Article

Development of a network model and investigation of hub proteins for asthma exacerbation

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Abstract

Asthma is a long-term inflammatory disease known to affect the airways in the lungs with variable and recurring symptoms. A large number of genes, transcription factors and proteins are involved in this process, which makes it polygenic. We investigated the responsible proteins for asthma by conducting in-depth analysis in the database of asthma proteins and subsequently examining their differential role in disease progression following a computational biological approach. Firstly, we constructed a protein-protein interaction network among 1152 proteins, and identified top 20 high degree nodes (known as hubs); considering threshold score of ≥100 by using Cytoscape 3.1.0 software package. Also we identified seven asthma signal transduction pathways from KEGG database and compared them with the pathways derived from NetWalker platform to determine the constituted proteins. Secondly, we conducted MCODE (molecular complex detection) analysis that divided the network into 27 clusters having threshold score of ≥4.0. These individual clusters of constituted proteins were compared with the hubs and the results demonstrated their functional role in asthma. We also identified the proteins involved in the regulatory, reactome and metabolic reaction interaction for asthma exacerbation, classified different lung functional roles of these proteins, and found hyper-geometric *p*-value of ≤0.05. Thus, our in-depth analysis suggests some important consequences for interpreting the resulting data significantly and gives more insight about asthma exacerbation.

Keywords asthma; protein interaction network; molecular complex; signal transduction pathways.

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1 Introduction

Asthma is a chronic airway inflammatory obstruction which affects more than 300 million people worldwide with emerging prevalence (Sullivan et al., 2016). Understanding asthma in human is very challenging although some molecular and immunological studies have already started to reveal the underlying mechanism of asthma using animal models and cell culture techniques (Zosky and Sly, 2007). But in human, asthmatic process is not entirely speculated; therefore, molecular mechanism of diseases and identification of their interaction is highly necessary (Elias et al., 1999). This complex polygenic disease is regulated by multiple gene-gene and gene-environment interactions (Ober and Vercelli, 2011; Holloway et al., 2010). Genome-wide association study (GWAS) identified that over 100 genes and 150 genes are interconnected with asthma in human and animal models respectively (Ober and Hoffjan, 2006) which indicates that asthma is a complex polygenic disease (Van Eerdewegh et al., 2002; Xu et al., 2002). The genome wide association study and candidate gene study have already attempted to reveal the complexity of this disease (Dahlin and Kelan, 2012). The perturbation of high throughput data profiling and computational modeling through network biology approach can enrich the underlying principle of this disease more advantageously (Ijaz et al., 2014).

Previously a single gene or protein was thought to be responsible for a specific disease. But nowadays not a single gene but a set of interacting genes and proteins are readily used to characterize the pathways and pathological processes of a disease (Strohman et al., 2002). A plethora of computational data analysis methods has emerged to minimize the complexity of determining the biomolecular interaction of diseases.

Considering network biology approach it is revealed that a complex network is formed by multiple numbers of nodes which are connected through edges (Zhang, 2012, 2016a, 2016b, 2018). This network is scale-free and contains low number of densely connected nodes (hubs) and large number of poorly connected nodes (non-hubs) (Barabasi et al., 1999); in Han et al. (2004), the degree cutoff with greater than 5 were considered as hubs; in Ekman et al. (2004), it is greater than 8; in Aragues et al. (2007) nodes with degree larger than 20 were defined as hub proteins. In Jin et al. (2007), hubs were defined if one protein had more than 12 partners in their top 20% ranking. A highly connected object of a network (hub protein) can be very crucial to play a central role in regulatory process of a disease (Kugler et al., 2011). The genome studies revealed that removing a hub protein from an organism is more likely to be lethal than removing a non-hub, a process widely known as centrality-lethality rule (He and Zhang, 2006). Therefore, hub proteins are indispensable part of a global network architecture (Batada et al., 2006). The notion of system biology also indicates that hub proteins are evolutionary conserved (Wuchty and Almaas, 2005) and play a central role for modular organization in a protein interaction network (Albert et al., 2000; Han et al., 2004).

The network biology approach aims to integrate complex biological data holistically whereas the classical analysis was done by reductionism philosophy (Frank and Dehmer, 2011). The biological network also decodes the functional interaction among the biomolecules and analysis of these networks helps in discovering new therapeutics and designing new protocol for early diagnosis of diseases (Pavlopoulos et al., 2011; Csermely et al., 2013).

In biological system, most abundant type of biomolecule is protein which interacts with DNA, RNA, metabolites and other protein of interest. But, protein-protein interactions (PPIs) build up a functional network among all biological molecular pathways (Ibrahim et al., 2011; Paris and Bazzoni, 2011; Zhang and Feng, 2017; Habib et al., 2018). Consequently, these PPIs help us to understand the inner complexity of an organism and lead to decipher the underlying pathogenic mechanisms of diseases (Bahadur, 2010).

In the present investigation, we established protein-protein interaction network episodes of asthma disease using a computational biological approach. We identified asthma signal transduction pathways by searching database, and the constituted proteins of those pathways were sorted out by software analysis. Moreover, we

classified different lung functional role of the proteins involved in regulatory interaction, reactome interaction and metabolic reaction.

2 Material and Methods

The Phenopedia database (Yu et al., 2009) was used for retrieval of proteins responsible for asthma disease. This analysis was performed with 1195 proteins reported for Asthma so far. The possible interacting proteins were identified from STRING database (Szklarczyk et al., 2015) and there we found 1152 proteins (nodes) and 19406 connections (edges). And 11330 protein-protein interactions (PPIs) were discovered having high confidence score of 7.0. Next, a network was constructed using Cytoscape 3.1.0 (Shannon et al., 2003) software package. A cytoscape plugin MCODE (molecular complex detection) was used for identification of densely connected region of this network based on ranking orders. During MCODE analysis we fixed the analytical value default such as - find clusters: in whole network; network scoring (advanced option) - a) include loops: turn off, b) degree cutoff: 2; cluster finding - a) haircut: turn on, b) fluff: turn off, c) node score cutoff: 0.2, d) K-core: 2, e) max. depth: 100.

To identify the asthma signal transduction pathways (STPs) we used KEGG (Kyoto Encyclopedia of Genes and Genomes) database (Kanehisa et al., 2016; Kanehisa and Goto, 2000). Here we discovered seven STPs which are associated with asthma disease progression. Furthermore, NetWalker 1.0 software package (Zhang et al., 2011) was used for identification of responsible proteins for asthma STPs. Also a network was constructed and later split into four cluster networks i.e. protein-protein interaction network, gene regulation network, reactome interaction network and metabolic reaction network. Default parameter values were used to construct and split the networks.

3 Results and Discussion

3.1 Network construction and identification of hubs

The Protein-protein interaction network was visualized by using Cytoscape 3.1.0 platform (Shannon et al., 2003). Cytoscape mapped 956 nodes with 11330 connections as edges. From this network analysis we identified top 20 high degree nodes having threshold score of ≥100 (Supplementary material file 1), which is separately highlighted in Fig. 1. These nodes were densely connected and consisted of huge number of edges compared with the other nodes in this network. These types of densely connected nodes are considered as hubs and they play pivotal role in a complex network. The network analyzer plotted degree against number of nodes which showed that the amount of high degree nodes is fewer than the low degree ones (Fig. 2). So it is apparent that in a complex network, hubs are the main control unit that is associated with so many objects compared with the non-hubs.

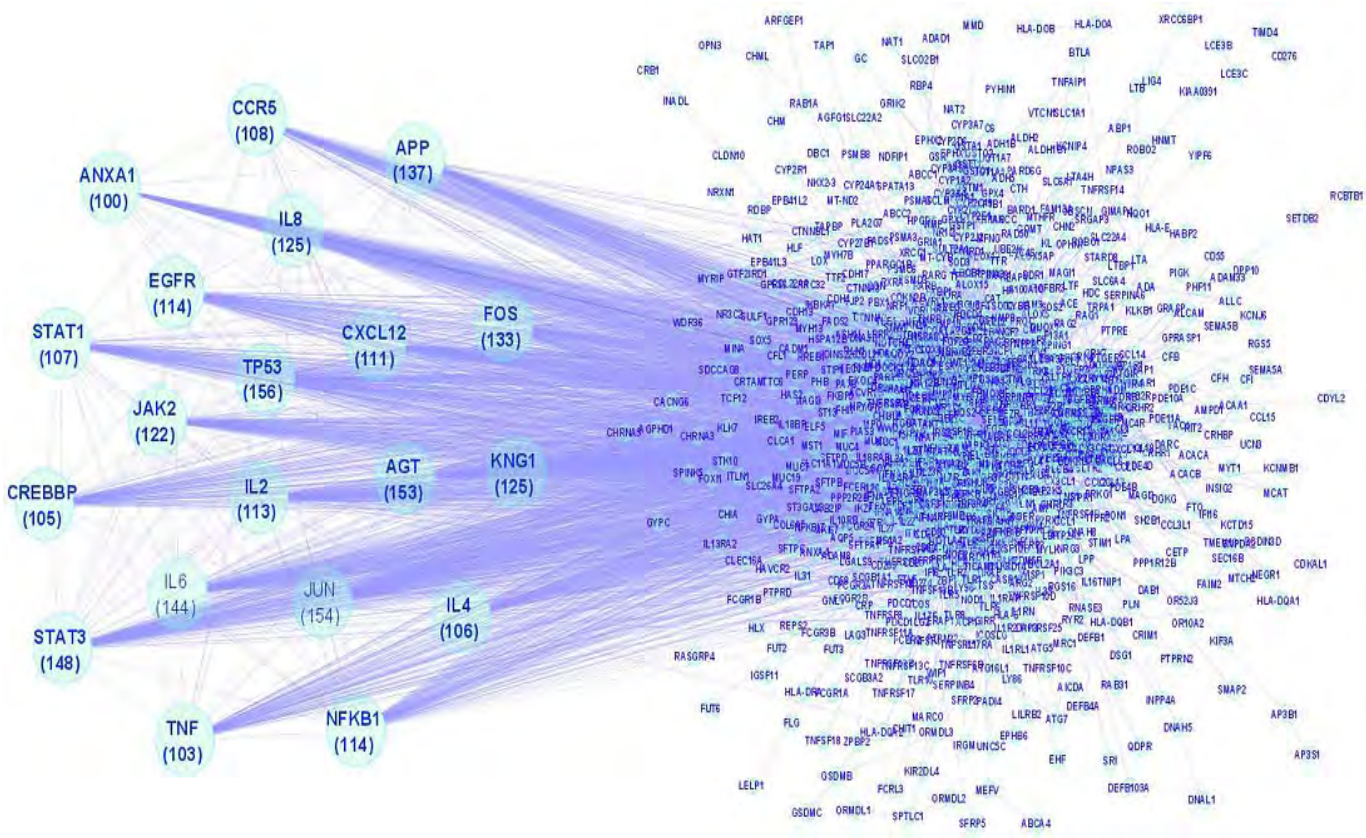

Fig. 1 Construction of asthma protein-protein interaction network in identifying hubs.

3.2 Identification of asthma signal transduction pathways

The asthma signal transduction pathways (STPs) were identified from KEGG pathway database (Kanehisa et al., 2016; Kanehisa and Goto, 2000). We retrieved seven pathways mainly responsible for asthma disease progression. These pathways were regulated by several proteins and they played crucial role for the development of asthma disease. To find out the best possible proteins of asthma STPs, we worked with NetWalker 1.0 software package (Zhang et al., 2011). Collaborating with this analysis we identified seven STPs by searching autogroup option in NetWalker where 91 proteins were involved in the cytokine receptor binding STP. Likewise, cell adhesion molecules, JAK-STAT cascade, antigen processing & presentation, and T cell receptor signaling pathway contained 36, 33, 27 and 24 proteins respectively. The B cell receptor signaling pathway contained 5 proteins whereas the Fc epsilon R1 signaling pathway had only 2 proteins in it (Supplementary material file 2).

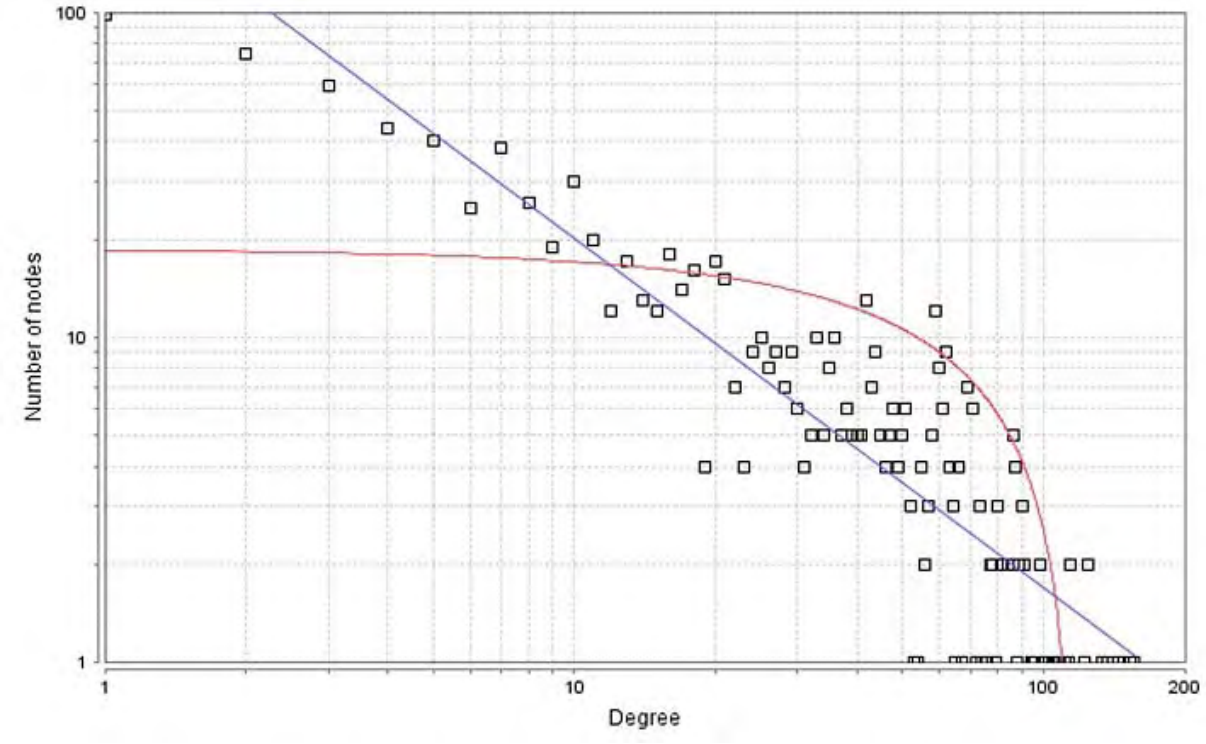

Fig. 2 Network plot parameter of degree against number of nodes.

However, cytokines play a crucial role for the development of asthmatic inflammatory process, which leads to inappropriate immune response in the body. This insidious role is mainly orchestrated by T helper 2 (Th2) cells and these Th2 cells primarily regulate the pathogenic effects of an asthmatic response. Both human and mice model had been employed to explain the involvement of Th2 cell in the pathophysiology of asthma (Wills-Karp, 1999). The inflammatory asthmatic response controlled by Th2 cytokines (IL-4, IL-5, and IL-13 proteins) (Pernis et al., 2004). Our network model depicted that these three proteins have high degree distribution of 106, 52 and 69 respectively (supplementary file 1). We investigated and found that there are 6 proteins (TNF, IL-2, IL-4, IL-6, IL-8, and CXCLI2) commonly expressed both in top degree nodes and cytokine-cytokine receptor interaction. In JAK-STAT signaling pathway AGT, STAT1, STAT3, IL-2, IL-4, IL-6, and JAK2 was found to be common.

Interestingly, the IL-4 and IL-12 activate the critical JAK-STAT signaling cascade which is a major pathway for asthma disease progression. In particular, the cytokines IL-4 and IL-13 induce high production of IgE that binds to high-affinity IgE receptors on mast cells and trigger the inflammatory asthmatic response (Jiang et al., 2000). On the contrary, IL-12 can block the synthesis of IgE (Yssel et al., 1998). The adequate number of nodes (proteins) can link-up with single high degree nodes as per the sensitivity of proteins. For example, the IL-4 and IL-6 possessed high degree and can regulate other nodes easily by connectivity of edges (interactions line).

The NFkB is commonly expressed in both T cell receptor signaling pathways and antigen processing and presentation STPs; and plays a critical role in asthmatic inflammatory process (explained in next section). Wegner and his colleagues (Wegner et al., 1990) first claimed that the cell adhesion molecules are majorly responsible for lung inflammation during asthma; though the function, fate and origin of these molecules are still unrevealed (Gearing and Newman, 1993). The two-integrin family's intercellular adhesion molecules (ICAM-1) and vascular cell adhesion molecules (VACM-1) are considered as the major markers for asthmatic

inflammation. Some study reported that after antigen challenge, soluble ICAM-1 and E-selectin were found in BAL fluid of asthma patients (Montefort et al., 1994). The VACM-1 expression in the lung also increases during asthma inflammation with several other parameters (Fukuda et al., 1996; Ohkawara et al., 1995; Gosset et al., 1995). In our predicted network model we detected 36 cell adhesion molecules which are majorly responsible for asthma inflammation. In the study we identified that ICAM-1 and VACM-1 were densely connected with high degree of 86 and 58 respectively. In B cell receptor signaling pathway we found MAPK1, NFATC2, PRKCB, CTLA4 and PTPN22 molecules; and for Fc Epsilon R1 signaling pathway MS4A2 and FCER1G are the main responsible molecules for asthma progression.

3.3 Molecular complex detection

Molecular Complex Detection (MCODE) method was applied for assessment of yeast-protein interaction network by existing established molecular complex data from mass spectrometry of the proteome. This study found that highly connected regions of a network form clusters based on their ranking order and they can be considered as a molecular complex (Islam et al., 2013). In our analysis the considered molecular complex threshold was ≥4.0 and we identified 27 clusters (Fig. 3a, 3b) on the basis of their ranking order. The rank 1 cluster was highest scoring 59 and in comparison with the top degree nodes AGT, APP, KNG1, IL-8, CXCLI2, CCR5 and ANXA1 were noticed to express commonly. Here, the AGT had highest degree distribution of 153 and also a common node in comparison with the nodes of JAK-STAT cascade. It is claimed that angiotensinogen gene (AGT) tremendously increased the effect of bronchoconstrictors and thereby produce a peptide. This peptide gradually accumulates in the airway and leads to progression of asthma in the body of the patients (Ying et al., 1991). Apparently, AGT expression has been connected to the lung fibrosis and also expressed in pulmonary cells (Uhal et al., 2012). So, AGT is considered as a good candidate hub in our network model. Therefore, the role of AGT in asthma exacerbation can be a good experimental tool for future studies. Second common node from the top hubs and JAK-STAT cascade was identified STAT1 (degree distribution 107) and also it was encountered from MCODE cluster rank 4. In asthmatic subject, STAT1 is selectively activated in bronchial epithelial cells and this STAT1 increased the expression of its target proteins: ICAM-1, IRF-1, and STAT1 (Sampath et al., 1999). STAT1 is considered as a key regulator of IFN-stimulated genes (ISGs) expression (Shornick et al., 2008; Holtzman et al., 2011). The ISGs encode proteins that control or defeat high level viral production by activating immune cells (Holtzman et al., 2012).

The IL-2, IL-4, IL-6 and JAK2 commonly expressed both in cluster rank 5 and JAK-STAT cascade. Except JAK2, these three proteins are commonly expressed in cytokine receptor binding STP. Here, IL-6 contains high degree distribution of 144 whereas IL-2, IL-4 and JAK2 are 113, 106 and 122 respectively. In cluster rank 6 of MCODE analysis TP53, STAT3 and EGFR were commonly expressed with the top 20 nodes. Here, STAT3 (degree distribution 148) and EGFR (degree distribution 114) are commonly expressed with JAK-STAT cascade and cell adhesion molecule. So, STAT3 can be considered as a good candidate hub in this network model. Ying and his colleagues claimed that STAT3 is a novel epithelial regulator of allergic response and targeting this molecule could be a novel and effective way for the development of therapeutics for asthma (Ying et al., 1991). In cluster rank 7, TNF-α (degree distribution 103) was commonly expressed in the top 20 hubs, JAK-STAT cascade and cell adhesion molecule. TNF-α is a master key regulator for asthma induction. It was reported that the airways of asthmatic patients contain TNF-α, which may deregulate the inflammatory response in the asthmatic airways and regulates disease progression (Ying et al., 1991; Bradding et al., 1994). There are some other key features of TNF-α including induction of histamine release (van Overveld et al., 1991), enhancement of the cytotoxic effects of eosinophils on endothelial cells (Slungaard et al., 1990), activation and cytokine release by T cells (Scheurich et al., 1987) and development of airway hyper responsiveness by increasing epithelial expression of adhesion molecules (Walter et al., 2002). Some studies

suggest that refractory asthma can be occurred by recruitment of neutrophils (Thomas et al., 1995), induction of glucocorticoid resistance (Franchimont et al., 1999), and myocyte proliferation (Desmoulière et al., 1993). Finally, in cluster rank 9, NFkB was commonly expressed in top 20 hubs, antigen processing and presentation, and T cell receptor-signaling pathways. This molecule is densely connected in our developed network with degree distribution of 114. It is presumed that, the cell adhesion molecules (e.g. ICAM-1, VCAM-1) are regulated by the action of NFkB. Thus, NFkB leads to fix the order of inflammatory gene expression and immune response in asthmatic airways in order to amplify and perpetuate the inflammatory process (Barnes et al., 1997).

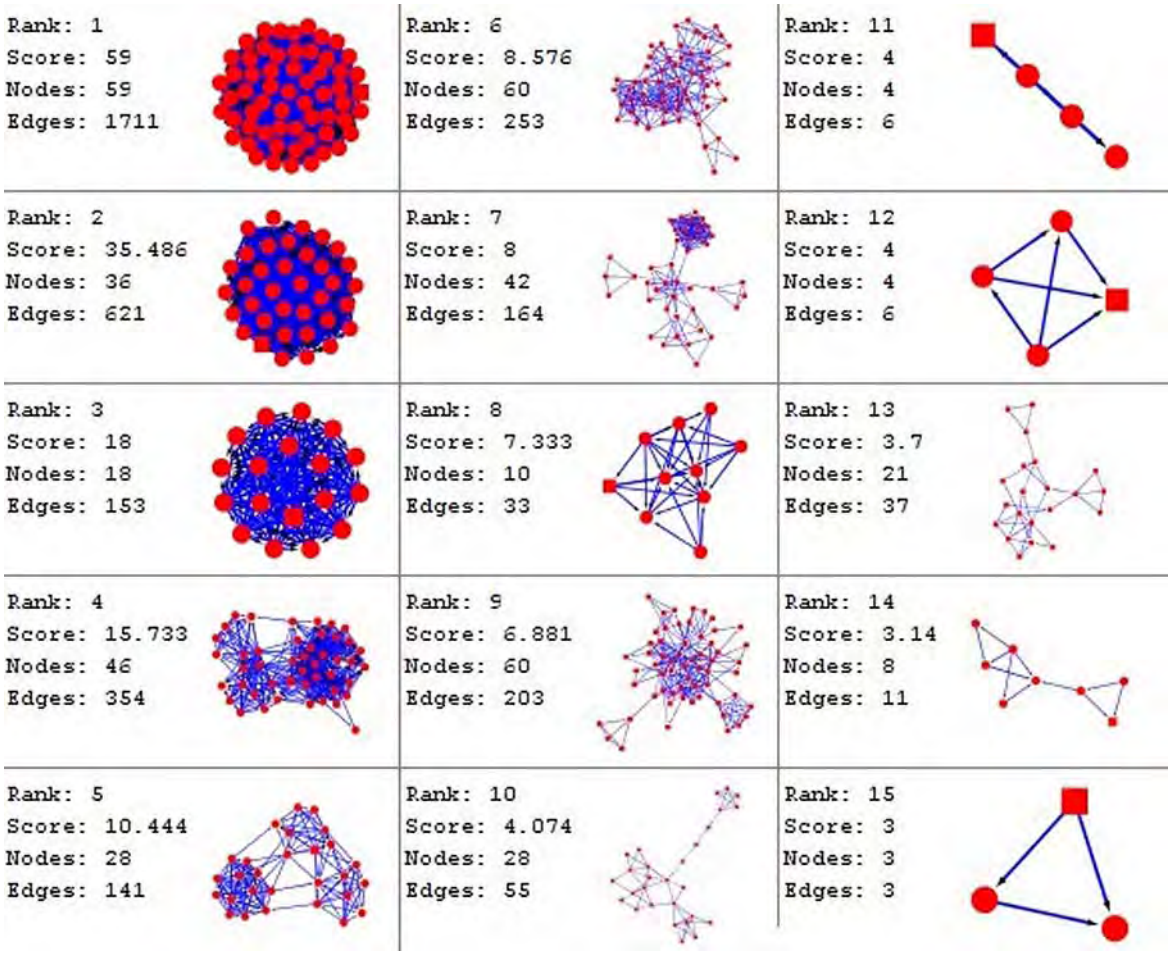

Fig. 3a Molecular complex detection analysis of asthma exacerbated protein-protein interaction based on ranking order (rank order 1-15).

3.4 Identification of regulatory interaction, reactome interactions and metabolic reaction proteins of asthma exacerbation

The blue color PPIs network showed the interaction of regulatory proteins of asthma (Fig. 4a). The asthma exacerbation related proteins are involved in deactivating the defense mechanism and increasing the external stimuli, therefore giving protection against immune response. This type of association may disappear when exacerbation related proteins are excluded. It is possible to differentiate whether the proteins are disease inducing or not by quantifying real-time PCR (RT-PCR) (Aoki et al., 2009). Identification of these types of

proteins is important for further target specification. The NetWalker software was used in our analysis to separate asthma gene regulatory network from the whole genes constitute network. Based on this software analysis, of 956 nodes, 262 were identified crucial for asthma disease progression (Supplementary material file 3).

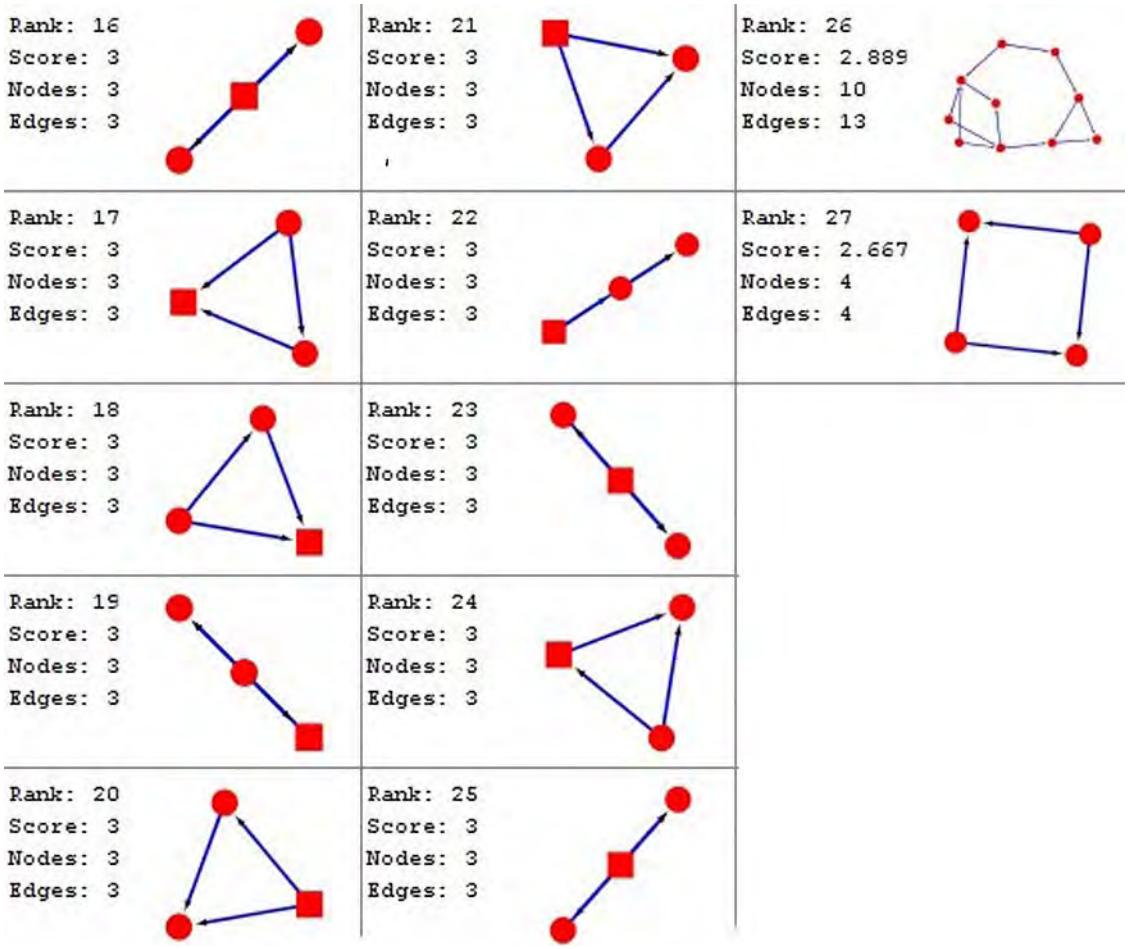

Fig. 3b Molecular complex detection analysis of asthma exacerbated protein-protein interaction based on ranking order (rank order 16-25).

Previously, our network model discovered top 20 high degree nodes (hubs); and in comparison with these hubs (except KNG1, JAK2, and CXCL12), all the 17 nodes were found similar. So it can be predicted that the hubs are playing major role in every possible reaction of disease progression. We also described the role of proteins for different functional stages of lung development with statistically significant data (table 1). Among all lung functional proteins, TNF, CTNNB1, FOXP3 and FOXA1 were very crucial as they had high degree distribution of 103, 77, 61 and 21 respectively in the predicted network model. We assume these could be the possible target proteins for further studies.

The aquacolor PPIs network showed reactome interaction of asthma (Fig. 4b). The neighboring reaction interaction proteins were separated and 297 proteins were identified involved in reactome interaction. Incomparison with the 20 hubs we identified 12 which were commonly expressed in reactome interaction. Here, we constructed a table (Table 1) based on the proteins having significant lung functional roles and found the two pairs of proteins are responsible for metabolic reaction. Here we demonstrated that 99 proteins had been playing potential role for metabolic reaction in asthma. The yellow color PPIs showed metabolic reaction (Fig. 4c). A very few proteins are responsible for lung functional roles in case of metabolic reaction. Only the LTA4H is common for all lung functional roles with significant hyper-geometric *p*-value showed in Table 1. Only TNF as common; it is suggested that other proteins should not be neglected as they have relatively high degree distribution. The CTNNB1 is responsible for various lung functional roles having high degree distribution of 77 which could also be regarded as a hub in a complex network. We can speculate from the fact that the reactome interacted TNF and CTNNB1 are important for further investigation.

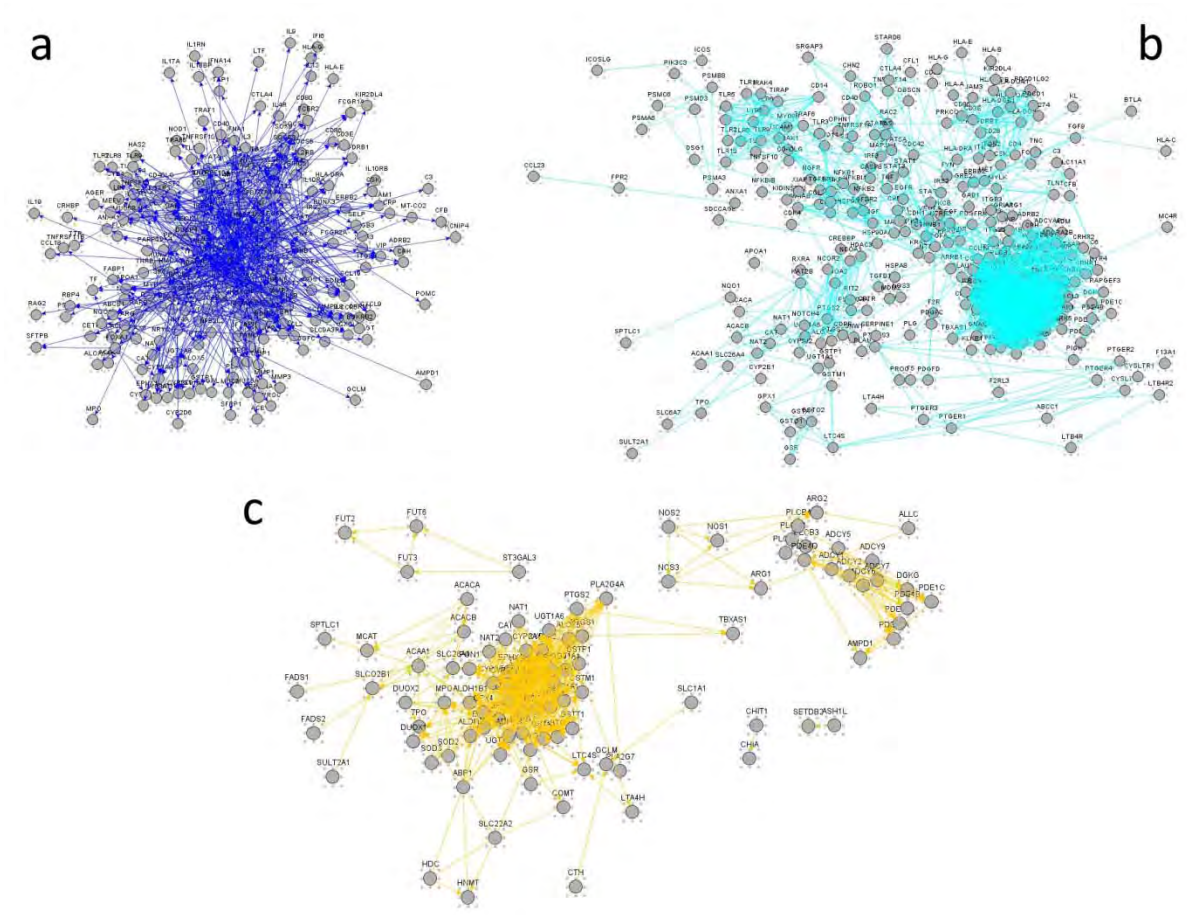

Fig. 4 NetWalker software constructed regulatory interaction (a), reactome interaction (b) and metabolic reaction (c) network of asthma exacerbation.

Some birth control cohorts claimed that the deficit of lung function development of neonates might initially develop asthma or a cause of this disease progression (Turner et al, 2004; Haland et al., 2006). Here, we demonstrated an integrative relationship between proteins and various lung functions development of asthma and found several responsible proteins for asthma disease progression. The TNF, FOXP3, FOXA1 and CTNNB1 are playing critical role for regulation of asthma. In parallel, TNF and CTNNB1 are responsible for reaction interaction of asthma whereas, LTA4H is important for metabolic reaction of asthma disease

progression. In a complex network model, data generation through genome-wide association, transcriptome, epigenome, microbiome, and metabolome analysis can assist us to understand the complex molecular mechanism of diseases. A complex biological network of asthma disease responsible proteins can help us to develop a casual relationship among the molecules within a cell or tissue or among themselves. Therefore, an integrative system wide data association can help us with the accurate prediction of important proteins responsible for disease progression that can be considered as a future reference to understand the disease assessment, risk monitoring factors, and therapeutic intervention.

Table 1 Classified lung functional role of regulatory interaction, reactome interaction and metabolic reaction proteins of asthma exacerbation.

annotation ID	Functional annotation	hyper-geometric p-value			Proteins		
		Regulatory interaction	Reactome interaction	Metabolic reaction	Regulatory interaction	Reactome interaction	Metabolic reaction
GO:0048286	lung alveolus development	0.003	0.03	0	VEGFA, PDGFA, FOXP3	VEGFA, PDGFA	No
GO:0061048	negative regulation of branching involved in lung morphogenesis	0	0	0	TNF	TNF	No
GO:0061047	positive regulation of branching involved in lung morphogenesis	0.0003	0.0004	0	CTNNB1	CTNNB1	No
GO:0061046	regulation of branching involved in lung morphogenesis	0.00006	0.0001	0	TNF, CTNNB1	TNF, CTNNB1	No
GO:0060916	mesenchymal cell proliferation involved in lung development	0.001	0.001	0	CTNNB1	CTNNB1	No
GO:0060502	epithelial cell proliferation involved in lung morphogenesis	0.007	0	0	FOXP3		No
GO:0060501	positive regulation of epithelial cell proliferation involved in lung morphogenesis	0.005	0	0	FOXP3		No
GO:0060492	lung induction	0.001	0.001		CTNNB1	CTNNB1	
GO:0060487	lung epithelial cell differentiation	0.02	0.02	0.003	FOXA1	LTA4H	LTA4H
GO:0060441	epithelial tube branching involved in lung morphogenesis	0.0009	0.01	0	TNF, CTNNB1, FOXA1	TNF, CTNNB1	No
GO:0060479	lung cell differentiation	0.0017	0.002	0.004	CTNNB1, FOXA1	CTNNB1, LTA4H	LTA4H
GO:0060484	lung-associated mesenchymal development	0.009	0.0005	0	CTNNB1	CTNNB1, FGF9	No
GO:0060425	lung morphogenesis	0.0006	0.04	0	TNF, CTNNB1, FOXA1, FOXP3	TNF, CTNNB1	No
GO:0060424	lung field specification	0.001	0	0	CTNNB1		No
GO:0060431	primary lung bud formation	0.003	0.004	0	CTNNB1	CTNNB1	No
GO:0060428	lung epithelium development	0.008	0	0	FOXA1, FOXP3		No

GO:0030324	lung development	0.0000007	0.000003	0.0003	NOS3, TNF, CFTR, CTNNB1, CRH, VEGFA, PDGFA, FOXA1, FOXP3, CEBPA, AGER, RBP4	NOS3, TNF, CFTR, CTNNB1, CRH, VEGFA, PDGFA, LTA4H, ARG1, TGFBR1, FGF9, PDGFRA	NOS3, LTA4H, ARG1, CYP1A2, ARG2
GO:0060428	lung epithelium development	0	0	0.01	No	No	LTA4H

4 Conclusions

The system biology approach is not widely validated yet but the phenomenon has rapidly escaped from its boundary. A network-based model can define meaningful information on the complex relationship of interacting proteins. This systematic approach outlines to design a higher order combination of target proteins of disease-associated pathways or interaction networks. In order to understand the asthma phenotypic expression completely, this promising approach can help in identifying large profiling and well-characterized asthma cohort's proteins by constructing a protein-protein interaction network related to disease progression. In accordance to our whole analysis, we have explained a predictive scenario of asthma disease responsible proteins network and their subsequent role in terms of this disease progression. Although different computational approaches had already been placed to design novel therapeutics against this disease but in this study we attempted to link up this picture as a whole. In this study, hub proteins were determined based on limited connections while it is possible to study with ample of hubs by reducing the degree distribution threshold. The large-scale real time data from patients is crucial to build up a complementary system wide profiling of asthma disease responsible proteins. Overall from this analysis it can be predicted that TNF-α played a critical role for asthma exacerbation and our network model found this molecule as a hub which can be a good target for novel drug design. As computational data analysis is very challenging, the refinement of real time software and tools is necessary for providing the knowledge of network engineering activities in cellular and molecular level of asthma disease episode.

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