

Original article

Genes regulating molecular and cellular functions in noninfectious nonallergic rhinitis

Background: Chronic noninfectious, nonallergic rhinitis (NINAR) is a complex syndrome with a principally unknown pathophysiology. New technology has made it possible to examine differentially expressed genes and according to network theory, genes connected by their function that might have key roles in the disease.

Methods: Connectivity analysis was used to identify NINAR key genes. mRNA was extracted from nasal biopsies from 12 NINAR patients and 12 healthy volunteers. Microarrays were performed using Affymetrix chips with 54 613 genes. Data were analysed with the Ingenuity Pathway System for organization of genes into annotated biological functions and, thereafter, linking genes into networks due to their connectivity. The regulation of key genes was confirmed with reverse transcription-polymerase chain reaction (RT-PCR).

Results: In all, 43 genes were differentially expressed. The functional analysis showed that these genes were primarily involved in cellular movement, haematological system development and immune response. Merging these functions, 10 genes were found to be shared. Network analysis generated three networks and two of these 'shared genes' in key positions, *c-fos* and cell division cycle 42 (*Cdc42*). These genes were upregulated in both the array and the RT-PCR analysis.

Conclusion: Ten genes were found to be of pathophysiological interest for NINAR and of these, *c-fos* and *Cdc42* seemed to be of specific interest due to their ability to interact with other genes of interest within this context. Although the role of *c-fos* and *Cdc42* in upper airway inflammation remains unknown, they might be used as potential disease markers.

**L. O. Cardell¹, M. Andersson²,
A. Cervin², Å. Davidsson³,
J. Hellgren⁴, M. Holmström⁵,
L. Lundblad¹, P. Stiernä¹, P. Stjärne¹,
M. Adner⁶, the SRFA study group**

¹Division of Otorhinolaryngology, Karolinska Institutet, Stockholm; ²Department of Otorhinolaryngology, Head and Neck Surgery, Lund University, Lund; ³Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Örebro, Örebro; ⁴Department of Ear, Nose, and Throat and Head and Neck Surgery, Capio Lundby Hospital, Gothenburg; ⁵Department of Otorhinolaryngology, Uppsala University Hospital, Uppsala; ⁶National Institute of Environmental medicine, Karolinska Institutet, Stockholm, Sweden

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Prof. L. O. Cardell
Head of the ENT Division
Department of Clinical Sciences
Intervention and Technology
Karolinska Institutet
Karolinska University Hospital
Huddinge
S-141 86 Stockholm
Sweden

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Rhinitis is caused by chronic or acute inflammation of the mucous membrane of the nose due to viruses, bacteria or irritants (1). It is characterized by itching, sneezing, rhinorrhoea and nasal blockage. The self-limiting common cold constitutes its most frequent form, whereas chronic rhinitis symptoms are among the most common problems presenting to physicians. When approaching the latter, the diagnostic challenge is to determine the aetiology, specifically whether it is allergic, nonallergic or perhaps an overlap of both conditions. Estimates of the prevalence of allergic rhinitis range from as low as 9% to as high as 42%. The prevalence of noninfectious/nonallergic rhinitis (NINAR) has not been studied definitively, but it appears to be very common, affecting more than 200 million people worldwide (2). The incidence is known to increase steadily with age (3).

Environmental irritants and changing weather conditions are the common triggers along with emotional and physical stress factors. Several mechanisms have been postulated to explain the pathophysiology of NINAR, of which neurogenic disorders and non-IgE-mediated inflammatory responses are among the most plausible (4, 5). An increase in nasal epithelial permeability might contribute to the disease development by enabling irritants, infection and cellular components such as eosinophil MBP/ECP to reach sensory nerve endings, vessels and secretory glands (6). It is also interesting to observe that several nasal provocation studies in NINAR patients have demonstrated that a positive response may exist despite a negative skin test and/or serum specific IgE. These reactions have been attributed to presence or synthesis of IgE locally in the nasal mucosa (7, 8).

However, despite vigorous research efforts, the aetiology of NINAR remains unknown and reliable diagnostic markers are still lacking (2).

Regardless of its cause, NINAR is most likely to be the consequence of an altered interplay between multiple genes. To target genes that might be involved in the pathogenesis, we performed microarray analysis of biopsies from NINAR patients and healthy control subjects. This kind of investigation usually results in a set of differently expressed genes and the question arises how to identify which of these genes that might have key functions in the disease. One approach to this problem is to treat differentially expressed genes as nodes in a network (9–11). Interacting nodes are connected by their function to describe the architecture of the network. Highly connected nodes are generally considered more important for the network function (12). Network analysis can be formed with the help of special computer-based tools like the Ingenuity Pathway System (IPA). Ingenuity Pathway System is based on extracting mammalian gene interactions from articles in the PubGene co-citation literature network. The aim of the present study was to use the IPA for identification of potentially important NINAR genes to generate information that might increase our pathophysiological insights.

Method

Subjects

The study included 12 patients (eight women and four men) with a history of NINAR (median age of 35, range: 22–59) and 12 (six women and six men) healthy volunteers (median age of 36, range: 20–50). Patients were admitted to the study if they had a history of nasal complaints such as nasal obstruction and rhinorrhoea for a period of over 1 year, which could not be attributed to allergic, nasal or paranasal infection, anatomical disorders affecting nasal function, pregnancy or lactation and/or systemic disorders. Some patients reported intermittent periods of sneezing. Both patients and controls were nonsmokers and displayed negative skin prick tests to a standard panel of allergens (described below). All participants were free of medication at the time of inclusion, except for the use of contraceptive pills. Before inclusion, all subjects were evaluated by an ear, nose and throat consultant who performed endoscopic examination of the nasal cavities, and subjects with nasal polyps were excluded. Individual nasal swabs to reveal eosinophilia were not performed; hence, patients suffering from NINAR with eosinophilia might be included as one of several phenotypic sub-groups. Elderly patients with rhinitis and patients suffering from rhinitis medicamentosa and CRS were excluded. The exclusion criteria for both groups also included upper airway infection during or 1 month prior to the investigation as well as treatment with local or systemic corticosteroids during the last 2 months prior to the potential inclusion. This study was approved by the Ethics Committee of the Medical Faculty, Lund University.

Nasal biopsy procedure

A single nasal biopsy, 2 × 2 × 2 mm, was taken from the inferior turbinate after topical application of local anaesthesia containing

lidocainhydrochloride : nafazoline (34 : 0.17 mg/ml) for 20 min. The biopsies were immediately placed in RNA-later (Qiagen, GmbH, Hilden, Germany) for 24 h at 4°C and then frozen at –20°C.

Skin prick test

Skin prick tests were performed with a standard panel of 10 common airborne allergens (ALK, Copenhagen, Denmark) including pollen (birch, timothy and mugwort), house dust mites (*Dermaphagoides pteronyssinus* and *D. farinae*), molds (*Cladosporium* and *Alternaria*) and animal allergens (cat, dog and horse). These tests were performed on the volar side of the forearm with saline buffer as negative and histamine chloride (10 mg/ml) as positive control. The wheal reactions were measured after 20 min. All participants, NINAR patients as well as controls displayed a negative skin prick test (wheal diameter < 2 mm).

Preparation of cRNA

RNA was isolated using the RNeasy kit (Qiagen). RNA concentrations were determined spectrophotometrically. For the microarrays, the RNA from patients in each group was pooled into three samples, each from four subjects. Double-stranded cDNA was prepared using Life Technologies Superscript Choice system (Life Technologies, Paisley, UK). Gel electrophoresis was performed to verify expected size distribution of cDNA, cRNA and fragmented cRNA and hybridization cocktails were prepared.

In vitro amplification

The quantity and quality of RNA were analysed with Nanodrop ND-1000 UV Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). As a result of the small amount of RNA, an *in vitro* amplification of two cycles was performed in accordance with the protocol for Small Sample Target Labeling Assay Version II (Affymetrix, Santa Clara, CA, USA) before the hybridization.

Oligonucleotide array hybridization and scanning

Washing and staining was performed using a fluidics station and a confocal scanner essentially according to the manufacturer's instructions (Affymetrix).

Analysis of DNA microarray data

To allow comparison of gene expression between patients and controls, the arrays were scaled to an average intensity of 500. RNA expression was quantitatively estimated by computing the average difference between a set of oligonucleotides that perfectly matched the gene and a set of mismatch control oligonucleotides. The average difference value will subsequently be referred to as the gene expression level and given in arbitrary units. Signal values and arrays were background corrected and normalized by using the robust multiarray average. A quantitative estimate of the difference in gene expression is given by the signal log ratio with 2 as the base of the log scale. Thus, a signal log ratio of 1.0 indicates an increase in the transcript level by twofold and –1.0 indicates a decrease by twofold. A signal log ratio of zero would indicate no change. This study follows the MIAME microarray standards (13).

IPA analysis

The limits for this analysis was set to a twofold up- or downregulation and that the mean expression on the array was significant ($P < 0.05$). A two-tailed Student's *t*-test was used. The IPA was used to organize differentially expressed genes into networks of interacting genes and to find modules of functionally related genes that correspond to pathways. Interactions were also inferred algorithmically by combining functional and structural information about proteins. Every interaction can be referenced back to a MedLine ID within the application. Identification of networks formed by the differentially expressed genes was performed in a stepwise manner:

1. Differentially expressed genes are scored on the basis of the number of specific interactions found within the IPA global network. Focus genes were identified as those having direct or indirect interactions with other genes in the IPA database.
2. The specificity of connections for each focus gene was calculated by the percentage of its connections to other differentially expressed genes. A network was then constructed, starting with the gene with the highest specificity of connections. Each network contained a maximum of 35 genes. This process was repeated for the remaining genes to construct new networks. As the number of focus genes is reduced as a result of their inclusion in previous networks, genes from the global network not in the original list but with significant specific connectivity to the growing network can be included into those new networks.
3. A statistical algorithm based on the Fischer's exact test was used to rank the networks. Identification of pathways was based on using canonical pathways in the IPA as templates for the differentially expressed genes. Again using the Fischer's exact test, the resulting pathways were ranked according to their correlation with the template in the IPA.

RT-PCR confirmation

Reverse transcription (RT) of total RNA to cDNA was carried out using OmniscriptTM reverse transcriptase kit (Qiagen) in 20 µl volume reaction at 37°C for 1 h by using Mastercycler personal PCR machine (Eppendorf AG, Hamburg, Germany). To quantify mRNA, real-time polymerase chain reaction (real-time PCR) was performed with the QuantiTectTM SYBR[®] Green PCR kit (Qiagen GmbH, Hilden, Germany) in Smart Cycler[®] II system (Cepheid, Sunnyvale, CA, USA).

The gene expression was assessed using the comparative cycle threshold (C_t) method (<http://docs.appliedbiosystems.com/pebio-docs/04303859.pdf>). The relative amounts of mRNA for the target genes were determined by subtracting the C_t values for these genes from the C_t value for the housekeeping gene β -actin (ΔC_t). Specific primers for the human *c-fos*, cell division cycle 42 (*Cdc42*) and house keeping gene β -actin were designed by using PRIME EXPRESS[®] 2.0 software (Applied Biosystem, Foster city, CA, USA) and synthesized by DNA Technology A/S (Aarhus, Denmark). *t*-test was used for the comparisons of the mRNA data, which were expressed as mean $\pm$ SEM of 100 000 molecules mRNA of β -actin. $P < 0.05$ was accepted as statistically significant. n equals the number subjects in each group.

Results

Forty-three out of 54 613 genes differed more than twofold in expression between the NINAR patients and

the healthy controls. The majority, 30 genes, were found to be upregulated whereas the rest were downregulated (Table 1). An IPA analysis demonstrated that these genes were associated with 10 different types of molecular and cellular functions. Of these 'cellular movement', 'haematological system development' and 'immune response' were shown to have the highest significance involving 15, 13 and 16 of our focus genes respectively (Table 2, Fig. 1). Ten of these genes were found in all three groups (Fig. 2) and therefore being of particularly interest in our quest for key disease regulating genes. However, when the relationship between these genes was analysed, very few connections were found both within and among the different functional groups.

The IPA was then used to build up networks that involved our differentially expressed focus genes. Three such network consisting of 35 genes that contained at least 10 focus genes were found. This means that at any rate, 25% of the genes differently expressed in NINAR could be related to at least one of three networks created on the basis on co-citations in PubGene. The network with most focus genes (15) were then merged with the genes found in the three most prominent functional groups described above, resulting in a new network of 36 genes (Fig. 3A). Seventy-one connections were found within the network, with a relation to gene expression, protein-DNA interactions (23) and protein-protein interactions (15). According to network theory and studies of yeast cells (10), genes that are connected with several other genes appear to have key regulatory roles. In our network, *v-myc* (1.83 downregulated, with 19 interactions), followed by *c-fos* (2.40 upregulated, with 15 interactions), SP-1 transcription factor (1.35 upregulated with 13 interactions) and CCAAT/enhancer binding protein α (1.40 downregulated with 10 connections), were found to be most prominent. Eleven genes were not connected at all and therefore dismissed from the network. None of the dismissed genes belonged to any of the three major functional groups.

The second network, created along the same principals as the first, highlighted the importance of three genes *catenin β 1*, CREB binding protein and amyloid beta (A4) precursor, although none of them was included in the group of 10 genes shared between the functional groups (Fig. 3B). The third network showed three highly connected genes, E2F transcription factor 1, retinoblastoma 1 and *Cdc42* (Fig. 3C), the latter belonging to the group of 10 genes shared between the functional groups.

Judging the overall picture, the two genes *c-fos* and *Cdc42* were found to be central both in a functional and network perspective. Their upregulation, seen in the arrays (2.40 and 3.39, respectively) was confirmed with RT-PCR (2.60 and 2.06 respectively; Fig. 4). The increase in *Cdc42* (2293 ± 238 in healthy individuals and 4730 ± 869 in NINAR patients relative to 100 00

Table 1. Genes significantly expressed on the arrays that were either up- or downregulated (≤ 2 -fold)

Description	Name	Normalized ratio	Entrez Gene ID
Amine oxidase (flavin containing) domain 2	AOF2	-2.136	23028
Chromosome 20 open reading frame 18	C20ORF18	2.189	10616
Chemokine (C-C motif) ligand 2	CCL2	2.154	6347
Cell division cycle 42 (GTP-binding protein, 25 kDa)*	CDC42	3.391	998
Chloride channel, calcium activated, family member 2	CLCA2	2.022	9635
Collagen, type X, alpha 1	COL10A1	-2.238	1300
Chondroitin sulphate proteoglycan 2 (versican)	CSPG2	2.055	1462
Cystatin SN	CST1	4.274	1469
Cystatin S	CST4	2.432	1472
Cytochrome P450, family 2, subfamily A	CYP2A6	-2.072	1548
DEAH (Asp-Glu-Ala-His) box polypeptide 9	DHX9	-2.084	1660
Ectonucleotide pyrophosphatase/phosphodiesterase 3	ENPP3	2.030	5169
Fc fragment of IgG, low affinity IIb, receptor (CD16b)	FCGR3B	2.038	2215
v-fos FBJ murine osteosarcoma viral oncogene homolog*	FOS	2.403	2353
Glycoprotein 2 (zymogen granule membrane)	GP2	2.773	2813
Major histocompatibility complex, class II, DP beta 1	HLA-DPB1	-2.115	3115
Major histocompatibility complex, class II, DQ alpha 1	HLA-DQA1	-2.045	3117
Major histocompatibility complex, class II, DQ beta 2	HLA-DQB2	-2.194	3120
Haptoglobin	HP	2.337	3240
Kinesin family member 9	KIF9	-2.057	64147
Kinetochore associated 2	KNTC2	2.042	10403
Lymphotoxin beta (TNF superfamily, member 3)*	LTB	-2.147	4050
LY6/PLAUR domain containing 3	LYPD3	2.216	27076
Microtubule-associated protein 1B	MAP1B	2.023	4131
Multimerin 1	MMRN1	2.200	22915
Nexilin (F actin binding protein)	NEXN	2.097	91624
Phosphoinositide-3-kinase, class 2, beta polypeptide	PIK3C2B	-2.158	5287
Phospholamban	PLN	2.917	5350
Pogo transposable element with ZNF domain	POGZ	-2.485	23126
Protease, serine, 21 (testisin)	PRSS21	2.039	10942
Prostaglandin-endoperoxide synthase 2*	PTGS2	2.354	5743
Repetin	RPTN	3.459	126638
S100 calcium binding protein A8 (calgranulin A)*	S100A8	2.506	6279
Serum amyloid A1*	SAA1	2.261	6288
Secretoglobin, family 2A, member 2	SCGB2A2	2.463	4250
Selectin E (endothelial adhesion molecule 1)*	SELE	2.244	6401
Serpin peptidase inhibitor, clade A*	SERPINA3	2.233	12
Serpin peptidase inhibitor, clade B, member 2	SERPINB2	2.902	5055
Serpin peptidase inhibitor, clade B, member 13	SERPINB13	2.833	5275
Small proline-rich protein 1B (cornifin)	SPRR1B	3.794	6699
Sterol regulatory element binding transcription factor 1*	SREBF1	-2.108	6720
Tudor domain containing 3	TDRD3	2.082	81550
Tyrosinase-related protein 1	TYRP1	-2.672	7306

*Those genes that are shared between the three most significant pathways (see below).

Table 2. The 10 most significant functions

Function	Significance	No. genes
Cellular movement	1,17E-05-4,86E-02	15
Haematological system development	1,17E-05-4,72E-02	13
Immune response	1,17E-05-4,44E-02	16
Cell-to-cell signaling and interaction	4,86E-04-4,86E-02	15
Immune and lymphatic system development and function	4,86E-04-4,72E-02	10
Neurological disease	5,38E-04-4,3E-02	8
Cellular compromise	1,03E-03-4,72E-02	4
Inflammatory disease	1,04E-03-3,03E-02	7
Immunological disease	1,06E-03-4,09E-02	5
Dermatological diseases and conditions	1,12E-03-3,6E-02	6

molecules β -actin mRNA) was significant ($P = 0.017$), whereas the increase in c-fos (1369 ± 414 in healthy individuals and 3554 ± 1338 in NINAR patients relative to 100 00 molecules β -actin mRNA) was not ($P = 0.17$).

Discussion

Microarray analysis was assessed with mRNA extracted from nasal biopsies from 12 patients with NINAR and 12 healthy control subjects. The IPA was used to identify genes of special interest. Three groups of genes, related to cellular movement, haematological system development

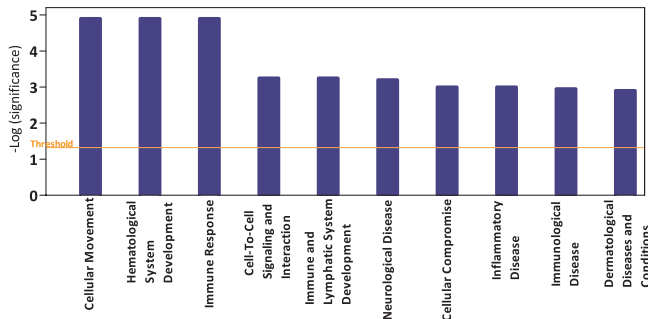


Figure 1. Genes, differently expressed in NINAR, organized into annotated biological functions using the IPA. Columns represent groups of genes associated with specific molecular or cellular functions (x-axis), with a statistical analysis of the probability for a significant role for each gene groups on the y-axis. The yellow line represents the threshold of $P < 0.05$.

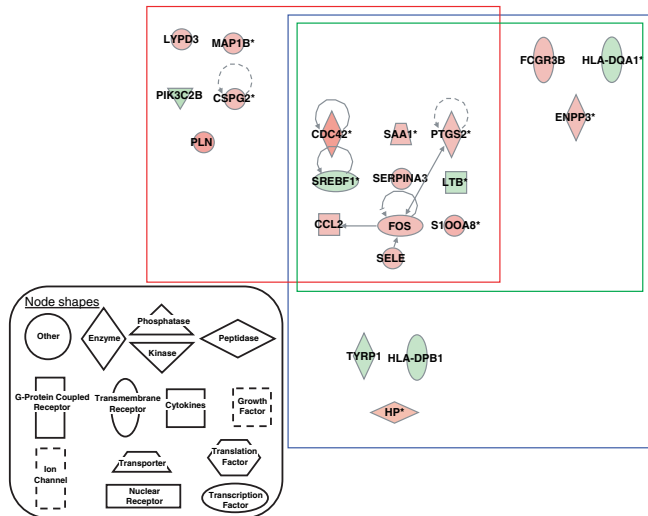


Figure 2. A graphic representation of genes involved in the three major functional groups described in Fig. 1. The red boxes represent genes connected with cellular movement, the green boxes genes associated with the haematological system development and the blue boxes genes involved in the immune response. As can be seen, 10 genes are shared by all three groups. Red and green symbols represent upregulated genes and downregulated genes respectively.

and immune response, appeared to be clearly involved in NINAR. Ten genes were found to be shared between these functions, eight that were upregulated and two that were downregulated. Two genes of importance, c-fos and Cdc42, were identified among the three pathways that were used to construct a network comprising to be of pivot importance for NINAR. Their array based upregulation was confirmed with RT-PCR.

Bioinformatical approaches have been used to discover and refine molecular pathways and functional

gene categories. In general, the approach is to find gene and protein interactions that agree with the patterns seen in various experiments including microarray studies. Human experts can formulate rules about gene interactions, e.g. gene A is a transcription factor and upregulates gene B. However, with the advent of massive amounts of data, it is necessary to have computational tools that further extend the human expert. The methods used to extract biological information from data include fuzzy logic (14, 15), genetic algorithms (16), neural networks (17) and an approach for biological pathway modelling based on hybrid intelligent systems (18). Those pathways and gene categories previously found by human interpretation and bioinformatical approaches can then be used to interpret new experiments, which in this study was performed by use of IPA.

Forty-three out of 54 613 genes were twofold up- or downregulated, at least twofold, when the NINAR patients were compared to the healthy controls. The 43 differently expressed genes were further analysed using IPA, organizing them into annotated biological functions. Three functions, related cellular movement, haematological system development and immune response respectively emerged as being of particular importance. Together these functions suggest that recruitment of white blood cells may be of significance for the immunological response in NINAR, thereby reflecting the chronic nature of an inflammatory disorder (19). No signs of genes involved in neurogenic disorders were detected in our investigation, leaving the hypothesis of a neurological imbalance as a primary cause for NINAR (20) without support. However, the presented analysis is based on nasal biopsies, known to be too superficial to include major neural elements. Hence, a role for a disturbance in the neural input as a contributing factor behind the development of local nasal inflammation with recruitment of white blood cells cannot be ruled out. However, regardless of the exact nature of the pathophysiology, all 43 genes found to be differentially expressed during NINAR might be used as potential disease markers and even targets for novel therapeutic approach.

To refine the search for pivot NINAR genes, two strategies were adopted within the use of IPA. The first was based on merging the three major functional gene groups described above and the second on network building based on co-citation of articles mentioning the 43 genes in the PubGene. When merging the functions, 10 genes were found to be shared among the three functional groups. Eight of these were upregulated: the transcription factor Rho, the GTPase Cdc42 (21), the regulator of prostaglandin synthesis prostaglandin-endoperoxide synthase 2 (also known as cyclooxygenase 2) (22), the calcium-binding protein calgranulin A (23), the proteolytic cleavage product of an acute phase reactant serum amyloid A1 (24), secretoglobin 2A2 that is abundantly

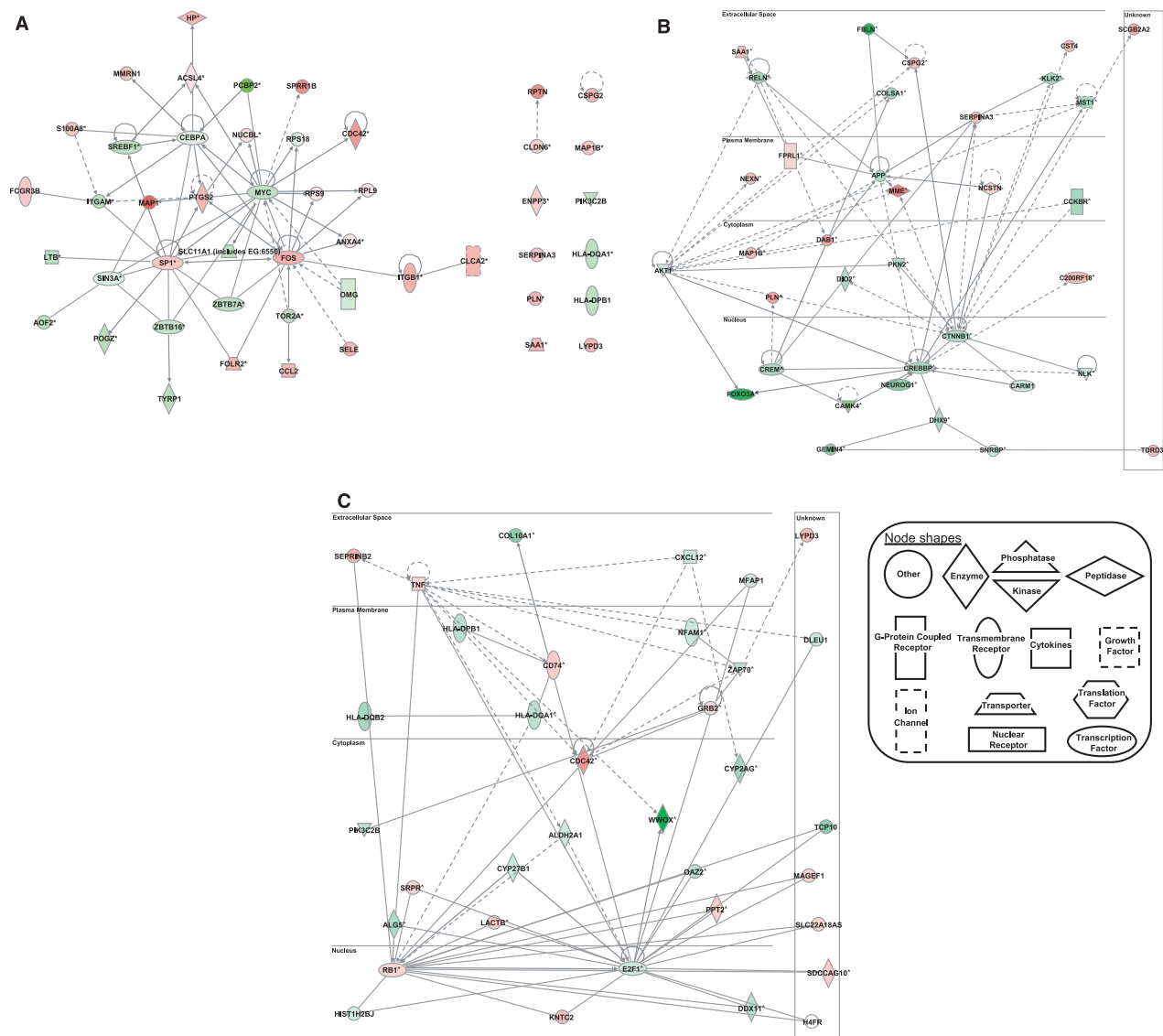


Figure 3. The result of an IPA based search for networks involving our 10 focus genes (marked in Fig. 2). (A–C) represents the three networks that involved as many as our focus genes as possible. c-fos and Cdc42 can be seen in key positions in network A and C respectively representing the most well connected genes in each network. Red symbols represent upregulated genes and green symbols downregulated genes.

expressed in a breast tumour (25), the plasma protease inhibitor serpin peptidase inhibitor A (26) and the endothelial adhesion molecule selectin E (27). Sterol regulatory element binding transcription factor 1 that control cholesterol homeostasis (28) and the TNF-related protein lymphotoxin β (29) were downregulated. A detailed analysis found only limited connections in-between them, thereby failing to single out any of them as key genes in NINAR. The second IPA approach based on network construction yield three networks with 10 or more genes. Assessment of these revealed two of these ‘shared genes’ in key positions (several connections to other genes), c-fos and Cdc42. Both c-fos and Cdc42

were found to be upregulated, suggesting increased activity.

c-fos, v-fos FBJ murine osteosarcoma viral oncogene homologue, is a proto-oncogene that mediates intracellular signals induced by various external mitogenic and toxic stimuli. It regulates gene expression involved in cell proliferation and differentiation, transformation, apoptosis, pulmonary defense, inflammation, immune responses (30). In airways, c-fos is known to be induced by different toxicants (31). It is intriguing to speculate in a role for c-fos in mediating general and/or specific external stimuli in NINAR. Furthermore, glucocorticoids are known to decrease the induction of c-fos (32). Local

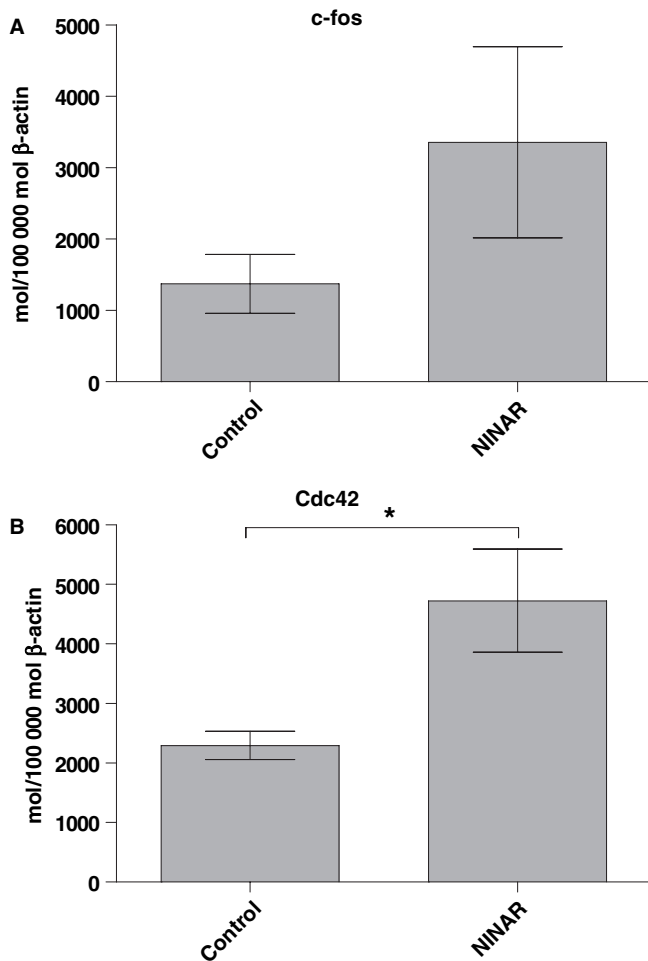


Figure 4. The mRNA expression of (A) c-fos and (B) Cdc42 in nasal biopsies from healthy individuals and NINAR patients. RT-PCR data are expressed in mean $\pm$ SEM in relation to 100 000 molecules β -actin; $n = 11$ – 12 ; * $P < 0.05$.

steroid treatment is the most commonly used NINAR therapy and its effects on c-fos expression support the presented idea of c-fos as key gene in NINAR. Comparatively little is known about Cdc42. It is a member of the Rho family of small GTP-binding protein found in airway epithelial cells. Cell division cycle 42 appears to be essential in the transduction of intracellular signals that induce actin–cytoskeleton reorganization and, furthermore, the control of endocytosis and secretion (33, 34), the latter which can have implication in NINAR.

References

1. Vignola AM, Bousquet J. Rhinitis and asthma: a continuum of disease? Clin Exp Allergy 2001;**31**:674–677.
2. Bousquet J, Fokkens W, Burney P, Durham SR, Bachert C, Akdis CA et al. Important research questions in allergy and related diseases: nonallergic rhinitis: a GA2LEN paper. Allergy 2008;**63**: 842–853.
3. Sanico A, Togias A. Noninfectious, nonallergic rhinitis (NINAR): considerations on possible mechanisms. Am J Rhinol 1998;**12**:65–72.

Previous studies have demonstrated that nasal biopsies have a complex structure, consisting not only of epithelial cells but also of mucosal cells and various types of leukocytes (35). The exact composition of may vary somewhat in-between samples. However, pooling reduces such differences. Pooling is a technique often used as a cost-saving strategy, but in the present study, it was used due to the limited size of each biopsy. It is reasonable to assume that the pooling procedure might result in loss of information, but recent studies have shown that this assumed loss of efficiency has no practical consequences (36). This notion is also supported by the present investigation. Individual samples analysed with PCR-based technique demonstrate a clear correlation with the corresponding data points obtained from the pools using microarrays. However, the complexity of the biopsies must urge us to interpret the results from the IPA analysis with some care, as genes emanating from various cell populations might be involved and the networks mainly relate to connection between intracellular targets. Thus, the network suggested may actually not exist. Further studies are needed, not only to confirm the role of c-fos and Cdc42 in NINAR and usefulness as disease markers, but also in which cell types they are activated. The latter might provide us with important information on what can be used to improve our understanding of the mechanisms behind the disease. This is in turn essential for the development of novel therapeutic strategies in NINAR.

In conclusion, 10 genes were found to be of pathophysiological interest for NINAR and of these, c-fos and Cdc42 seem to be worth special attention due to their ability to interact with other genes of interest within this context. These results demonstrate that microarrays could be a useful tool to characterize NINAR and they should be looked upon as a way to map potential candidates that need to be further investigated to establish markers for the disease.

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4. Sigsgaard T, Bonefeld-Jørgensen EC, Kjaergaard SK, Mamas S, Pedersen OF. Cytokine release from the nasal mucosa and whole blood after experimental exposures to organic dusts. *Eur Respir J* 2000;**16**:140–145.
5. Carney AS, Jones NS. Idiopathic rhinitis: idiopathic or not? *Clin Otolaryngol Allied Sci* 1996;**21**:198–202.
6. Fokkens WJ. Thoughts on the pathophysiology of nonallergic rhinitis. *Curr Allergy Asthma Rep* 2002;**2**:203–209.
7. Huggins KG, Brostoff J. Local production of specific IgE antibodies in allergic rhinitis patients with negative skin tests. *Lancet* 1975;**2**:148–150.
8. Rondón C, Romero JJ, López S, Antúnez C, Martín-Casañez E, Torres MJ et al. Local IgE production and positive nasal provocation test in patients with persistent nonallergic rhinitis. *J Allergy Clin Immunol* 2007;**119**:899–905.
9. Benson M, Steenhoff Hov DA, Clancy T, Hovig E, Rudemo M, Cardell LO. Connectivity can be used to identify key genes in DNA microarray data: a study based on gene expression in nasal polyps before and after treatment with glucocorticoids. *Acta Otolaryngol* 2007;**127**:1074–1079.
10. Lu X, Jain VV, Finn PW, Perkins DL. Hubs in biological interaction networks exhibit low changes in expression in experimental asthma. *Mol Syst Biol* 2007;**3**:98.
11. Camargo A, Azuaje F. Linking gene expression and functional network data in human heart failure. *PLoS ONE* 2007;**2**:e1347.
12. Jeong H, Mason SP, Barabási AL, Oltvai ZN. Lethality and centrality in protein networks. *Nature* 2001;**411**:41–42.
13. Barabási AL, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet* 2004;**5**:101–113.
14. Datta S, Sokhansanj BA. Accelerated search for biomolecular network models to interpret high-throughput experimental data. *BMC Bioinformatics* 2007;**8**:258.
15. Ho SY, Hsieh CH, Chen HM, Huang HL. Interpretable gene expression classifier with an accurate and compact fuzzy rule base for microarray data analysis. *Biosystems* 2006;**85**:165–176.
16. Pan KH, Lih CJ, Cohen SN. Analysis of DNA microarrays using algorithms that employ rule-based expert knowledge. *Proc Natl Acad Sci USA* 2002;**99**:2118–2123.
17. Chen CF, Feng X, Szeto J. Identification of critical genes in microarray experiments by a Neuro-Fuzzy approach. *Comput Biol Chem* 2000;**30**:372–381.
18. Bosl WJ. Systems biology by the rules: hybrid intelligent systems for pathway modeling and discovery. *BMC Syst Biol* 2007;**1**:13.
19. Philip G, Togias AG. Nonallergic rhinitis. Pathophysiology and models for study. *Eur Arch Otorhinolaryngol* 1995;**252**:S27–S32.
20. Sarin S, Udem B, Sanico A, Togias A. The role of the nervous system in rhinitis. *J Allergy Clin Immunol* 2006;**118**:999–1016.
21. Shinjo K, Koland JG, Hart MJ, Narasimhan V, Johnson DI, Evans T et al. Molecular cloning of the gene for the human placental GTP-binding protein Gp (G25K): identification of this GTP-binding protein as the human homolog of the yeast cell-division-cycle protein CDC42. *Proc Natl Acad Sci USA* 1990;**87**:9853–9857.
22. Hla T, Neilson K. Human cyclooxygenase-2 cDNA. *Proc Natl Acad Sci USA* 1992;**89**:7384–7388.
23. Wilkinson MM, Busuttill A, Hayward C, Brock DJ, Dorin JR, Van Heyningen V. Expression pattern of two related cystic fibrosis-associated calcium-binding proteins in normal and abnormal tissues. *J Cell Sci* 1988;**91**:221–230.
24. Bausserman LL, Saritelli AL, Herbert PN, McAdam KP, Shulman RS. NH₂-terminal analysis of four of the polymorphic forms of human serum amyloid A proteins. *Biochim Biophys Acta* 1982;**704**:556–559.
25. Watson MA, Darrow C, Zimonjic DB, Popescu NC, Fleming TP. Structure and transcriptional regulation of the human mammaglobin gene, a breast cancer associated member of the uteroglobin gene family localized to chromosome 11q13. *Oncogene* 1998;**16**:817–824.
26. Chandra T, Stackhouse R, Kidd VJ, Robson KJ, Woo SL. Sequence homology between human alpha 1-antichymotrypsin, alpha 1-antitrypsin, and antithrombin III. *Biochemistry* 1983;**22**:5055–5061.
27. Watson ML, Kingsmore SF, Johnston GI, Siegelman MH, Le Beau MM, Lemons RS et al. Genomic organization of the selectin family of leukocyte adhesion molecules on human and mouse chromosome 1. *J Exp Med* 1990;**172**:263–272.
28. Hua X, Wu J, Goldstein JL, Brown MS, Hobbs HH. Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11.2 and 22q13. *Genomics* 1995;**25**:667–673.
29. Browning JL, Ngam-ek A, Lawton P, DeMarinis J, Tizard R, Chow EP et al. Lymphotoxin beta, a novel member of the TNF family that forms a heteromeric complex with lymphotoxin on the cell surface. *Cell* 1993;**72**:847–856.
30. Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta* 1991;**1072**:129–157.
31. Reddy SP, Mossman BT. Role and regulation of activator protein-1 in toxicant-induced responses of the lung. *Am J Physiol Lung Cell Mol Physiol* 2002;**283**:L1161–L1178.
32. Baraniuk JN, Wong G, Ali M, Sabol M, Troost T. Glucocorticoids decrease c-fos expression in human nasal polyps in vivo. *Thorax* 1998;**53**:577–582.
33. Ridley AJ. Rho: theme and variations. *Curr Biol* 1996;**6**:1256–1264.
34. Chant J, Stowers L. GTPase cascades choreographing cellular behavior: movement, morphogenesis, and more. *Cell* 1995;**81**:1–4.
35. Fransson M, Benson M, Erjefält JS, Jansson L, Uddman R, Björnsson S et al. Expression of toll-like receptor 9 in nose, peripheral blood and bone marrow during symptomatic allergic rhinitis. *Respir Res* 2007;**8**:17.
36. Faraggi D, Reiser B, Schisterman EF. ROC curve analysis for biomarkers based on pooled assessments. *Stat Med* 2003;**22**:2515–2527.