_HUMAN	ENTK_HUMAN	AT6B_HUMAN
NPT4_HUMAN	PGK1_HUMAN	CBP2_HUMAN
CB2R_HUMAN	EL3A_HUMAN	ANL2_HUMAN
SRR_HUMAN	ITIH4_HUMAN	CRTC_HUMAN
RN122_HUMAN	RAB26_HUMAN	ENSP00000366160
ARBK1_HUMAN	NEK1_HUMAN	PIS_HUMAN
LRC4_HUMAN	GRHR_HUMAN	OPH1_HUMAN
PP2AA_HUMAN	O60502	PGRP4_HUMAN
ENSP00000334319	SPUF_HUMAN	ZM10_HUMAN
MAPA_HUMAN	LCB2_HUMAN	I2C1_HUMAN
Q9NRD8	NLP_HUMAN	Q9NXH3
PDA5_HUMAN	SATT_HUMAN	CN10_HUMAN
PLCG2_HUMAN	WEE1_HUMAN	CD59_HUMAN
GP27_HUMAN	RL38_HUMAN	Q93052
G3BP_HUMAN	EST2_HUMAN	EAA3_HUMAN
Q9BQ90	NID1_HUMAN	RL18_HUMAN
IVD_HUMAN	ASNS_HUMAN	P2Y4_HUMAN
PCM1_HUMAN	KCS3_HUMAN	PRI0_HUMAN
INAF_HUMAN	Q9UBU6	RET_HUMAN
TBX6_HUMAN	Q9Y6E2	SAA4_HUMAN
L7L2_HUMAN	HOM3_HUMAN	NTCP_HUMAN
TNIK_HUMAN	Q96SI1	O75762
AD21_HUMAN	CN045_HUMAN	I23O_HUMAN
TRL2_HUMAN	CHST4_HUMAN	XDH_HUMAN
CRCM_HUMAN	TX3B_HUMAN	CC101_HUMAN
FXL7_HUMAN	RIPA_HUMAN	N214_HUMAN
NEP_HUMAN	IP13_HUMAN	SNA1_HUMAN
Q92521	IASPP_HUMAN	THTM_HUMAN
ENSP00000332105	FHR2_HUMAN	A8A2_HUMAN
CEA7_HUMAN	SRRM2_HUMAN	PPOX_HUMAN
G6PE_HUMAN	SSH3_HUMAN	

Supplement Table 2. 182 differentially expressed transcripts were enriched using GO slim into biological process, cellular component and molecular functions. The most enriched categories are displayed. These results are calculated using the hyper geometric distribution in the GO-TermFinder module with the Bonferroni correction. The p-value cut-off was: 0.05. The minimum number of gene products: 3. There were 83 result(s) clearing the threshold values and 1039 not.

Biological Process

GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0007165 signal transduction	2.12e-02	32/172 (18.6%)	2623/20270 (12.9%)	CRCM_HUMAN PLCG2_HUMAN TNFL8_HUMAN PENK_HUMAN GPR27_HUMAN TMPS6_HUMAN NCTR2_HUMAN CD59_HUMAN ACHB3_HUMAN RET_HUMAN DUOX2_HUMAN EFNB3_HUMAN TNIK_HUMAN CLC1B_HUMAN ATF6B_HUMAN SF17A_HUMAN OPHN1_HUMAN LIFR_HUMAN GNRHR_HUMAN GRM6_HUMAN EPHA1_HUMAN MPP3_HUMAN CNTF_HUMAN P2RY4_HUMAN ARBK1_HUMAN SH3G3_HUMAN PP2AA_HUMAN GBGT2_HUMAN HOME3_HUMAN DOCK1_HUMAN CNR2_HUMAN SSR4_HUMAN
GO:0007166 cell surface receptor linked signal transduction	2.33e-02	16/172 (9.3%)	1090/20270 (5.4%)	PLCG2_HUMAN CD59_HUMAN DUOX2_HUMAN EFNB3_HUMAN CLC1B_HUMAN LIFR_HUMAN GNRHR_HUMAN GRM6_HUMAN P2RY4_HUMAN ARBK1_HUMAN PP2AA_HUMAN GBGT2_HUMAN HOME3_HUMAN DOCK1_HUMAN CNR2_HUMAN SSR4_HUMAN
GO:0009100 glycoprotein metabolic process	4.16e-02	4/172 (2.3%)	153/20270 (0.8%)	MA2B1_HUMAN NCOAT_HUMAN KLK6_HUMAN PIGB_HUMAN
GO:0007155 cell adhesion	4.24e-02	9/172 (5.2%)	543/20270 (2.7%)	SAA_HUMAN ITA10_HUMAN GLCNE_HUMAN EMR1_HUMAN CHST4_HUMAN GPV_HUMAN ARVC_HUMAN CHSTA_HUMAN PP2AA_HUMAN

Cellular Component

GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0005887 integral to plasma membrane	1.60e-06	28/172 (16.3%)	1228/20270 (6.1%)	SATT_HUMAN TNFL8_HUMAN NCTR2_HUMAN NPT4_HUMAN ACHB3_HUMAN KCMB4_HUMAN TRPM2_HUMAN ITA10_HUMAN NTCP_HUMAN EFNB3_HUMAN CLC1B_HUMAN EMR1_HUMAN GPV_HUMAN NEP_HUMAN LIFR_HUMAN MIP_HUMAN CEAM5_HUMAN GNRHR_HUMAN TRPA1_HUMAN GRM6_HUMAN NAR1_HUMAN EPHA1_HUMAN MPP3_HUMAN RHAG_HUMAN P2RY4_HUMAN EAA3_HUMAN CNR2_HUMAN SSR4_HUMAN

Molecular Function

GO Term	P-value	Sample frequency	Background frequency	Genes
GO:0022891 substrate-specific transmembrane transporter activity	1.05e-03	13/172 (7.6%)	562/20270 (2.8%)	SATT_HUMAN NPT4_HUMAN ACHB3_HUMAN VA0E1_HUMAN KCMB4_HUMAN TRPM2_HUMAN NTCP_HUMAN SC5A5_HUMAN SCNBA_HUMAN RHAG_HUMAN KCMB1_HUMAN EAA3_HUMAN KCNS3_HUMAN
GO:0004252 serine- type endopeptidase activity	5.01e-03	4/172 (2.3%)	81/20270 (0.4%)	GRAH_HUMAN TMPS6_HUMAN KLK6_HUMAN ELA3A_HUMAN
GO:0004872 receptor activity	1.02e-02	15/172 (8.7%)	904/20270 (4.5%)	CRCM_HUMAN NCTR2_HUMAN RET_HUMAN EFNB3_HUMAN CLC1B_HUMAN EMR1_HUMAN EDAR_HUMAN LIFR_HUMAN PGRP4_HUMAN GNRHR_HUMAN GRM6_HUMAN OGFR_HUMAN CRLF1_HUMAN CNR2_HUMAN SSR4_HUMAN

Supplement Table 3. 22 receptors with upregulated transcript expression levels in allergic versus healthy conjunctival specimens and their characterization in SwissProt. Lipid raft and/or caveola associated proteins are marked bold (26-33).

1. SSR4_HUMAN	9. TNFL8_HUMAN	17. NID1_HUMAN
2. EMR1_HUMAN	10. CD59_HUMAN	18. IPO13_HUMAN
3. O10H3_HUMAN	11. TRPM2_HUMAN	19. ARBK1_HUMAN
4. CNR2_HUMAN	12. EPHA1_HUMAN	20. RET_HUMAN
5. GPR27_HUMAN	13. EFNB3_HUMAN	21. TRPA1_HUMAN
6. GP153_HUMAN	14. CLC1B_HUMAN	22. ACHB3_HUMAN
7. P2RY4_HUMAN	15. EDAR_HUMAN	
8. NCTR2_HUMAN	16. ITA10_HUMAN	

SSR4_HUMAN

Protein name: Somatostatin receptor type 4
Gene name: SSTR4

FUNCTION: Receptor for somatostatin-14. The activity of this receptor is mediated by G proteins which inhibits adenylyl cyclase. It is functionally coupled not only to inhibition of adenylyl cyclase, but also to activation of both arachidonate release and mitogen-activated protein (MAP) kinase cascade. Mediates antiproliferative action of somatostatin in tumor cells.

SUBCELLULAR LOCATION: Cell membrane; Multi-pass membrane protein.

TISSUE SPECIFICITY: Specifically expressed in fetal and adult brain, lung tissue, stomach, and in lesser quantities in the kidney, pituitary and adrenals.

Molecular class: G protein coupled receptor
Molecular function: G protein coupled receptor activity
Biological process: cell communication; signal transduction

EMR1_HUMAN

Protein name: EGF-like module-containing mucin-like hormone receptor-like 1
Gene name: EMR1

FUNCTION: Could be involved in cell-cell interactions.

SUBCELLULAR LOCATION: Cell membrane; Multi-pass membrane protein.

TISSUE SPECIFICITY: Wide expression; increased levels in peripheral blood mononuclear cells.

Molecular class: G protein coupled receptor
Molecular function: G protein coupled receptor activity
Biological process: cell communication; signal transduction

O10H3_HUMAN

Protein name: Olfactory receptor 10H3
Gene name: OR10H3

FUNCTION: Odorant receptor

SUBCELLULAR LOCATION: Cell membrane; Multi-pass membrane protein.

Molecular class: G protein coupled receptor
Molecular function: G protein coupled receptor activity
Biological process: cell communication; signal transduction

CNR2_HUMAN

Protein name: Cannabinoid receptor 2
Gene name: CNR2

FUNCTION: Involved in cannabinoid-induced CNS effects through G-protein mediated inhibition of adenylate cyclase. Could be a receptor for anandamide.

SUBCELLULAR LOCATION: Cell membrane; Multi-pass membrane protein.

TISSUE SPECIFICITY: Not found in brain. Found in spleen.

Molecular class: G protein coupled receptor
Molecular function: G-protein coupled receptor activity
Biological process: cell communication; signal transduction

GPR27_HUMAN

Protein name: Probable G-protein coupled receptor 27
Gene name: GPR27

FUNCTION: Orphan receptor. Possible candidate for amine-like G-protein coupled receptor.

SUBCELLULAR LOCATION: Cell membrane; Multi-pass membrane protein

TISSUE SPECIFICITY: Highly expressed as a 3.0 kb transcript in brain, ovary, testis, heart, prostate and peripheral Leukocytes. Lower levels in pancreas and small intestine. A 2.3 kb transcript was also found in peripheral Leukocytes. In brain regions, detected as a 3.0 kb transcript in all regions tested. Highest levels in the caudate nucleus, putamen, hippocampus and subthalamic nucleus. Lowest level in the cerebellum.

Molecular class: G protein coupled receptor
Molecular function: G protein coupled receptor activity
Biological process: cell communication; signal transduction

GP153_HUMAN

Protein name: Probable G-protein coupled receptor 153
Gene name: GPR153

FUNCTION: Orphan receptor.

SUBCELLULAR LOCATION: Cell membrane; Multi-pass membrane protein.

Molecular class: G protein coupled receptor
Molecular function: G protein coupled receptor activity
Biological process: cell communication; signal transduction

P2RY4_HUMAN

Protein name: P2Y purinoceptor 4
Gene name: P2RY4

FUNCTION: Receptor for UTP and UDP coupled to G-proteins that activate a phosphatidylinositol-calcium second messenger system. Not activated by ATP or ADP.

SUBCELLULAR LOCATION: Cell membrane; Multi-pass membrane protein.

TISSUE SPECIFICITY: Pancreas.

PTM: Phosphorylation of Ser-333 and Ser-334 is a key step in agonist-dependent desensitization and loss of surface P2RY4. This phosphorylation does not involve PKC, nor other calcium activated kinases.

Molecular class: G protein coupled receptor

Molecular function: G-protein coupled receptor activity

Biological process: cell communication; signal transduction

NCTR2_HUMAN

Protein name: Natural cytotoxicity triggering receptor 2 [Precursor]

Gene name: NCR2

FUNCTION: Cytotoxicity-activating receptor that may contribute to the increased efficiency of activated natural killer (NK) cells to mediate tumor cell lysis.

SUBCELLULAR LOCATION: Cell membrane; Single-pass type I membrane protein

TISSUE SPECIFICITY: Selectively expressed by activated NK cells and by activated TCRg/d lymphoid cells.

Molecular class: cell surface receptor

Molecular function: receptor activity

Biological process: immune response

TNFL8_HUMAN

Protein name: Tumor necrosis factor ligand superfamily member 8

Gene name: TNFSF8

FUNCTION: Cytokine that binds to TNFRSF8/CD30. Induces proliferation of T-cells.

SUBCELLULAR LOCATION: Membrane; Single-pass type II membrane protein.

Molecular class: ligand

Molecular function: receptor binding

Biological process: immune response

CD59_HUMAN

Protein name: CD59 glycoprotein [Precursor]

Gene name: CD59

FUNCTION: Potent inhibitor of the complement membrane attack complex (MAC) action. Acts by binding to the C8 and/or C9 complements of the assembling MAC, thereby preventing incorporation of the multiple copies of C9 required for complete formation of the osmolytic pore. This inhibitor appears to be species-specific. Involved in signal transduction for T-cell activation complexed to a protein tyrosine kinase.

SUBCELLULAR LOCATION: Cell membrane; Lipid-anchor, GPI-anchor.

PTM: N- and O-glycosylated. The N-glycosylation mainly consists of a family of biantennary complex-type structures with and without lactosamine extensions and outer arm fucose residues. The predominant O-glycans are mono-sialylated forms of the disaccharide, Gal-beta-1,3GalNAc, and their sites of attachment are probably on Thr-76 and Thr-77.

PTM: Glycated. Glycation is found in diabetic subjects, but only at minimal levels in nondiabetic subjects. Glycated CD59 lacks MAC-inhibitory function and confers to vascular complications of diabetes.

Molecular class: cell surface receptor

Molecular function: receptor activity

Biological process: immune response

TRPM2_HUMAN

Transient receptor potential cation channel subfamily M member 2

Gene name: TRPM2

FUNCTION: Nonselective, voltage-independent cation channel mediating sodium and calcium ion influx in response to oxidative stress. Extracellular calcium passes through the channel and acts from the intracellular side as a positive regulator in channel activation. Activated by ADP-ribose, nicotinamide adenine dinucleotide (NAD(+)), reactive nitrogen species and arachidonic acid. Inactivated by intracellular ATP. Confers susceptibility to cell death following oxidative stress. Isoform 2 does not seem to be regulated by ADPR. Has ADP-ribose pyrophosphatase activity.

Cellular component: integral to plasma membrane

Molecular function: calcium channel activity

Biological process: calcium ion transport

EPHA1_HUMAN

Protein name: Ephrin type-A receptor 1

Gene name: EPHA1

FUNCTION: Receptor for members of the ephrin-A family. Binds with a low affinity to ephrin-A1.

CATALYTIC ACTIVITY: ATP + a [protein]-L-tyrosine = ADP + a [protein]-L-tyrosine phosphate.

SUBCELLULAR LOCATION: Membrane; Single-pass type I membrane protein.

TISSUE SPECIFICITY: Overexpressed in several carcinomas.

Molecular class: receptor tyrosine kinase

Molecular function: transmembrane receptor protein tyrosine kinase activity

Biological process: cell communication; signal transduction

EFNB3_HUMAN

Protein name: Ephrin-B3 [Precursor]

Gene name: EFNB3

FUNCTION: May play a pivotal role in forebrain function. Binds to, and induce the collapse of, commissural axons/growth cones *in vitro*. May play a role in constraining the orientation of longitudinally projecting axons

SUBCELLULAR LOCATION: Membrane; Single-pass type I membrane protein.

TISSUE SPECIFICITY: Highly expressed in brain; expressed in embryonic floor plate, roof plate and hindbrain segments.

Molecular class: membrane bound ligand

Molecular function: receptor binding

Biological process: cell communication; signal transduction

CLC1B_HUMAN

Protein name: C-type lectin domain family 1 member B

Gene name: CLEC1B

FUNCTION: Acts as a receptor for the platelet-aggregating snake venom protein rhodocytin. Rhodocytin binding leads to tyrosine phosphorylation and this promotes the binding of spleen tyrosine kinase (Syk) and initiation of downstream tyrosine phosphorylation events and activation of PLC-gamma-2. Acts as an attachment factor for human immunodeficiency virus type 1 (HIV-1) and facilitates its capture by platelets.

SUBCELLULAR LOCATION: Membrane; Single-pass type II membrane protein

TISSUE SPECIFICITY: Expressed preferentially in the liver. Also expressed in immune cells of myeloid origin and on the surface of platelets.

PTM: Glycosylated.

PTM: Phosphorylated on tyrosine residue in response to rhodocytin binding.

Molecular class: cell surface receptor

Molecular function: receptor activity

Biological process: cell communication; signal transduction

EDAR_HUMAN

Protein name: Tumor necrosis factor receptor superfamily member EDAR [Precursor]

Gene name: EDAR

FUNCTION: Receptor for EDA isoform A1, but not for EDA isoform A2. Mediates the activation of NF-kappa-B and JNK. May promote caspase-independent cell death.

SUBUNIT: Binds to EDARADD. Associates with TRAF1, TRAF2, TRAF3 and NIK.

SUBCELLULAR LOCATION: Membrane; Single-pass type I membrane protein (Probable).

TISSUE SPECIFICITY: Detected in fetal kidney, lung, skin and cultured neonatal epidermal keratinocytes. Not detected in lymphoblast and fibroblast cell lines.

DEVELOPMENTAL STAGE: Found in craniofacial tissues from embryonic day 42-53. Expressed in fetal skin 11 and 15 weeks after gestation.

DISEASE: Defects in EDAR are a cause of hypohidrotic ectodermal dysplasia (HED); also known as anhidrotic ectodermal dysplasia (EDA). Ectodermal dysplasias (EDs) constitute a heterogeneous group of developmental disorders affecting tissues of ectodermal origin. EDs are characterized by abnormal development of two or more ectodermal structures such as hair, teeth, nails and sweat glands, with or without any additional clinical sign. Each combination of clinical features represents a different type of ectodermal dysplasia. HED is characterized by sparse hair (atrachosis or hypotrichosis), abnormal or missing teeth and the inability to sweat due to the absence of sweat glands. Inheritance can be autosomal dominant or recessive.

Molecular class: cell surface receptor

Molecular function: receptor activity

Biological process: cell communication; signal transduction

ITA10_HUMAN

Protein name: Integrin alpha-10 [Precursor]

Gene name: ITGA10

FUNCTION: Integrin alpha-10/beta-1 is a receptor for collagen.

SUBUNIT: Heterodimer of an alpha and a beta subunit. Alpha-10 associates with beta-1.

SUBCELLULAR LOCATION: Membrane; Single-pass type I membrane protein.

TISSUE SPECIFICITY: Widely expressed with highest expression in muscle and heart. Found in articular cartilage.

Molecular class: cell surface receptor

Molecular function: receptor activity

Biological process: cell communication; signal transduction

NID1_HUMAN

Protein name: Nidogen-1

Gene name: NID1

FUNCTION: Sulfated glycoprotein widely distributed in basement membranes and tightly associated with laminin. Also binds to collagen IV and perlecan. It probably has a role in cell-extracellular matrix interactions.

SUBUNIT: Interacts with FBLN1 and LGALS3BP.

SUBCELLULAR LOCATION: Secreted, extracellular space, extracellular matrix, basement membrane.

PTM: N- and O-glycosylated.

Molecular class: extracellular matrix protein

Molecular function: extracellular matrix structural constituent

Biological process: cell growth and / or maintenance

IPO13_HUMAN

Protein name: Importin-13

Gene name: IPO13

FUNCTION: Functions in nuclear protein import as nuclear transport receptor. Serves as receptor for nuclear localization signals (NLS) in cargo substrates. Is thought to mediate docking of the importin/substrate complex to the nuclear pore complex (NPC) through binding to nucleoporin and the complex is subsequently translocated through the pore by an energy requiring, Ran-dependent mechanism. At the nucleoplasmic side of the NPC, Ran binds to the importin, the importin/substrate complex dissociates and importin is re-exported from the nucleus to the cytoplasm where GTP hydrolysis releases Ran. The directionality of nuclear import is thought to be conferred by an asymmetric distribution of the GTP- and GDP-bound forms of Ran between the cytoplasm and nucleus. Mediates the nuclear import of UBC9, the RBM8A/MAGOH complex, PAX6 and probably other members of the paired homeobox family. Also mediates nuclear export of eIF-1A, and the cytoplasmic release of eIF-1A is triggered by the loading of import substrates onto IPO13.

SUBUNIT: Interacts with UBC9, RBM8A, eIF-1A and PAX6.

SUBCELLULAR LOCATION: Cytoplasm. Nucleus.

TISSUE SPECIFICITY: Expressed in fetal brain, heart, intestine and kidney.

Molecular class:nuclear receptor

Molecular function: ligand-dependent nuclear receptor activity

Biological process: cell communication; signal transduction

ARBK1_HUMAN

Protein name: Beta-adrenergic receptor kinase 1

Gene name: ADRBK1

FUNCTION: Specifically phosphorylates the agonist-occupied form of the beta-adrenergic and closely related receptors, probably inducing a desensitization of them.

CATALYTIC ACTIVITY: $\text{ATP} + [\text{beta-adrenergic receptor}] = \text{ADP} + [\text{beta-adrenergic receptor}] \text{phosphate.}$

Molecular class: enzyme: Serine / threonine kinase

Molecular function: Protein serine / threonine kinase activity

Biological process: cell communication; signal transduction

RET_HUMAN

Protein name: Proto-oncogene tyrosine-protein kinase receptor ret [Precursor]

Gene name: RET

FUNCTION: Probable receptor with tyrosine-protein kinase activity; important for development.

CATALYTIC ACTIVITY: $\text{ATP} + \text{a} [\text{protein}]\text{-L-tyrosine} = \text{ADP} + \text{a} [\text{protein}]\text{-L-tyrosine phosphate.}$

SUBUNIT: Phosphorylated form interacts with the PBT domain of DOK2, DOK4 and DOK5

SUBCELLULAR LOCATION: Membrane; Single-pass type I membrane protein.

PTM: Phosphorylated.

PTM: Autophosphorylated on C-terminal tyrosine residues upon ligand stimulation.

POLYMORPHISM: The Cys-982 polymorphism may be associated with an increased risk for developing Hirschsprung disease.

DISEASE: Defects in RET are a cause of pheochromocytoma. The pheochromocytomas are catecholamine-producing, chromaffin tumors that arise in the adrenal medulla in 90% of cases. In the remaining 10% of cases, they develop in extra-adrenal sympathetic ganglia and may be referred to as "paraganglioma." Pheochromocytoma usually presents with hypertension. Approximately 10% of pheochromocytoma is hereditary. The genetic basis for most cases of non-syndromic familial pheochromocytoma is unknown.

DISEASE: Defects in RET are the cause of medullary thyroid carcinoma (MTC). MTC is a rare tumor derived from the C cells of the thyroid. Three hereditary forms are known, that are transmitted in an autosomal dominant fashion: (a) multiple neoplasia type 2A (MEN2A), (b) multiple neoplasia type IIB (MEN2B) and (c) familial MTC (FMTC), which occurs in 25-30% of MTC cases and where MTC is the only clinical manifestation.

DISEASE: Defects in RET are the cause of multiple neoplasia type IIA (MEN2A); also called multiple neoplasia type II (MEN2). MEN2A, the most frequent form of MTC, is an inherited cancer syndrome characterized by MTC, pheochromocytoma and/or hyperparathyroidism.

DISEASE: Defects in RET are the cause of multiple neoplasia type IIB (MEN2B). MEN2B is an uncommon inherited cancer syndrome characterized by predisposition to MTC and pheochromocytoma which is associated with marfanoid habitus, mucosal neuromas, skeletal and ophthalmic abnormalities, and ganglioneuromas of the intestine tract. Then the disease progresses rapidly with the development of metastatic MTC and a pheochromocytoma in 50% of cases.

DISEASE: Defects in RET are a cause of Hirschsprung disease (HSCR). HSCR is a genetic disorder of neural crest development characterized by the absence of intramural ganglion cells in the hindgut, often resulting in intestinal obstruction. Occasionally, MEN2A or FMTC occur in association with HSCR.

DISEASE: Chromosomal aberrations involving RET are a cause of thyroid papillary carcinoma (PACT). Inversion inv(10)(q11.2;q21) generates the RET/CCDC6 (PTC1) oncogene; inversion inv(10)(q11.2;q11.2) generates the RET/NCOA4 (PTC3) oncogene; translocation t(10;14)(q11;q32) with GOLGA5 generates the RET/GOLGA5 (PTC5) oncogene; translocation t(8;10)(p21.3;q11.2) with PCM1 generates the PCM1/RET fusion; translocation t(6;10)(p21.3;q11.2) with RFP generates the Delta RFP/RET oncogene; translocation t(1;10)(p13;q11) with TRIM33 generates the TRIM33/RET (PTC7) oncogene; translocation t(7;10)(q32;q11) with TIF1 generates the TIF1/RET (PTC6) oncogene. The PTC5 oncogene has been found in 2 cases of PACT in children exposed to radioactive fallout after Chernobyl.

DISEASE: Defects in RET are a cause of congenital central hypoventilation syndrome (CCHS); also known as congenital failure of autonomic control or Ondine curse. CCHS is a rare disorder characterized by abnormal control of respiration in the absence of neuromuscular or lung disease, or an identifiable brain stem lesion. A deficiency in autonomic control of respiration results in inadequate or negligible ventilatory and arousal responses to hypercapnia and hypoxemia.

DISEASE: Defects in RET may be a cause of colorectal cancer (CRC)

Molecular class: receptor tyrosine kinase

Molecular function: transmembrane receptor protein tyrosine kinase activity

Biological process: cell communication; signal transduction

TRPA1_HUMAN

Protein name: Transient receptor potential cation channel subfamily A member 1

Gene name: TRPA1

FUNCTION: Receptor-activated non-selective cation channel involved in detection of sensations such as cold, pain or sounds perception. Has a central role in the pain response to endogenous inflammatory mediators and to a diverse array of volatile irritants, such as mustard oil, garlic and acrolein, an irritant from tears gas and vehicle exhaust fumes. Also involved in pain sensation triggered by capsaicin, the pungent ingredient in chilli peppers. Acts also as a ionotropic cannabinoid receptor by being activated by delta(9)-tetrahydrocannabinol (THC), the psychoactive component of marijuana. Not involved in menthol sensation. May be a component for the mechanosensitive transduction channel of hair cells in inner ear, thereby participating in the perception of sounds. Probably operated by a phosphatidylinositol second messenger system

SUBCELLULAR LOCATION: Membrane; Multi-pass membrane protein.

TISSUE SPECIFICITY: Expressed at very low level. Expressed at very low level in human fibroblasts and at a moderate level in liposarcoma cells.

Molecular class: ion channel

Molecular function: ion channel activity

Biological process: transport

ACHB3_HUMAN

Protein name: Neuronal acetylcholine receptor subunit beta-3 [Precursor]

Gene name: CHRNA3

FUNCTION: After binding acetylcholine, the AChR responds by an extensive change in conformation that affects all subunits and leads to opening of an ion-conducting channel across the plasma membrane.

SUBUNIT: Neuronal AChR seems to be composed of two different type of subunits: alpha and beta.

SUBCELLULAR LOCATION: Cell junction, synapse, postsynaptic cell membrane; Multi-pass membrane protein. Cell membrane; Multi-pass membrane protein.

Molecular class: voltage gated channel

Molecular function: voltage-gated ion channel activity

Biological process: cell communication; signal transduction