TNF-α: Common Culprit of Inflammatory Diseases

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Abstract: *Inflammation is a necessary evil required for restorative activities and healing but is also involved in the onset of diseases. Inflammatory markers such as Interleukin 6 (IL-6), ACE, and Tumor Necrosis Factor-α (TNF-α) are reported to be vital in the onset of various diseases including coronary artery disease (CAD), obesity, and diabetes. These inflammatory markers are reported from the various populations and are also involved in the pathophysiology of many other diseases. Yet there is no explanation that how these genes controlling specific inflammatory pathways are responsible for all of these diseases. Therefore, pathways enrichment method is exploited to identify the role of inflammatory genes in the onset of CAD, obesity, and diabetes. The results are verified and analyzed using protein-protein interaction network and disease-gene network analysis. It is concluded that CAD, obesity, and diabetes are linked with each other through AGE/RAGE and HIF-1 Signaling Pathway. It is also found that TNF-α is the common inflammatory gene which is involved for CAD, obesity, and diabetes.*

Keywords: *Inflammatory pathways, Pathway analysis, Protein-protein interaction, Network analysis, Disease-gene networks.*

Introduction

Inflammation is a generic response to both internal and external threat signals, making it a necessary evil required for homeostasis, healing and restorative processes, but this process is also involved in the onset of various diseases such as coronary artery disease (CAD), obesity, diabetes and even cancer [28]. As a key contributing factor, the role of chronic inflammation is well appreciated and is currently receiving attention to understand its role not only in mechanisms of disease onset but also in the appearance of associated comorbidities [12].

In recent years, there has been extensive research supporting the involvement of inflammatory cells, inflammatory proteins and inflammatory responses from vascular cells and their role in atherosclerosis ultimately leading to CAD [19]. It is also being reported that chronic infections lead to initiation, progression, and destabilization of atherosclerotic plaques by enhancing T-cell activation as well as the response of the body to risk factors such as hyperlipidemia [27]. Obesity, therefore, is considered to be a fundamental cause of cardiovascular disorders as it is directly associated with elevated plasma triglyceride levels. Adipokines or inflammatory cytokines, secreted by adipose tissues located pericardially, alter the function of adipocytes and produce proinflammatory cytokines which efficiently contribute to cardio-metabolic complications [24]. More precisely the visceral adiposity upregulates the inflammatory cytokines and chronic elevation of these cytokines leads to atherosclerosis in particular and cardiovascular diseases in general [33] In case of diabetes, another key contributor of cardiac diseases, various cytokines associated with insulin

resistance are reported to be involved in the development of atherosclerosis [14]. Diabetes, obesity, hypertension, and insulin resistance are directly associated with inflammation [7] and overexpression of cytokines by adipose tissues. These cytokines are involved in the chronic inflammatory process and initiate accumulation of lipids and the development of atherosclerosis leading to CAD [38]. Identification of key inflammatory genes which are common in CAD, obesity, and diabetes could be a significant step in the identification of inflammation-related biomarkers for early and efficient diagnosis of these genes, which may also lead to personalized drugs tailored specifically to polymorphisms or genetic variants in these genes.

Inflammatory diseases

CAD is generally characterized by episodes of reversible mismatch in myocardial demand and supply, resulting in hypoxia associated with chest discomfort. Hypertension, obesity, diabetes, smoking, hypercholesterolemia and genetic susceptibility are considered common risk factors for CAD. Although CAD is a well-known lipid accumulation disease, but it is also well documented that various overlapping immune mechanisms along with risk factors, not only initiate but also activate lesions in the coronary artery.

Atherosclerosis is asymmetrical focal thickening of inner walls of arteries, where inflammatory and immune cells along with vascular epithelial and smooth muscle cells make a close mesh. Myocardial infarction occurs when these lesions block blood flow through the coronary artery. Atherosclerosis plaque makes these arteries susceptible to rupture, which is also triggered by the inflammatory regulators such as C-Reactive Protein (CRP), Interleukin 6 (IL-6), and fibrinogen. Tumor Necrosis Factor-α (TNF-α) is frequently reported to be involved in endothelial dysfunction and heart failure. A sensitive balance between inflammatory and anti-inflammatory processes controls the progression of atherosclerosis.

The inflammatory process in atherosclerosis increases the blood level of inflammatory cytokines, therefore, elevated levels of CRP and IL-6 are considered important indicators for CAD [4]. CRP is also considered a powerful biomarker for CAD as well as risk factors for obesity, hypertension, and diabetes [26].

Obesity, characterized as an excess accumulation of fat, is associated with dyslipidemia, vascular inflammation, and metabolic syndromes. Although it is a low-grade inflammation, if it becomes chronic and remains unmanaged, adipose tissues start to release inflammatory mediators or adipokines including leptin and adiponectin [9]. Interleukins and tumor necrosis factors are reported to further upregulate these adipokines [8, 34]. Adiposity also increases reactive oxygen species and endoplasmic reticulum stress, which becomes an important link between obesity, inflammation, diabetes and CAD [5].

Diabetes, on the other hand, causes hyperglycemia, increased oxidative stress, advanced glycation end products, dyslipidemia, hyperinsulinemia, and many other conditions which lead to artery damages [13]. For instance, insulin resistance leads to an increased level of free fatty acids in plasma (also involved in myocardial dysfunction) [13, 29]. Free fatty acids along with TNF-α, activate inflammatory pathways including inflammation of vessels, leukocyte chemotaxis and angiogenesis [38]. Amount of adiponectin reduces with the increase in obesity and diabetes and this reduction is also reduced with cardiovascular dysfunction [40]. Leptin, involved in regulation of energy expenditure and food intake, has also been reported to participate in atherosclerosis by processes such as oxidative stress, platelet aggregation and nitric oxide production by cardiomyocytes, etc. [41].

Materials and methods

Although CAD, obesity, and diabetes are non-communicable diseases they co-exist due to a susceptible common cause, i.e., inflammation [1, 2, 11, 33]. Therefore, to have a better understanding of genes and pathways involved in each of these diseases and to dig out the common pathway responsible for these comorbid conditions, pathways enrichment methods were used (Fig. 1).

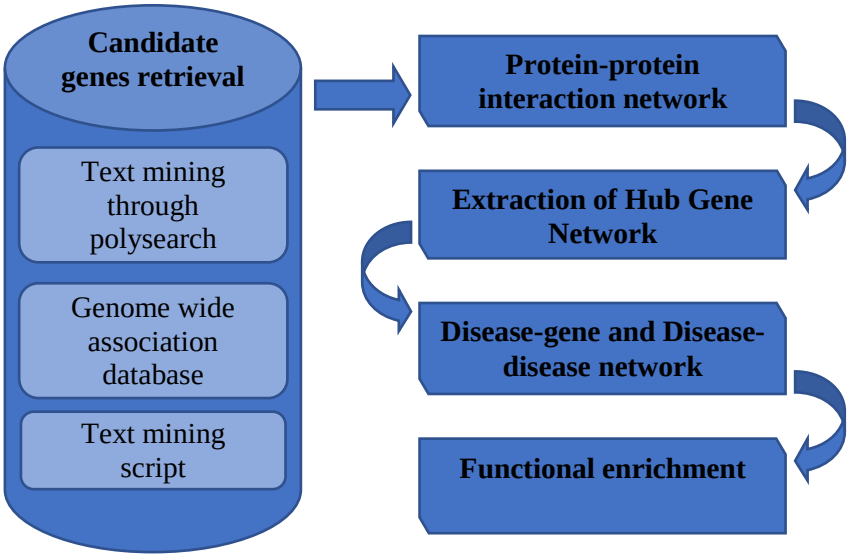

Fig. 1 Graphical summary of the project

Retrieval of candidate genes

In order to construct a gene association network, genes reported to be involved in CAD, diabetes and obesity were selected from three different sources, including PolySearch, (http://polysearch.ca/) which provides data from various sources such as, PubMed, OMIM, DrugBank [20], Genome-Wide Association Studies Database (GWAS) (http://jjwanglab.org/gwasdb), to retrieve information related to genome variant (such as genome mapping information, amino acid substitution, evolution), [18] and a self-written Java Script.

PPI network analysis

The selected candidate genes are converted into seed proteins and protein-protein interactions are downloaded from precomputed STRING database version 10.5 (https://string-db.org/cgi/input.pl). This database provides information about experimental and predicted interactions. Hub genes were extracted using Pejak Tool.

Disease-disease and disease-gene network analysis

Human disease network analysis for the CAD, obesity, and diabetes is performed through DisGeNET Plugin of Cytoscape. Disease projections are selected and independent sub-networks are extracted. Subnetworks are later merged to get a giant network.

Results and discussion

CAD, acute myocardial infarction, diabetes, hypertension, and obesity are inflammatory pathologies which involve interleukins (ILs), such as IL-1β, IL-6 and TNF-α, as well as acute phase proteins production, such as CRP. Genetic linkage and association studies have

discovered the involvement of genetic variations and polymorphisms important in inflammation and inflammatory diseases. Inflammation itself is a complicated process involving various stages, i.e., hematopoiesis, sensing of danger, mobilization of immune cells, activation of immune and non-immune cells for functional responses. All these changes are thoroughly controlled by a different set of genes and proteins [21]. In addition to inflammatory diseases, these genes also contribute in the onset of various other diseases such as cancer [10]. Various stages of inflammation involve interaction between different pathways selecting candidate inflammatory genes for a particular disease difficult. Therefore, selection of these candidate inflammatory genes become more difficult due to the potential genetic risk factors contributing to the inflammatory pathway in disease prognosis. By selecting query type as "Disease-gene/Protein Association" on PolySearch 1142, 169 and 175 hits were retrieved against keyword "coronary artery disease", "obesity" and "diabetes", respectively. The retrieved data was then manually scrutinized and hits with Z score >1 were considered, shrinking the list to 100, 59 and 175 literature hits against CAD, obesity, and diabetes respectively. GWAS was used to extract associated genes [18] and 1206, 137 and 201 hits were found against CAD, obesity and diabetes respectively. After removal of duplicates, final dataset was 30, 16, and 19 genes. For further enrichment of extracted gene data, a java code was written which provided 70, 30, and 54 hits against CAD, obesity, and diabetes (Table 1). Redundancy in datasets retrieved from all three sources was removed by Weka Tool (www.cs.waikato.ac.nz/ml/weka/).

Table 1. Number of Genes retrieved and selected from various text mining approaches

Disease	PolySearch		GWAS		Indigenous search	
	Literature hits	Selected hits	Literature hits	Selected hits	Inflammation keywords containing paper	Inflammatory genes
CAD	1142	100	1206	30	230	70
Obesity	175	40	137	16	2750	30
Diabetes	169	59	201	19	712	54
Total		**199**		**65**		**154**

Understanding of interactions within a cell and complex biological processes along with their integration with the biological system has led to the major developments in Network Biology. Biological networks are graphs of connected nodes, (genes or proteins) and edges (lines with connecting nodes depicting their inter-relationship). These inter-relationships vary from physical or functional associations to regulatory interactions and metabolic pathways. Therefore, networks are used to predict the relationship between various components of biological systems including genes, proteins, transcription factors and metabolites [17]. Biological systems could be understood well through the knowledge of interactions and these networks provide information for better understanding of disease processes and identification of disease-causing genes.

Proteins cannot work independently to perform their desired function efficiently. Therefore, proteins involved in similar cellular processes are usually found to be interacting with each other. Protein-protein interaction networks could be used to infer protein function within a cell. Cytoscape v3.5.1 [36] is used to construct and visualize protein-protein interaction network, 231 nodes, and 2612 edges along with the values of connectivity degree (k), betweenness centrality (BC), and closeness centrality (CC) are retrieved (Fig. 2).

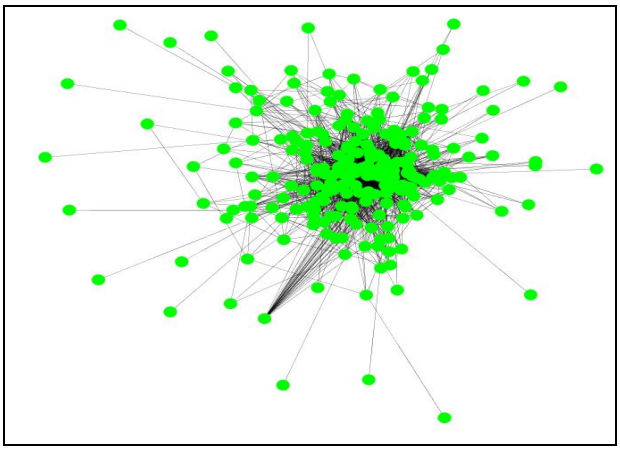

Fig. 2 Giant PPIs network in CAD, obesity and diabetes mellitus
retrieved from STRING and visualized in Cytoscape,
where nodes represent genes and edges represent the interaction between genes

Nodes with high BC and *k* values are the backbone of the network which controls the whole network in general. By using the cutoff value of 0.04 BC and 50 K, five genes were selected for further analysis. Pejak tool was used to measure the accuracy and validate the extracted hub genes. The test network was constructed through the logic of leaving one out from selected hub genes, and the whole process was repeated for all top 31 nodes (Fig. 3, Table 2).

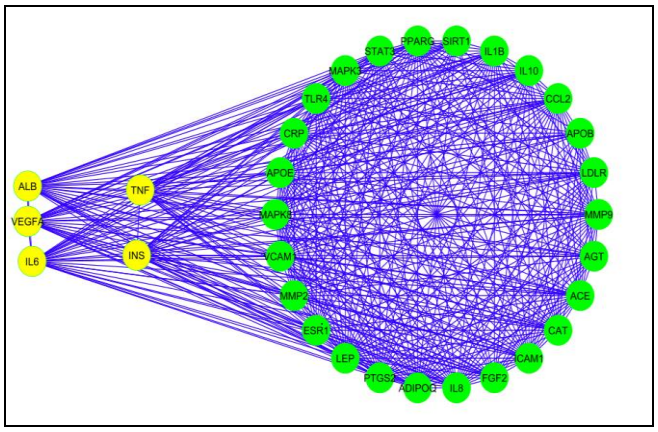

Fig. 3 Hub genes network of CAD, obesity and diabetes mellitus
retrieved from Giant Network based on highest BC value and highest degree.
Yellow nodes represent the genes with the highest degree and
BC value while green nodes represent other associated genes.

The disease could be an outcome of mutations or genetic variations in more than one gene or the variations within the same gene that could result in different disease, therefore, disease gene associations are essential to explain the fundamental questions such as how disease and the gene are related, what are the mechanisms by which the same gene could cause different phenotypes etc. Human diseasome, based on network biology, created a foundation to explore the cohesive relationship between disease genes and their outcomes by integrating all the datasets of recorded genetic disorders as well as complete list of known disease-causing genes [15].

Table 2. List of parameters for each of the selected genes

Sr. No	Gene Symbol	Name	Degree	Betweenness centrality	Disease nodes	Edges	Connected diseases
1	INS	Insulin	141	0.167539	59	139	diabetes, obesity, metabolic disorders, hypotension
2	ALB	Albumin	124	0.085201	63	125	cerebrovascular accident, kidney diseases, nephritis
3	IL-6	Interleukin 6	111	0.05481	102	149	inflammation, cancer, heart diseases
4	TNF-α	Tumor necrosis factor-α	105	0.047748	160	295	Inflammation, CAD, liver, kidney
5	VEGFA	Vascular endothelial growth factor A	94	0.047222	65	97	myocardial ischemia, CAD, kidney failure, diabetes, obesity

Network-based models have revealed that how genes contribute to common disorders and the interaction of their products [30]. Disease-focused networks are required to validate the biological markers and comprehend pathophysiology of various diseases. Network pharmacology can help to analyze the action of drugs on systems, identification of drug targets, the development of therapeutic targets with fewer side effects, and prediction of toxic effects [31]. Human disease network analysis for the CAD, obesity, and diabetes was performed through DisGeNET Plugin of Cytoscape. Disease projections were selected and three independent sub-networks were extracted. Subnetworks were later merged into a giant network. The nearest neighbors of each disease were detected and to understand the interaction of hub genes with disease, Gene-disease network was constructed. The interactions of each Hub gene were projected separately and highly connected diseases with the particular gene were analyzed. All these interactions were merged into the giant Gene-disease network (Fig. 4).

It was found that TNF-α and IL-6, both inflammatory genes [6, 23, 35, 37, 39], represent maximum connectivity while a non-inflammatory gene ALB represents the lowest. ALB gene was also found to have no connections with other genes in the selected list. Disease-gene network depicted that an obese individual with mutations in INS, IL-6 and TNF genes will be more susceptible to develop CAD and diabetes, similarly diabetic individuals with mutations VEGFA will be prone to develop CAD.

Molecular functional enrichment analysis

Functional properties of Hub Genes were analyzed by pathways annotations enrichment using ClueGO and functionally related genes were clustered and visualized by using KEGG pathways with Kappa score of 0.3 (Fig. 5, Table 3). Insulin resistance process was found to be the key regulator through two main signaling pathways HIF-1 [22, 25] and AGE-RAGE pathway [32] in diabetic complications. The AGE-RAGE pathway is well documented to be involved with diabetes and cardiac dysfunction [16] while the HIF-1 pathway is involved in inflammation through hypoxia [1].

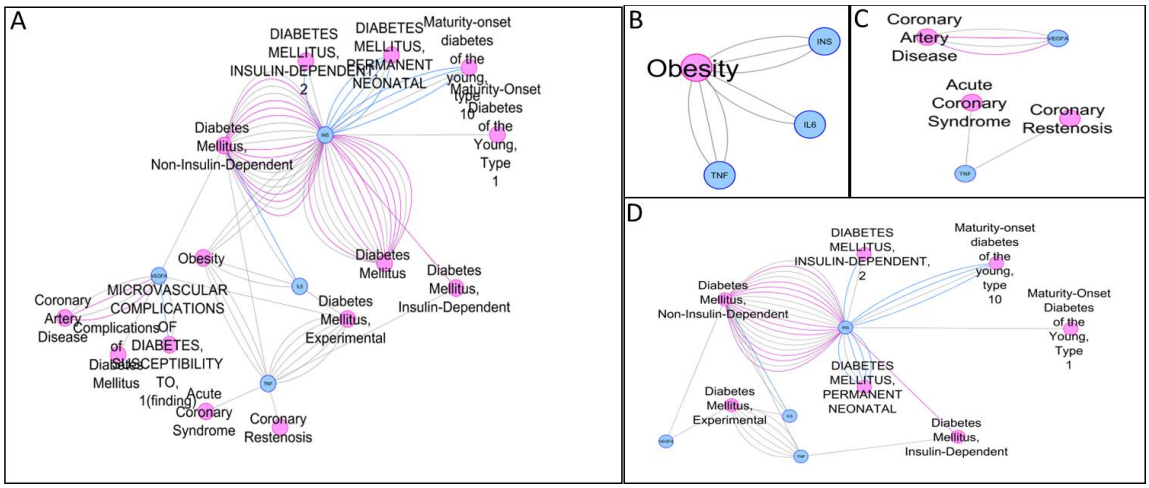

Fig. 4 (A) Giant network representing Disease-disease network and Gene-disease network of CAD, obesity, and diabetes, constructed through DisGenNET plugin of Cystoscope. Pink nodes represent associated disease and cyan nodes indicate five hub genes. (B), (C) and (D) represent sub-sub networks representing Gene-disease association of obesity, CAD and diabetes, respectively.

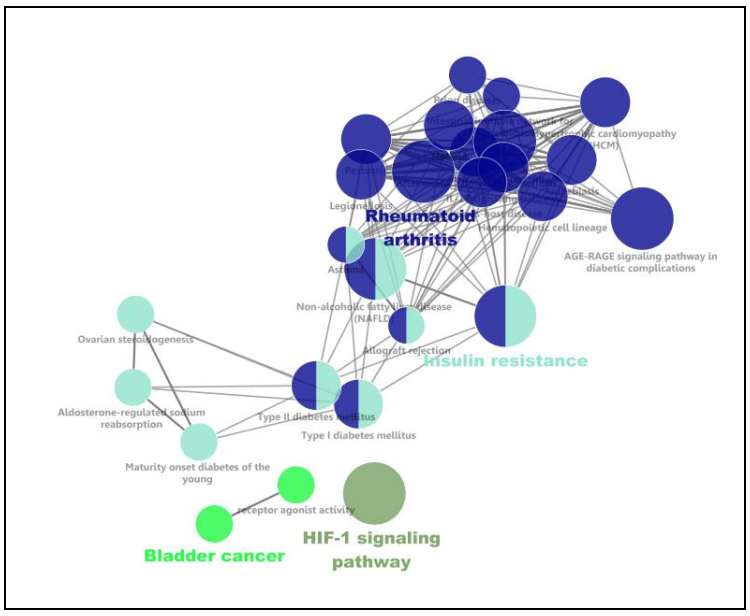

Fig. 5 Functionally grouped network of enriched genes association with pathways

Table 3. Grouping of networks based on GO term and pathways

GO ID	GO term	Genes	Association, %	*p*-value	Associated genes found
KEGG 04933	AGE-RAGE signaling pathway in diabetes complications	3	3.03	6.69E-10	IL-6, TNF, VEGFA
KEGG 04066	HIF-1 signaling pathway	3	3.03	6.69E-7	IL-6, INS, VEGFA
KEGG 04931	Insulin resistance	3	2.8	8.46E-7	IL-6, INS, TNF
GO: 0048018	Receptor against activity	1	3.84	5.80E-3	VEGFA

Conclusion

Inflammation is frequently reported to be a risk factor for coronary artery disease, obesity and diabetes. It is also well documented that the individual suffering from obesity, diabetes or coronary artery disease tends to develop other diseases. Pathway-based analysis revealed that the key inflammatory genes, i.e., INS, ALB, TNF, IL-6 and VEGFA through AGE-RAGE and HIF-1 signaling pathway regulate this prognosis and onset of this disease.

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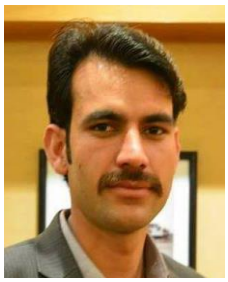

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