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A protein interaction network associated with asthma

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Abstract

Identifying candidate genes related to complex diseases or traits and mapping their relationships require a system-level analysis at a cellular scale. The objective of the present study is to systematically analyze the complex effects of interrelated genes and provide a framework for revealing their relationships in association with a specific disease (asthma in this case). We observed that protein–protein interaction (PPI) networks associated with asthma have a power-law connectivity distribution as many other biological networks have. The hub nodes and skeleton substructure of the result network are consistent with the prior knowledge about asthma pathways, and also suggest unknown candidate target genes associated with asthma, including GNB2L1, BRCA1, CBL, and VAV1. In particular, GNB2L1 appears to play a very important role in the asthma network through frequent interactions with key proteins in cellular signaling. This network-based approach represents an alternative method for analyzing the complex effects of candidate genes associated with complex diseases and suggesting a list of gene drug targets. The full list of genes and the analysis details are available in the following online supplementary materials: http://biosoft.kaist.ac.kr:8080/resources/asthma_ppi.

Keywords: Disease network; Protein–protein interaction; Microarray expression; System biology

1. Introduction

Asthma is one of the most complex diseases characterized by specific patterns of inflammation in the airway mucosa, infiltration of eosinophils, increased numbers of T-helper-2 (Th2) cells relative to Th1 cells, and increased numbers of activated mast cells (Tattersfield et al., 2002). In addition, there are characteristic structural changes to the airways including subepithelial fibrosis, airway smooth muscle hypertrophy and hyperplasia, angiogenesis, and increased mucus secretory cells (Payne et al., 2003). These pathophysiological symptoms of asthma result from interactions between inflammatory cells, mediators, and inflammatory proteins that are controlled by many

Abbreviations: ADRB2, beta-2 adrenergic receptor; BCAR1, breast cancer antiestrogen resistance 1; BRCA1, breast cancer 1 early onset; CBL, cas-Br-M ectopic retroviral transforming sequence; CREBBP, cAMP-response-element-binding binding protein; EGFR, epidermal growth factor receptor; GNB2L1, guanine-nucleotide-binding protein beta polypeptide 2-like 1; GRB2, growth factor receptor-bound protein 2; ICAM1, intercellular adhesion molecule 1; IL, interleukins; IRAK1, interleukin-1 receptor-associated kinase 1; JAK, janus kinase; LYN, v-yes-1 yamaguchi sarcoma viral-related oncogene homolog; MAPK, mitogen-activated protein kinase; MYD88, myeloid differentiation primary response gene 88; NFKB1, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; PCNA, proliferating cell nuclear antigen; PDE, phosphodiesterase; PRKACA, protein kinase, cAMP-dependent, catalytic, alpha; PRKCE, protein kinase c, epsilon; PRKDC, protein kinase DNA-activated catalytic polypeptide; PTPRC, protein tyrosine phosphatase, receptor type c; RB1, retinoblastoma 1; SP1, Sp1 transcription factor; SRC, v-src sarcoma viral oncogene homolog; STAT, signal transducers and activators of transcription; SYK, spleen tyrosine kinase; TLR4, toll-like receptor 4; VAV1, vav1 oncogene; VCAM1, vascular cell adhesion molecule 1; VIL2, villin 2.

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proinflammatory and cellular signaling genes (Barnes, 2004). Therefore, identifying candidate genes that could play important roles as drug targets for asthma requires systematic analyses of the complicated interrelations between the genes responsible for the progress of asthma.

Various methods have been employed to identify candidate genes to be targeted by drugs in complex diseases (Freudenberg and Propping, 2002; Perez-Iratxeta et al., 2002; Tiffin et al., 2005). Most of these approaches have not extensively considered the molecular biological pathways or the systematic interactions between components, even though previous studies have highlighted the importance of interactions between biological components such as proteins and metabolic substrates (Jeong et al., 2000, 2001). Hub proteins having numerous interactions with other proteins are strongly related to the lethality of an organism. The complicated interactions and identifying hub nodes or central nodes can be analyzed by representing the components and their relations in a network.

In this paper, we introduce a new approach involving the network analysis of disease-related protein–protein interactions (PPIs) that we have applied to asthma. We adopted PPIs in constructing a biological network since proteins execute nearly all cell functions associated with enzymes, channels, and transporters (Alberts et al., 2002). The systematic approach proposed here is useful for analyzing the complex effects of these genes and building a framework of such relations to genes associated with asthma.

2. Materials and methods

Our protocol involved three main steps: (1) finding candidate genes from the literature database and analyzing the results of microarray experiments; (2) using these genes and PPI information to construct PPI networks; and (3) analyzing these networks qualitatively by visualization.

2.1. Finding candidate genes

Genes associated with asthma were found by searching the Online Mendelian Inheritance in Man (OMIM) database, considered to be more authentic than published papers (Hamosh et al., 2005), with a keyword 'asthma' and from the differentially expressed genes of microarray experiments of the Gene Expression Omnibus (GEO) database (Edgar et al., 2002). In the GEO database, all the data from microarray experiments related to asthma were used: GEO series (GSE) 470 and GSE473. The GSE470 (HG_U95a, Affymetrix) experiment is a comparison of epithelial cells derived from asthmatic and normal airways, and the GSE473 experiments (HG_U133a and HG_U133b, Affymetrix) are investigations of CD4+ lymphocytes from patients with and without atopy in combination with asthma. We used combined gene expression data since we wanted to construct a total framework network of asthma, and not one that reflects only the active status. The Wilcoxon rank-sum test was used to extract the significant genes associated with asthma from the microarray experiments (Walpole and Myers, 1993). The microarray data we used do not follow the normal distribution, as checked by a quantile–quantile plot and a Pearson chi-square normality test (Moore, 1986; Thode Jr., 2002). The Wilcoxon rank-sum test is a nonparametric test of the equality of means of two samples that are non-normal, and it appears to be a robust choice for microarray data since it operates on rank-transformed data (Walpole and Myers, 1993; Troyanskaya et al., 2002). We selected genes for which the probability value was $p<0.05$. The list of the selected genes is provided on the online supplementary materials with their references.

2.2. PPI networks

The construction of the PPI networks associated with asthma was based on the protein expressed from a gene being a functional molecule in a biological system. Although the mRNA expression level does not necessarily represent the true protein abundance, several studies have found mRNA and protein expression levels to be correlated (Griffin et al., 2002; Cardozo et al., 2003; Greenbaum et al., 2003; Mijalski et al., 2005). Therefore, we chose the proteins as nodes of the network corresponding to candidate genes obtained from the OMIM and GEO microarray data, and then adopted the PPIs between candidate proteins as links, which were extracted from the Human Protein Reference Database (HPRD) (Peri et al., 2004) during April 2005. The HPRD provides human PPIs found from researching of biological research papers. The reliability and quality of the interactions were ascertained by the subcellular localization information of SwissProt (http://www.ebi.ac.uk/swissprot).

2.3. Network analysis

The following measurements were used to reveal target proteins that play important roles in the network: (1) degree (or connectivity); (2) the betweenness centrality (BC); (3) the edge BC; and (4) the closeness centrality (CC). The degree tells us how many links a node has to other nodes and the degree distribution is obtained by counting the number of nodes with a given degree and dividing by the total number of nodes. The degree distribution reveals a relatively small number of highly connected nodes that are known as hubs and they play a major role as a local property in the network (Barabasi and Oltvai, 2004). On the other hand, the BC was measured to find non-hub proteins that still play important roles as a global property since the BC is a useful measurement for detecting bottlenecks in a network. For node k BC is defined as

$$b(k)=\sum_{i,j} b_{i\to j}(k)=\sum_{i,j}\frac{g^k_{i\to j}}{g_{i\to j}},$$

where $g_{i\to j}$ is the number of shortest geodesic paths from node i to node j, and $g^k_{i\to j}$ is the number of geodesic paths among $g_{i\to j}$ from node i to node j that pass through node k (Freeman, 1977; Brandes, 2001). Since we have to investigate all nodes of the network to calculate the BC, it has global properties in the network compared to the degree that can be obtained by investigating its nearby nodes, and therefore has local properties. The edge BC is defined in the same way as BC, but refers to an edge rather than a node, and the CC is the inverse of the network diameter defined as the average number of hops (jumps) through the shortest geodesic paths from node k to all other nodes. The diameter characterizes the ability of two nodes to communicate with each other: the smaller the diameter (the larger CC) is, the shorter is the expected path between them. Therefore, a large CC indicates that the node is close to the topological center of the network (Sabidussi, 1966; Freeman, 1977).

From the results of network analysis, we chose the high-degree nodes and high-centrality (BC and CC) nodes as the *key nodes* that are considered playing an important role in a network. Half of the maximum degree of the network was used as the critical point of high-degree nodes. The selected key nodes are about 10% of the largest connected cluster of the network. All key nodes are connected to each other by a link that has a large edge BC, and they form a skeleton of the network (Kim et al., 2004).

2.4. Biological function analysis of asthma

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database was searched to elucidate the function of target proteins in the biological pathway (Ogata et al., 1999), gene ontology (GO) information being used to translate lists of candidates into the associated biological phenomena (Harris et al., 2004). The statistical significance of each biological process (i.e., the probability value) was estimated by adopting the core algorithm of Onto-Express (Draghici et al., 2003) according to

$$p = 1 - \sum_{i=0}^{x-1} \binom{K}{i} \left(\frac{M}{N}\right)^i \left(1 - \frac{M}{N}\right)^{K-i}.$$

Let us consider a microarray involving N genes that are in one of two biological processes: F and non-F and among N genes, M genes are F. A subset of K genes is selected, and we observe that x of these K genes are F and want to determine the probability of this happening by chance. The probability that a certain category occurs x times just by chance in the list of differentially regulated genes is appropriately modeled by a hypergeometric distribution with parameters N, M, and K (Tavazoie et al., 1999). Based on this, the probability of having x genes or fewer in F can be calculated by the summation of the probabilities of a random list of K genes having from 1 to x genes of category F. It is well known that the hypergeometric distribution tends to the binomial distribution when N is large. Therefore, if a binomial distribution is used, the probability of having x genes in F in a set of K randomly picked genes is given by the classic formula for a binomial probability: the probability of extracting a gene from F is estimated by M/N, the ratio of F genes present on the microarray according to the above formula (Draghici et al., 2003).

3. Results and discussion

3.1. PPI network of asthma

We constructed two types of PPI network: a core network and an extended network. The nodes of the core network represent asthma-associated candidate proteins corresponding to genes obtained from a text database and microarray data, and the links between the nodes represent the PPIs. The nodes of the extended network represent not only candidate proteins but also interacting proteins, with the links again representing interactions.

The core network comprises 606 nodes: 269 isolated nodes and 337 nodes in 28 clusters, with the largest cluster containing 269 nodes. These clustered 337 nodes are connected via 406 PPIs, which corresponds to an effective mean degree of 2.4. Degree is the number of nearest neighbors of a node and effective mean degree is the average degree of all nodes except isolated ones. A search of a text database yielded 107 nodes, and 506 nodes were obtained from differentially expressed genes of microarray experiments; 7 nodes overlapped in both methods. The 337 nodes of the core network are illustrated in Fig. 1a, where the color of each node represents the subcellular location, indicating whether the PPIs are reliable based on the proximity of two interacting proteins. The extended network comprises 2438 nodes and 4029 links, with a mean degree of 3.3. The network comprises 78 clusters, including a single giant cluster that contains 2256 (92.5%) nodes.

Our PPI network associated with asthma includes several known signaling pathways of asthma (Amrani et al., 2000; Litonjua et al., 2005; Kabesch et al., 2006; Nakashima et al., 2006). First, signals from ADRB2 are delivered to various key molecules: PRKCE, PRKACA, MAPK1, and MAPK3. In this pathway, PRKCE causes airway hyper-responsiveness, PRKACA causes a relaxation to breathe, and MAPK1 and MAPK3 cause airway remodeling (Litonjua et al., 2005). This pathway is indicated with a black arrow in Fig. 1a. Second, the TLR4 signaling pathway controls an immune response through MYD88 and IRAK1 (Nakashima et al., 2006). The core network (Fig. 1a) contains the only interaction between TLR4 and MYD88 but the extended network includes its signaling pathway. Third, ILs activate the JAK-STAT pathway (Jiang et al., 2000; Kabesch et al., 2006). Since it is a well-known pathway to induce inflammation in asthma (Pernis and Rothman, 2002), we describe its mechanism revealed in our PPI network.

In our PPI network, IL4, IL13, and IL9 interact through their receptors with JAK1, which is a component of the

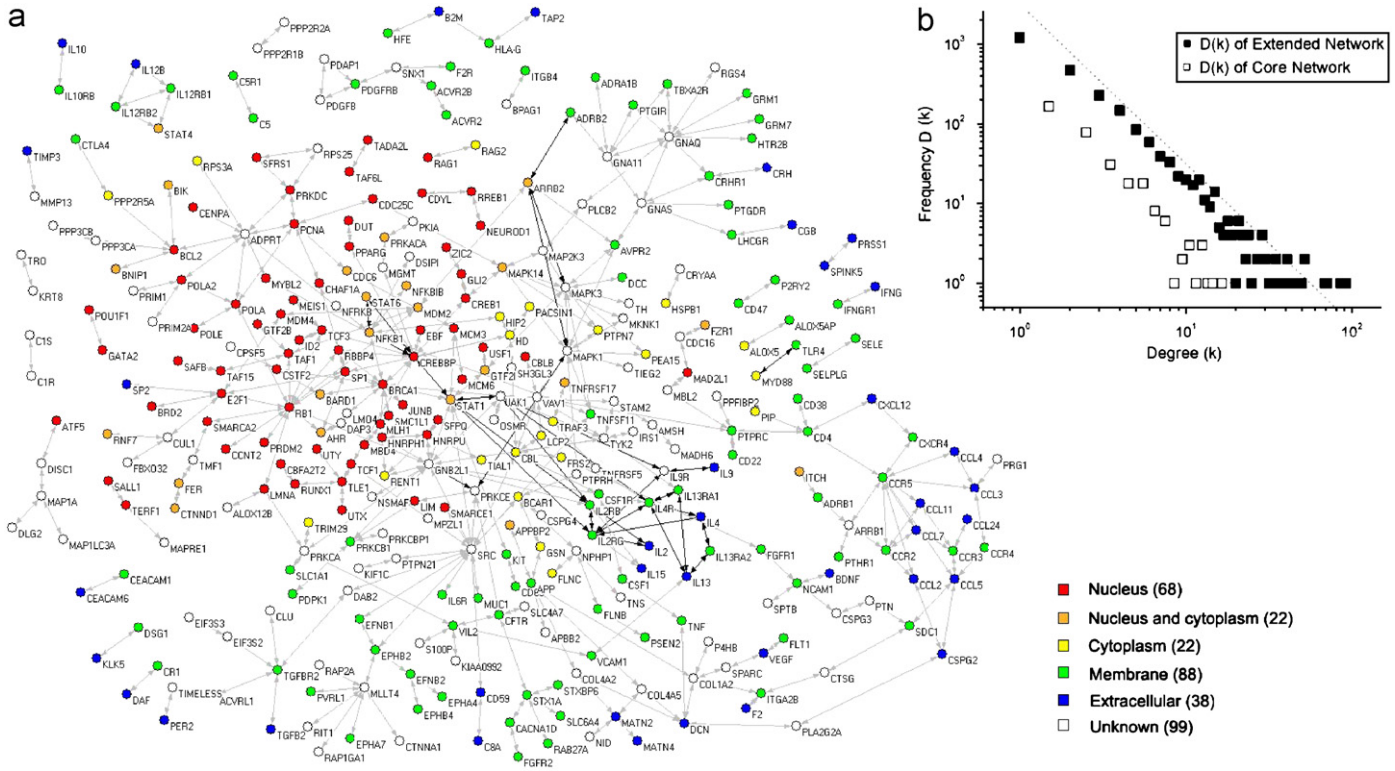

Fig. 1. Topological view of the asthma network. (a) The core network of the PPIs of asthma. The black arrows indicate the routes of the JAK-STAT pathway activated by interleukins, ADRB2 pathway, and TLR4 pathway. The color of the nodes represents the subcellular location of proteins as follows: nuclear proteins (red), proteins moving through the nuclear membrane (orange), cytoplasmic proteins (yellow), cellular membrane proteins and proteins moving through the cellular membrane (green), and extracellular proteins and peripheral membrane proteins (blue). White represents proteins for which there is no information on their subcellular locations. Mitochondrial and Golgi organelle proteins are not colored. Detailed information of the localization of proteins is available in the online supplementary materials. And the graph rendering software is Pajek (Batagelj and Mrvar, 1998). (b) The degree distributions $D(k)$ of the extended and core networks follow a power-law degree distribution, where the dotted line indicates an exponent of -2.

JAK-STAT pathway. This pathway provides one of the most direct routes of signaling ligands from cell-surface receptors to the nucleus, and recruits several genes related to the induction of allergic inflammation (Alberts et al., 2002); the signaling ligands are interferons, hormones, and ILs (including IL4, IL13, and IL9) (Darnell et al., 1994). IL4, IL13, and IL9 are all proinflammatory cytokines (Barnes, 2004). IL4 induces eosinophilic inflammation and promotes the differentiation of Th2 cells, acting at a proximal and crucial point in the allergic response (Gavett et al., 1997). IL13 and IL4 are closely related cytokines because they share a surface receptor, IL4R alpha (Jiang et al., 2000). IL13 produces immunoglobulin E (IgE) from B lymphocytes (Wills-Karp et al., 1998). IL9, a Th2 cytokine, enhances mast-cell mediator release and IgE production, and induces mucus hypersecretion (Levitt et al., 1999). Our resulting network, shown in Fig. 1a, contains these ILs and the JAK-STAT pathway (indicated by the black arrows).

3.2. The key nodes of PPI network of asthma

Both the core and extended networks follow a power-law ($D(k)\sim k^{-\gamma}$) degree distribution (Fig. 1b), where γ is the degree exponent and $\sim$ indicates 'proportional to' (Barabasi and Oltvai, 2004). Our results are consistent with other researchers' results of other biological networks (Pastor-Satorras et al., 2003; Lee et al., 2006; Diao et al., 2007; Kim and Jeong, 2007). Moreover, we checked that the overall source PPI network also follows a power-law degree distribution with the exponent about -1.8 (Stelzl et al., 2005), which is similar with the exponents of our sampled networks: the exponent of the extended network is 2.0 (standard error $= 0.1$ and coefficient of determination $R^2 = 0.94$) and that of the core network is -2.1 (standard error $= 0.2$ and $R^2 = 0.91$) by the least square fit (Son and Jeong, 2006; Kim and Jeong, 2007). Therefore, the overall PPI network and our two sampled networks have characters of scale-free networks whose degree distribution approximates a power law.

With the same number of nodes and links, the probability that a node is highly connected is statistically more significant in a scale-free network than in a random graph. The properties of such a network are often determined by a relatively small number of highly connected nodes, which are called hubs (Barabasi and Oltvai, 2004). Therefore, in a scale-free network, the hubs are more important than other less connected nodes. The fact that the hub proteins in the cell are the most important for its survival was verified in a *Saccharomyces*

cerevisiae PPI network (Jeong et al., 2001). In the simulation of attack tolerance of complex networks, when hub nodes were attacked, the scale-free network was broken into many isolated fragments (Albert et al., 2000). In the asthma core network, we also found the same results. When we removed the 13 largest degree nodes (about 4%) among all nodes, the largest cluster with 269 nodes broke into 68 fragments among which the largest one had only 24 nodes.

Table 1
Hub nodes and nodes with large betweenness centrality (BC)

Gene	Hub node	Large-BC node
SRC	Yes	Yes
GNB2L1	Yes	Yes
RB1	Yes	Yes
STAT1	Yes	
CCR5	Yes	
VAV1		Yes
MAPK1		Yes
PTPRC		Yes
BCAR1		Yes

A hub node is statistically significant in a scale-free network, and a node with a large BC is near the global center of a network. SRC, GNB2L1, and RB1 are hub nodes that have a large BC.

The hub nodes and nodes with a large BC are summarized in Table 1. The BC is used to determine the global central node. The effect of removing nodes of a large BC is similar to that of removing hub nodes because nodes with a large BC are usually hub nodes (Son et al., 2004). However, when nodes with a large BC and a small degree are removed, the network was broken into several modules, not many isolated fragments (Girvan and Newman, 2002). Although they are not hub nodes, they have a role to connect several modules like a bridge in a network. Therefore, we used the BC to determine key nodes. We confirmed that the hub and large-BC nodes are located in the topological center of the network by examining their CC. A skeleton is formed from the *hot links* (which have a

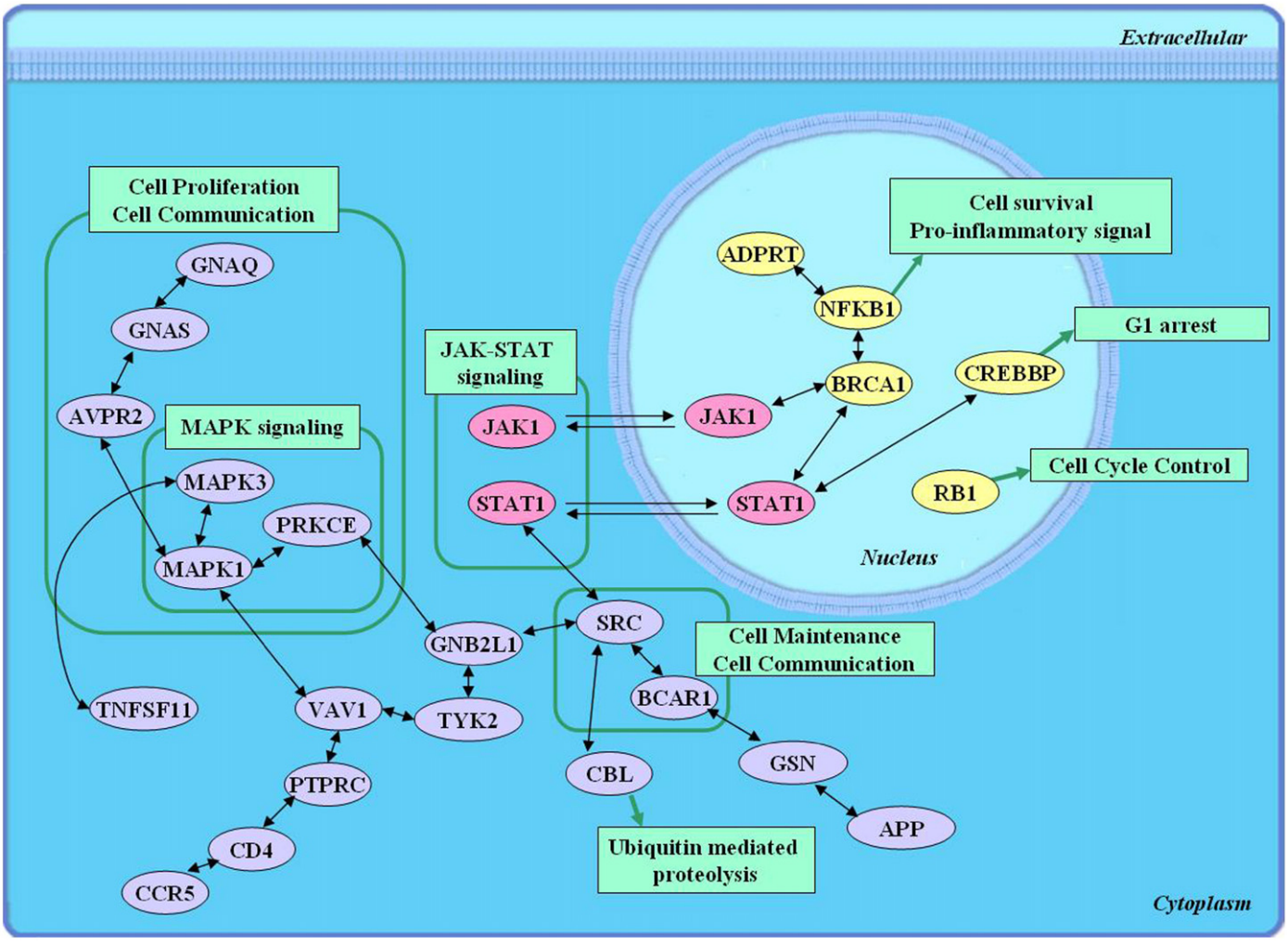

Fig. 2. The key nodes of the core asthma network and the skeleton of the core network of the PPIs of asthma. Each node represents a protein, where the node name follows the name of the gene for convenience. The yellow, lavender, and hot-pink bubbles are proteins that are in the nucleus, cytosol, and moving into nuclear membranes, respectively. Therefore, the hot-pink proteins link the networks present in the nucleus and cytosol.

large edge BC) that connect the key nodes of the hubs and large-BC nodes (Kim et al., 2004). Fig. 2 is a schematic diagram of the key nodes from the core network of asthma using only the *hot links*.

The key nodes play an important role in constructing the network with *hot links*. The roles of the key nodes of our PPI network are cellular proliferation, maintenance, and communication (Fig. 2). The cellular proliferation is controlled by three genes: NFKB1, CREBBP, and RB1 (Martinon et al., 2000; Alberts et al., 2002; MacPartlin et al., 2005). NFKB1 is a proinflammatory signaling molecule that is associated with cell survival (Martinon et al., 2000). CREBBP plays a role in controlling G1 arrest in the cell cycle, with RB1 controlling the cell cycle (Alberts et al., 2002; MacPartlin et al., 2005). SRC and BCAR1 in the focal adhesion pathway play roles in cell maintenance (Lee and Juliano, 2004; Mitra et al., 2005). With dynamin, CBL forms a SRC kinase-sensitive complex that is important in the assembly and remodeling of the actin cytoskeleton in cell communication (Bruzzaniti et al., 2005). The MAPK signaling pathway and SRC also play roles in cell communication (Alberts et al., 2002).

The results of searching the KEGG pathway database and analyzing GO annotation information of two sets of microarray data also agree with the above results. KEGG pathways are related to several aspects of cell communication and signal transduction: MAPK signaling, JAK-STAT, calcium signaling, tight junctions, adherens junctions, and focal adhesion. The GO analysis was applied to 509 genes extracted from two sets of microarray data (GSE 470 and GSE473). In all microarrays, the signal transduction and cellular physiological processes are highly significant (see Table 2). For example, the metabolism and regulation of physiological processes are significant in the GSE473 (HG_U133b array) and GES470, neurophysiological processes are significant in the GSE473 (HG_U133b array), and development is significant in the GSE470.

Table 2
Gene ontology analysis of genes extracted from microarray data

Biological process	*p*-Value	GSE473	
	GSE470	(HG_U133a)	(HG_U133b)
Cellular process	1.0E–6	1.0E–6	1.0E–6
Cell communication	1.0E–6	1.4E–3	1.0E–6
Signal transduction	2.6E–4	4.4E–3	1.0E–6
Cellular physiological process	1.0E–6	1.9E–4	1.0E–6
Development	1.0E–6	8.5E–4	7.7E–3
Metabolism	1.0E–6	Not significant	1.0E–6
Regulation of biological process	1.0E–6	3.5E–2	1.0E–6
Neurophysiological process	Not significant	Not significant	9.7E–4

Gene Ontology information was used to translate lists of genes differentially expressed in the microarray data of asthma patients into pertinent biological phenomena. The following three microarrays were analyzed: HG_U133a, HG_U133b, and HG_95a.

3.3. Putative target genes for asthma

The protein nodes in a biological network do not play an equally important role: hub proteins in the cell are the most important for its survival and the communication of protein nodes (Albert et al., 2000; Jeong et al., 2001). Since the degree and the BC reflect the local and global importance of the node, respectively, both variables need to be measured in order to find target genes. Therefore, we hypothesize that the nodes corresponding to target genes with large degrees and large BC have especially important roles in biological systems.

We identified seven nodes as target genes: SRC, CREBBP, MAPK1, GNB2L1, VAV1, CBL, and BRCA1. In Table 3, we confirmed that these proteins are both biologically important and related to asthma by checking the relevant literature for PPIs with known asthma drug targets (Kurosaki et al., 1994; Turki et al., 1995; Luttrell et al., 1999; Yarwood et al., 1999; Mullen et al., 2001; Barnes, 2004). Among them, CREBBP, VAV1, and CBL are so-called date hubs that participate in a wide range of interconnections required for the global organization of biological modules in the entire proteome network (Han et al., 2004). The remaining four proteins could not be confirmed as either party hubs or date hubs because the sets of microarray data did not provide the correlation values of the PPIs for each hub. Party hubs represent integral elements within distinct modules (Han et al., 2004).

Table 3
The nodes corresponding to the target genes with a large degree and large BC

Gene	Description	Drug target
SRC	ADRB2 binds beta-arrestin-1, which then binds SRC at its amino terminus	SYK
CREBBP	Flow cytometric analysis showed that IL12 enhances and prolongs interferon gamma expression in Tbet-differentiated Th1 cells through an interaction with CREBBP	
MAPK1	A key molecule of the MAPK signaling pathway	
GNB2L1	GNB2L1 plays a very important role in the network, interacting with key proteins in signaling such as protein kinase C, JAK1, STAT1, and SRC	IL 4 receptor, PDE4D
VAV1	Vav1 oncogene	
CBL	Ubiquitin ligase	
BRCA1	Tumor suppressor	NFKB1

If a target gene has links to already known drug-target genes of asthma as nearest neighbors, the genes and their descriptions are noted in the third column.

Table 4
There are six interesting nodes that are not important or do not appear in the core network, but have a large BC in the extended network

Gene	Description	Drug target
GRB2	IL5 activates a signal transduction pathway utilizing the adaptor protein GRB2 in human eosinophil	ADRB2, CD28 antigen
EGFR	EGFR was reported as an asthma-related gene and its transactivation of EGFR through CCR3 is a critical pathway that elicits mitogen-activated protein kinase activation and cytokine production in bronchial epithelial cells	
PCNA	In response to DNA damage, this protein is ubiquitinated and is involved in the RAD6-dependent DNA repair pathway.	
SP1	Phorbol 12-myristate 13-acetate induces MUC5AC respiratory mucin in human bronchial epithelial cells, via SP1-dependent mechanisms	
PRKDC		LYN
VIL2	This protein serves as an intermediate between the plasma membrane and the actin cytoskeleton. It plays a key role in cell surface adhesion, migration, and organization	ICAM1, VCAM1

If an interesting gene has links to already known drug-target genes of asthma as nearest neighbors, the genes and their descriptions are noted in the third column.

As well as target proteins, there are six interesting protein nodes that are not important or do not appear in the core network but which are large-BC nodes in the extended network: GRB2, EGFR, PCNA SP1, PRKDC, and VIL2 (Table 4). While they are not central proteins in the core network, they are important in the communication and connections between both well-known and unknown proteins, and they could be useful in identifying other candidate target genes.

3.4. The biological importance of putative target genes for asthma

We identified seven nodes as target genes (SRC, CREBBP, MAPK1, GNB2L1, VAV1, CBL, and BRCA1) and six nodes as interesting genes (GRB2, EGFR, PCNA, SP1, PRKDC, and VIL2). The biological importance of the three target proteins is as follows: SRC relays the activating signaling pathway. ADRB2 agonists are the most widely used agents in the treatment of asthma, and a single-nucleotide polymorphism of ADRB2 (ARG16GLY) is genetically susceptible to nocturnal asthma (Turki et al., 1995; Barnes, 2004). Activated ADRB2 binds beta-arrest-in-1, which then binds SRC at its amino terminus. This interaction allows for beta-2-adrenegic activation of MAPK1 and MAPK3 (Luttrell et al., 1999). In addition, SRC interacts with SYK, which is an asthma drug target (Kurosaki et al., 1994; Barnes, 2004). SYK is pivotal in the signaling of the high-affinity IgE receptor in mast cells (Barnes, 2004). Through interaction with CREBBP, IL12 enhances and prolongs interferon gamma expression in Tbet-differentiated Th1 cells. Tbet is the Th1-specific T-box transcription factor that controls the expression of the Th1 cytokine, interferon gamma (Mullen et al., 2001). Therefore, we consider that SRC, CREBBP, and MAPK1 play biologically important roles in the asthma pathway in accordance with our hypothesis.

GNB2L1 has an especially important role in our PPI network. It interacts with key proteins in signaling such as protein kinase C, JAK1, STAT1, and SRC, and also with asthma drug targets: IL4 receptor and PDE4D (Yarwood et al., 1999; Usacheva et al., 2003). PDE4 inhibitors may be employed in anti-inflammatory treatment in asthma, particularly as there is some evidence for the overexpression of PDE4 in atopic patients (Barnes, 2004). GNB2L1 appears to play key roles in interacting with key proteins and asthma drug targets with many links and, moreover, it exhibits large degree and BC. However, research on the association of GNB2L1 with asthma is yet to be reported. Accordingly, it would be valuable to investigate the function of GNB2L1 in the asthma pathway. As for SRC, CREBBP, MAPK1, and GNB2L1, we consider that VAV1, CBL, and BRCA1 are important proteins despite them having received relatively little attention in asthma research. Therefore, we contend that these four proteins—VAV1, CBL, BRCA1, and GNB2L1—are also important target proteins in the asthma pathway, and should be investigated further.

The two interesting genes, GRB2 and EGFR, do not appear in the core network but are incorporated in the extended network through PPIs and have a large BC. The adaptor protein GRB2 is utilized in IL5 activation of a signal transduction pathway in human eosinophils (Bates et al., 1998). In addition, GRB2 interacts with ADRB2 and CD28, which are asthma drug targets (Karoor et al., 1998; Okkenhaug and Rottapel, 1998; Barnes, 2004). EGFR was reported as an asthma-related gene and its transactivation through chemokine receptor 3 is a critical pathway that elicits MAPK activation and cytokine production in bronchial epithelial cells (Adachi et al., 2004; Malerba and Pignatti, 2005). Even though GRB2 and EGFR did not appear in the core network, we considered them as target nodes that were identified during the analysis of the extended network. It has been confirmed that GRB2 and EGFR are related to asthma signaling due to PPIs with asthma drug targets (Bates et al., 1998; Karoor et al., 1998; Okkenhaug and Rottapel, 1998; Adachi et al., 2004; Barnes, 2004; Malerba and Pignatti, 2005).

Similar to GRB2 and EGFR, the expression of PCNA is correlated with the epithelium thickness in corticosteroid-dependent asthmatic subjects, and the expressions of PCNA, NFKB, and CD40-L are higher in

these subjects than in inhaled corticosteroid-treated asthmatic, untreated asthmatic, and control subjects (Vignola et al., 2001). In the case of SP1, phorbol 12-myristate 13-acetate induces MUC5AC respiratory mucin in human bronchial epithelial cells, via SP1-dependent mechanisms (Hewson et al., 2004). PRKDC interacts with the asthma drug targets LYN (Kumar et al., 1998). VIL2 also interacts with the asthma drug targets ICAM1, and VCAM1 (Kumar et al., 1998; Barreiro et al., 2002; Barnes, 2004). Therefore, we suggest that PCNA, SP1, PRKDC, and VIL2 as well as GRB2 and EGFR constitute useful target proteins for asthma research.

3.5. Dependence on prior knowledge

To extract candidate proteins related to asthma, our approach requires both the OMIM database to be read and experimental results (microarray data) to be obtained in order to decipher the entire interaction structure. When we constructed the asthma network using only microarray data (i.e., without OMIM data), the single giant cluster was fragmented into several parts. This fragmentation also occurred when constructing the network with only protein nodes found from OMIM. Furthermore, constructing the network from the microarray data alone reduced the number of nodes from 2438 to 2153 (88.3%). To examine the dependence on the data, we investigated how many nodes overlapped with OMIM data when the network was constructed with only microarray data: there were 45 (42.1%) overlapped nodes among the 104 nodes that were already known in the OMIM database. Eleven of the remaining 62 genes were not tested in the microarray experiments, and the others were not significant since their probability values were larger than the significant level. Therefore, we believe that our systematic combined data analysis method can shed new light on asthma.

4. Conclusions

We have extracted data related to asthma—text data from the OMIM database, microarray experiments from the GEO database, and PPIs from the HPRD—and subsequently constructed PPI networks that displayed degrees with a power-law distribution, with an exponent of approximately −2. The PPI networks represent the well-known knowledge, such as the proinflammatory and cellular proliferation signaling pathways of asthma. Applying the network analysis to the constructed networks revealed putative target genes (SRC, CREBBP, MAPK1, GNB2L1, VAV1, CBL, and BRCA1) and other genes (GRB2, EGFR, PCNA, SP1, PRKDC, and VIL2) that are biologically significant and topologically centered in asthma networks. Putative target genes with a large degree and a large BC are locally and globally important nodes in the core network. They play biologically important roles in the asthma network like relaying the activating signals along the pathways. Other genes do not appear in the core network but they have a large BC in the extended network. While they are not central nodes, they play important roles in the communication and connections between both well-known drug targets and unknown genes.

The network approach has these advantages: it easily represents the complex relationship of proteins by PPI links, helping us to understand how signaling pathways associated with a disease are connected, and making it possible to find disease target genes such as putative target genes and other genes by network analysis. Therefore, this network approach represents an alternative method for analyzing the complex effects of candidate genes that are related to complex diseases, and is also useful in identifying target genes for research into new drug targets.

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References

Adachi, T., Cui, C.H., Kanda, A., Kayaba, H., Ohta, K., Chihara, J., 2004. Activation of epidermal growth factor receptor via CCR3 in bronchial epithelial cells. Biochem. Biophys. Res. Commun. 320, 292–296.

Albert, R., Jeong, H., Barabasi, A.L., 2000. Error and attack tolerance of complex networks. Nature 406, 378–382.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. Molecular Biology of the Cell. Garland Science.

Amrani, Y., Chen, H., Panettieri Jr.,, R.A., 2000. Activation of tumor necrosis factor receptor 1 in airway smooth muscle: a potential pathway that modulates bronchial hyper-responsiveness in asthma? Respir. Res. 1, 49–53.

Barabasi, A.L., Oltvai, Z.N., 2004. Network biology: understanding the cell's functional organization. Nat. Rev. Genet. 5, 101–113.

Barnes, P.J., 2004. New drugs for asthma. Nat. Rev. Drug. Discov. 3, 831–844.

Barreiro, O., Yanez-Mo, M., Serrador, J.M., Montoya, M.C., Vicente-Manzanares, M., Tejedor, R., Furthmayr, H., Sanchez-Madrid, F., 2002. Dynamic interaction of VCAM-1 and ICAM-1 with moesin and ezrin in a novel endothelial docking structure for adherent leukocytes. J. Cell Biol. 157, 1233–1245.

Batagelj, V., Mrvar, A., 1998. Pajek: program for large network analysis. Connections 21, 47–57.

Bates, M.E., Busse, W.W., Bertics, P.J., 1998. Interleukin 5 signals through Shc and Grb2 in human eosinophils. Am. J. Respir. Cell Mol. Biol. 18, 75–83.

Brandes, U., 2001. A faster algorithm for betweenness centrality. J. Math. Sociol. 25, 163–177.

Bruzzaniti, A., Neff, L., Sanjay, A., Horne, W.C., De Camilli, P., Baron, R., 2005. Dynamin forms a src kinase-sensitive complex with Cbl and regulates podosomes and osteoclast activity. Mol. Biol. Cell 16, 3301–3313.

Cardozo, A.K., Berthou, L., Kruhoffer, M., Orntoft, T., Nicolls, M.R., Eizirik, D.L., 2003. Gene microarray study corroborates proteomic findings in rodent islet cells. J. Proteome Res. 2, 553–555.

Darnell Jr.,, J.E., Kerr, I.M., Stark, G.R., 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264, 1415–1421.

Diao, Y., Li, M., Feng, Z., Yin, J., Pan, Y., 2007. The community structure of human cellular signaling network. J. Theor. Biol. 247, 608–615.

Draghici, S., Khatri, P., Martins, R.P., Ostermeier, G.C., Krawetz, S.A., 2003. Global functional profiling of gene expression. Genomics 81, 98–104.

Edgar, R., Domrachev, M., Lash, A.E., 2002. Gene expression omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30, 207–210.

Freeman, L.C., 1977. A set of measures of centrality based on betweenness. Sociometry 40, 35–41.

Freudenberg, J., Propping, P., 2002. A similarity-based method for genome-wide prediction of disease-relevant human genes. Bioinformatics 18 (Suppl. 2), S110–S115.

Gavett, S.H., O'Hearn, D.J., Karp, C.L., Patel, E.A., Schofield, B.H., Finkelman, F.D., Wills-Karp, M., 1997. Interleukin-4 receptor blockade prevents airway responses induced by antigen challenge in mice. Am. J. Physiol. 272, L253–L261.

Girvan, M., Newman, M.E., 2002. Community structure in social and biological networks. Proc. Natl. Acad. Sci. 99, 7821–7826.

Greenbaum, D., Colangelo, C., Williams, K., Gerstein, M., 2003. Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biol. 4, 117.

Griffin, T.J., Gygi, S.P., Ideker, T., Rist, B., Eng, J., Hood, L., Aebersold, R., 2002. Complementary profiling of gene expression at the transcriptome and proteome levels in *Saccharomyces cerevisiae*. Mol. Cell Proteomics 1, 323–333.

Hamosh, A., Scott, A.F., Amberger, J.S., Bocchini, C.A., McKusick, V.A., 2005. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res. 33, D514–D517.

Han, J.D., Bertin, N., Hao, T., Goldberg, D.S., Berriz, G.F., Zhang, L.V., Dupuy, D., Walhout, A.J., Cusick, M.E., Roth, F.P., Vidal, M., 2004. Evidence for dynamically organized modularity in the yeast protein–protein interaction network. Nature 430, 88–93.

Harris, M.A., Clark, J., Ireland, A., Lomax, J., Ashburner, M., Foulger, R., Eilbeck, K., Lewis, S., Marshall, B., Mungall, C., Richter, J., Rubin, G.M., Blake, J.A., Bult, C., Dolan, M., Drabkin, H., Eppig, J.T., Hill, D.P., Ni, L., Ringwald, M., Balakrishnan, R., Cherry, J.M., Christie, K.R., Costanzo, M.C., Dwight, S.S., Engel, S., Fisk, D.G., Hirschman, J.E., Hong, E.L., Nash, R.S., Sethuraman, A., Theesfeld, C.L., Botstein, D., Dolinski, K., Feierbach, B., Berardini, T., Mundodi, S., Rhee, S.Y., Apweiler, R., Barrell, D., Camon, E., Dimmer, E., Lee, V., Chisholm, R., Gaudet, P., Kibbe, W., Kishore, R., Schwarz, E.M., Sternberg, P., Gwinn, M., Hannick, L., Wortman, J., Berriman, M., Wood, V., de la Cruz, N., Tonellato, P., Jaiswal, P., Seigfried, T., White, R., 2004. The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res. 32, D258–D261.

Hewson, C.A., Edbrooke, M.R., Johnston, S.L., 2004. PMA induces the MUC5AC respiratory mucin in human bronchial epithelial cells, via PKC, EGF/TGF-alpha, Ras/Raf, MEK, ERK and Sp1-dependent mechanisms. J. Mol. Biol. 344, 683–695.

Jeong, H., Tombor, B., Albert, R., Oltvai, Z.N., Barabasi, A.L., 2000. The large-scale organization of metabolic networks. Nature 407, 651–654.

Jeong, H., Mason, S.P., Barabasi, A.L., Oltvai, Z.N., 2001. Lethality and centrality in protein networks. Nature 411, 41–42.

Jiang, H., Harris, M.B., Rothman, P., 2000. IL-4/IL-13 signaling beyond JAK/STAT. J. Allergy Clin. Immunol. 105, 1063–1070.

Kabesch, M., Schedel, M., Carr, D., Woitsch, B., Fritzsch, C., Weiland, S.K., von Mutius, E., 2006. IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma. J. Allergy Clin. Immunol. 117, 269–274.

Karoor, V., Wang, L., Wang, H.Y., Malbon, C.C., 1998. Insulin stimulates sequestration of beta-adrenergic receptors and enhanced association of beta-adrenergic receptors with Grb2 via tyrosine 350. J. Biol. Chem. 273, 33035–33041.

Kim, D.H., Noh, J.D., Jeong, H., 2004. Scale-free trees: the skeletons of complex networks. Phys. Rev. E Stat. Nonlinear Soft Matter Phys. 70, 046126.

Kim, P.J., Jeong, H., 2007. Reliability of rank order in sampled networks. Eur. Phys. J. B 55, 109–114.

Kumar, S., Pandey, P., Bharti, A., Jin, S., Weichselbaum, R., Weaver, D., Kufe, D., Kharbanda, S., 1998. Regulation of DNA-dependent protein kinase by the Lyn tyrosine kinase. J. Biol. Chem. 273, 25654–25658.

Kurosaki, T., Takata, M., Yamanashi, Y., Inazu, T., Taniguchi, T., Yamamoto, T., Yamamura, H., 1994. Syk activation by the Src-family tyrosine kinase in the B cell receptor signaling. J. Exp. Med. 179, 1725–1729.

Lee, J.W., Juliano, R., 2004. Mitogenic signal transduction by integrin- and growth factor receptor-mediated pathways. Mol. Cells 17, 188–202.

Lee, S.H., Kim, P.J., Jeong, H., 2006. Statistical properties of sampled networks. Phys. Rev. E Stat. Nonlinear Soft Matter Phys. 73, 016102.

Levitt, R.C., McLane, M.P., MacDonald, D., Ferrante, V., Weiss, C., Zhou, T., Holroyd, K.J., Nicolaides, N.C., 1999. IL-9 pathway in asthma: new therapeutic targets for allergic inflammatory disorders. J. Allergy Clin. Immunol. 103, S485–S491.

Litonjua, A., Thorn, C.F., Liggett, S.B., 2005. Beta-Agonist and Beta-Blocker Pathway. PharmGKB.

Luttrell, L.M., Ferguson, S.S., Daaka, Y., Miller, W.E., Maudsley, S., Della Rocca, G.J., Lin, F., Kawakatsu, H., Owada, K., Luttrell, D.K., Caron, M.G., Lefkowitz, R.J., 1999. Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. Science 283, 655–661.

MacPartlin, M., Zeng, S., Lee, H., Stauffer, D., Jin, Y., Thayer, M., Lu, H., 2005. p300 regulates p63 transcriptional activity. J. Biol. Chem. 280, 30604–30610.

Malerba, G., Pignatti, P.F., 2005. A review of asthma genetics: gene expression studies and recent candidates. J. Appl. Genet. 46, 93–104.

Martinon, F., Holler, N., Richard, C., Tschopp, J., 2000. Activation of a pro-apoptotic amplification loop through inhibition of NF-kappaB-dependent survival signals by caspase-mediated inactivation of RIP. FEBS Lett. 468, 134–136.

Mijalski, T., Harder, A., Halder, T., Kersten, M., Horsch, M., Strom, T.M., Liebscher, H.V., Lottspeich, F., de Angelis, M.H., Beckers, J., 2005. Identification of coexpressed gene clusters in a comparative analysis of transcriptome and proteome in mouse tissues. Proc. Natl. Acad. Sci. 102, 8621–8626.

Mitra, S.K., Hanson, D.A., Schlaepfer, D.D., 2005. Focal adhesion kinase: in command and control of cell motility. Nat. Rev. Mol. Cell Biol. 6, 56–68.

Moore, D.S., 1986. Goodness-of-Fit Techniques. Marcel Dekker, New York.

Mullen, A.C., High, F.A., Hutchins, A.S., Lee, H.W., Villarino, A.V., Livingston, D.M., Kung, A.L., Cereb, N., Yao, T.P., Yang, S.Y., Reiner, S.L., 2001. Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. Science 292, 1907–1910.

Nakashima, K., Hirota, T., Obara, K., Shimizu, M., Jodo, A., Kameda, M., Doi, S., Fujita, K., Shirakawa, T., Enomoto, T., Kishi, F., Yoshihara, S., Matsumoto, K., Saito, H., Suzuki, Y., Nakamura, Y., Tamari, M., 2006. An association study of asthma and related phenotypes with polymorphisms in negative regulator molecules of the TLR signaling pathway. J. Hum. Genet. 51, 284–291.

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., Kanehisa, M., 1999. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 27, 29–34.

Okkenhaug, K., Rottapel, R., 1998. Grb2 forms an inducible protein complex with CD28 through a Src homology 3 domain–proline interaction. J. Biol. Chem. 273, 21194–21202.

Pastor-Satorras, R., Smith, E., Sole, R.V., 2003. Evolving protein interaction networks through gene duplication. J. Theor. Biol. 222, 199–210.

Payne, D.N., Rogers, A.V., Adelroth, E., Bandi, V., Guntupalli, K.K., Bush, A., Jeffery, P.K., 2003. Early thickening of the reticular basement membrane in children with difficult asthma. Am. J. Respir. Crit. Care Med. 167, 78–82.

Perez-Iratxeta, C., Bork, P., Andrade, M.A., 2002. Association of genes to genetically inherited diseases using data mining. Nat. Genet. 31, 316–319.

Peri, S., Navarro, J.D., Kristiansen, T.Z., Amanchy, R., Surendranath, V., Muthusamy, B., Gandhi, T.K., Chandrika, K.N., Deshpande, N., Suresh, S., Rashmi, B.P., Shanker, K., Padma, N., Niranjan, V., Harsha, H.C., Talreja, N., Vrushabendra, B.M., Ramya, M.A., Yatish, A.J., Joy, M., Shivashankar, H.N., Kavitha, M.P., Menezes, M., Choudhury, D.R., Ghosh, N., Saravana, R., Chandran, S., Mohan, S., Jonnalagadda, C.K., Prasad, C.K., Kumar-Sinha, C., Deshpande, K.S., Pandey, A., 2004. Human protein reference database as a discovery resource for proteomics. Nucleic Acids Res. 32, D497–D501.

Pernis, A.B., Rothman, P.B., 2002. JAK-STAT signaling in asthma. J. Clin. Invest. 109, 1279–1283.

Sabidussi, G., 1966. The centrality index of a graph. Psychometrika 31, 581–603.

Son, S.W., Jeong, H., 2006. Reconstruction of a genetic network from gene perturbation data. J. Kor. Phys. Soc. 48, S208.

Son, S.W., Kim, D.H., Ahn, Y.Y., Jeong, H., 2004. Response network emerging from simple perturbation. J. Kor. Phys. Soc. 44, 628–632.

Stelzl, U., Worm, U., Lalowski, M., Haenig, C., Brembeck, F.H., Goehler, H., Stroedicke, M., Zenkner, M., Schoenherr, A., Koeppen, S., Timm, J., Mintzlaff, S., Abraham, C., Bock, N., Kietzmann, S., Goedde, A., Toksoz, E., Droege, A., Krobitsch, S., Korn, B., Birchmeier, W., Lehrach, H., Wanker, E.E., 2005. A human protein–protein interaction network: a resource for annotating the proteome. Cell 122, 957–968.

Tattersfield, A.E., Knox, A.J., Britton, J.R., Hall, I.P., 2002. Asthma. Lancet 360, 1313–1322.

Tavazoie, S., Hughes, J.D., Campbell, M.J., Cho, R.J., Church, G.M., 1999. Systematic determination of genetic network architecture. Nat. Genet. 22, 281–285.

Thode Jr.,, H.C., 2002. Testing for Normality. Marcel Dekker, New York.

Tiffin, N., Kelso, J.F., Powell, A.R., Pan, H., Bajic, V.B., Hide, W.A., 2005. Integration of text- and datamining using ontologies successfully selects disease gene candidates. Nucleic Acids Res. 33, 1544–1552.

Troyanskaya, O.G., Garber, M.E., Brown, P.O., Botstein, D., Altman, R.B., 2002. Nonparametric methods for identifying differentially expressed genes in microarray data. Bioinformatics 18, 1454–1461.

Turki, J., Pak, J., Green, S.A., Martin, R.J., Liggett, S.B., 1995. Genetic polymorphisms of the beta 2-adrenergic receptor in nocturnal and nonnocturnal asthma. Evidence that Gly16 correlates with the nocturnal phenotype. J. Clin. Invest. 95, 1635–1641.

Usacheva, A., Tian, X., Sandoval, R., Salvi, D., Levy, D., Colamonici, O.R., 2003. The WD motif-containing protein RACK-1 functions as a scaffold protein within the type I IFN receptor-signaling complex. J. Immunol. 171, 2989–2994.

Vignola, A.M., Chiappara, G., Siena, L., Bruno, A., Gagliardo, R., Merendino, A.M., Polla, B.S., Arrigo, A.P., Bonsignore, G., Bousquet, J., Chanez, P., 2001. Proliferation and activation of bronchial epithelial cells in corticosteroid-dependent asthma. J. Allergy Clin. Immunol. 108, 738–746.

Walpole, R.E., Myers, R.H., 1993. Probability and Statistics for Engineers and Scientists. Macmillan, New York.

Wills-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T.Y., Karp, C.L., Donaldson, D.D., 1998. Interleukin-13: central mediator of allergic asthma. Science 282, 2258–2261.

Yarwood, S.J., Steele, M.R., Scotland, G., Houslay, M.D., Bolger, G.B., 1999. The RACK1 signaling scaffold protein selectively interacts with the cAMP-specific phosphodiesterase PDE4D5 isoform. J. Biol. Chem. 274, 14909–14917.