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Gene Network Analysis of PPAR-γ: A Bioinformatics Approach Using STRING

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***Abstract*—**Gene networks present a graphical view at the level of gene activities and genetic functions and help us to understand complex interactions in a meaningful manner. In the present study, we have analyzed the gene interaction of PPAR-γ (peroxisome proliferator-activated receptor gamma) by search tool for retrieval of interacting genes. We find PPAR-γ is highly networked by genetic interactions with 10 genes: RXRA (retinoid X receptor, alpha), PPARGC1A (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha), NCOA1 (nuclear receptor coactivator 1), NR0B2 (nuclear receptor subfamily 0, group B, member 2), HDAC3 (histone deacetylase 3), MED1 (mediator complex subunit 1), INS (insulin), NCOR2 (nuclear receptor co-repressor 2), PAX8 (paired box 8), ADIPOQ (adiponectin) and it augurs well for the fact that obesity and several other metabolic disorders are inter related.

***Keywords*—**Gene networks, NCOA1, PPARγ, PPARGC1A, RXRA.

I. Introduction

THE functioning of biological systems is carried out through the concerted activity of many genes [1], [2]. Estimates suggest that there are more than 30,000 different genes in human beings. Although it is an idealization, it is common for biologists to think of genes as being turned "on" or "off". If a gene is "off" the associated protein would not be synthesized. Only a subset of genes will be expressed at any one time in any particular cell of the body. The expression of the genes in a cell is controlled by various diffusible factors, called transcription factors. A transcription factor is a protein whose sequence is in turn coded by a gene and whose expression is controlled by transcription factors [3]. Hence, activity of genes is regulated by proteins and metabolites, which are produced by proteins. But proteins are also gene products, thus genes can influence each other (induce or repress) through a chain of proteins and metabolites [1]. Proteins can interact with each other in a variety of functional complexes, regulatory interactions, and metabolic pathways. These interactions can be presented as a meaningful set of genetic functions when they are conceptualized as gene networks. Presently to increase the statistical power of human genetics, to assists drug discovery, to avoid gaps in metabolic enzyme knowledge and to predict gene functions the "network view" is more often presented [4], [5].

PPAR-γ is a gene that belongs to the superfamily of nuclear receptors (NRs) that are ligand-dependent transcription factors and are important regulators of lipid storage and metabolism [6]. PPAR-γ has been implicated in diseases associated with dysregulation of lipid levels, such as obesity, cardiovascular disease, and type 2 diabetes [6]. It has many pleiotropic functions; it plays a crucial role in the expression of key genes involved in adipogenesis [7], carbohydrates metabolism [7], inflammation [7], and cancer [7] and hence can form a complex network with different disease causing genes. Even though there are many reports [7] on the association of PPAR-γ with other disorders, there are no reports on the gene network analysis of PPAR-γ using bioinformatics tools. Hence ours is probably the first such report and our result will be useful for researchers working in the field of obesity and related disorders.

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II. Methodology

We have used the search tool for retrieval of interacting genes/protein (STRING 9.0) [4] that explains a comprehensive PPARγ gene-disease and gene-gene association involving associations from several sources such as databases of physical interactions and databases of curated biological pathway knowledge.

A. Confidence Scoring and Network Analysis.

STRING database was used to retrieve and construct disease-gene network of PPARγ. The functional interaction was analyzed by using confidence score, ranging from 0.5 to 1.0. Interactions with score < 0.3 are considered as low confidence, scores ranging from 0.3 to 0.7 are classified as medium confidence and scores > 0.7 yield high confidence [8].

B. Gene Ontology Annotations and Clustering

STRING imported information for PPARγ gene network from Gene Ontology (GO) data sources to understand the functional organization of gene networks. The criteria for gene ontology was based on the p-value and a value of ≤ 0.05 was found to be a significant one [9]. Also STRING provides clustered functional modules of genes in the network and thus explaining a closest coexpressive path.

III. Results and Discussion

A. Network Study and Confidence Scoring of PPARγ

In the PPARγ network analysis, links between genes signify various evidence type interaction data supporting the network. The evidence based representations of PPARγ associated with

several other functional genes are depicted in Fig. 1. Here PPARγ is the node and the functional partners are represented towards the edges. PPARγ network represent the existence of different types of evidence used in predicting the gene-gene associations. Each line of evidence represents co-occurrence or binding, co-expression, experiments, databases and text mining [10].

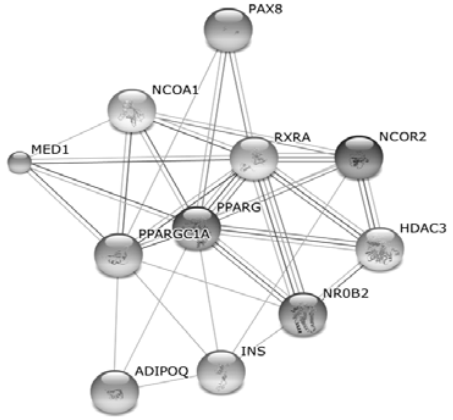

Fig. 1 Evidence view of PPARγ gene network showing functional association with 10 genes produced using multiple pieces of evidence

This functional interaction of PPARγ obtained is analyzed by confidence score. The result shows PPARγ is associated with RXRA not only through experimental evidence but also interacted by biochemical data which is co-mentioned in extended database and information in literature. Also, RXRA contributes to coronary heart disease [11] and it is found to be the most associated functional node of PPARγ with highest confidence scoring (0.999) as mentioned in Table I [12]-[26]. PPARGC1A is the next functional node associated with PPARγ and its association obtained from experimental data and literature evidence. Dysregulation of this gene is co-related with abdominal obesity and hypertrophic cardiomyopathy [27]. Several findings suggest that both NCOA1 and NR0B2 are associated with breast cancer [28]-[29]. Our result also depicts that NCOA1 and NR0B2 are networked with PPARγ with high confidence score. HDAC3 which is associated with the risk of colon cancer shows a good interaction with PPARγ and its association is obtained from experimental data and curated database. MED1 gene ranked as 6th in PPARγ gene network, pertains its association by experimental and literature evidence and is also associated with the disease risk of colorectal and ovarian cancer [30]. INS and ADIPOQ genes exhibit good interactions with PPARγ; this is most commonly found to be associated with diabetes mellitus [31] and coronary heart disease [32]. NCOR2 and PAX8 interacting with PPARγ, pertains their association by literature and data-mining evidence.

B. GO Annotation of PPARγ

Gene ontology for PPARγ network involving biological processes, molecular function and cellular components has been framed at different specificity levels to explore interaction patterns and its expression levels. The findings of GO annotation for PPARγ referred collectively from NCBI (Gene ID: 5468) along with STRING 9.0 GO dataset.

Our result demonstrates the functional organization and composition of ten genes in PPARγ network. Here, the PPARγ network emphasizes ten biological annotations of genes. Out of ten, eight genes (PPARG, RXRA, PPARGC1A, NR0B2, HDAC3, MED1, NCOR2, ADIPOQ) shows the lipid metabolic process, three genes (PPARG, RXRA, ADIPOQ) shows response to nutrient in a cell, two genes (PPARG, ADIPOQ) not only indicate negative regulation of macrophage derived foam cell differentiation but also negative regulation of receptor biosynthetic process. There are four genes (PPARGC1A, RXRA, NR0B2, and MED1) in PPARγ network which are associated with intracellular receptor mediated signaling pathway, three genes (PPARG, RXRA, INS) which respond to insulin stimulus. Glucose homeostasis is followed by four genes (PPARG, PPARGC1A, INS, and ADIPOQ) and lipid homeostasis by two genes (PPARG, INS). Lastly, five genes (PPARG, PPARG C1A, RXRA, MED1, and PAX8) involve positive regulation of transcription from RNA polymerase II promoter and three genes (PPARG, PPARG C1A, and INS) are associated with positive regulation of sequence-specific DNA binding transcription factor activity. PPARγ also demonstrates molecular functions of the genes that interact with it. Among ten genes, two of them (NCOR2, HDAC3) are associated with enzyme binding activity, three genes (PPARG, RXRA, NR0B2) are associated with ligand-dependent nuclear receptor activity and three genes (PPARG, RXRA, PAX8) indicate both sequence-specific DNA binding and transcription factor activity. It has been revealed that all the ten genes in the PPARγ network are integrated to protein binding activity and hence explains that they can either selectively or non-covalently bind with any proteins or its complex. Also there are three genes (PPARG, RXRA, and NCOR1) that involve the molecular function of transcription regulatory region of DNA binding activity. Later, in PPARγ network, the cytoplasmic activity is associated by five genes (PPARG, INS, ADIPOQ, PPARGC1A, and HDAC3) followed by nucleoplasm and nuclear activity association with eight genes (PPARG, PPARGC1A, NCOR2, HDAC3, PAX8, NR0B2, RXRA, MED1).

TABLE I
INTERACTION OF PPARG WITH FUNCTIONAL PARTNERS

S.no	Functional node	Interaction type	Types of evidence for the association.	Combined confidence score
1	RXRA (retinoid X receptor, alpha)	Binding, Reaction	Experimental/ Biochemical Data [12], Association in Curated Database (KEGG pathway) and co-mentioned in pubmed abstract [13]	0.999
2	PPARGC1A (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha)	Binding	Experimental/ Biochemical Data[14], Co mentioned in Pubmed abstract [15]	0.999
3	NCOA1 (nuclear receptor coactivator 1)	Binding,	Association in curated database (KEGG pathway), Co mentioned in pubmed abstract [16]	0.997
4	NR0B2 (nuclear receptor subfamily 0, group B, member 2)	Binding	Experimental/ Biochemical Data [17], Association in curated database (KEGG pathway), Co mentioned in pubmed abstract [18]	0.997
5	HDAC3 (histone deacetylase 3)	Binding,	Experimental/ Biochemical Data [19], Association in curated database (KEGG pathway), Co mentioned in pubmed abstract [20]	0.997
6	MED1 (mediator complex subunit 1)	Binding, post-translation modification	Experimental/ Biochemical Data [21], Co mentioned in Pubmed abstract [22]	0.926
7	INS (insulin)	Binding, post-translation modification.	Co mentioned in pubmed abstract [23]	0.994
8	NCOR2 (nuclear receptor co-repressor 2)	nil	Association in curated database (KEGG pathway), Co mentioned in pubmed abstract [24]	0.993
9	PAX8 (paired box 8)	Binding	Association in curated database (KEGG pathway), Co mentioned in pubmed abstract [25]	0.992
10	ADIPOQ (adiponectin)	Expression	Co mentioned in pubmed abstract [26]	0.992

C. Clustering of PPARγ Network

Clustering of genes in PPARγ network has been analyzed to find groups of genes that have similar functions. Fig. 2 explains PPARγ network involving two clusters which involve upregulated and downregulated genes.

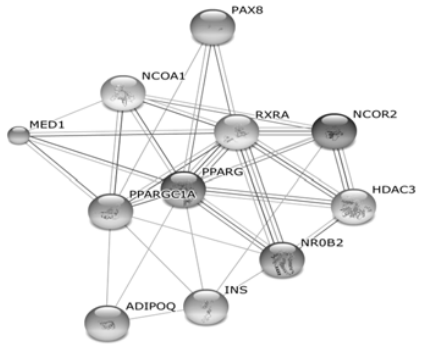

Fig. 2 Clustering of PPARγ network

Nine genes in the network represent upregulated genes (PPARγ, PPARGC1A, RXRA, MED1, NCOA1, PAX8, INS, ADIPOQ, and NR0B2) and two genes represents downregulated genes (NCOR2, HDAC3) [33]. These upregulated genes in clusters are associated by text-mining. Among them PPARγ, PPARGC1A, RXRA, MED1, NCOA1 and NR0B2 are associated by experimental evidence. PPARγ, RXRA, PAX8 and NR0B2 are found to be associated by databases. Again the downregulated genes (NCOR2, HDAC3) in the second cluster are associated by database, experimental and text-mining evidence.

On the whole, our study emphasize that out of ten genes in PPARγ network (PPARGC1A, RXRA, MED1, NCOA1, PAX8, INS, ADIPOQ, NR0B2, NCOR2 and HDAC3), five genes (PPARGC1A, NCOA1, NR0B2, HDAC3 and PAX8) are confederated by binding interaction pattern and one gene (RXRA) is associated not only by binding but involve in biochemical reaction patterns also. Two genes (MED1 and INS) are involved in binding and post-translation modification and ADIPOQ show expression patterns. Even though, NCOR2 does not show any interaction there is information in literature [24] to suggest its association with PPARγ as mentioned in Table I. Thus our computational analysis suggest that, PPARγ and its associated ten genes are well coordinated and any alteration in the network might result in obesity and associated diseases like coronary heart disease, cardiomyopathy, breast cancer, and diabetes mellitus. Finally, we believe that our results will be useful for researchers working in the field of genetic and metabolic disorders.

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