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Computational Systems Biology Sheds Insights on to Metabolic Syndrome and its Component Disorders

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Abstract: The interconnected physiological and metabolism factors, such as obesity, hypertension, insulin resistance, etc. that increases the risk of cardiovascular disorders (CVD), type2 diabetes mellitus (T2DM) that causes mortality is the description of metabolic syndrome (MetS). It remains unclear how molecular mechanisms are typical between MetS, CVD, T2DM, obesity, and hypertension. In this study, we compiled 27 common genes by mapping miRNAs and TFs as active seed nodes into the regulatory TF-miRNAs and TF-miRNA networks by the integration of target prediction. By merging these networks, the gene-based miRNA and TF mediated regulatory network common for MetS and its associated diseases were constructed. As a result, we obtained a potential active sub-network based on degree analysis. Next, by using the breadth-first-search approach, 46 regulatory pathways, which are the gene-based regulatory cascade of TFs and miRNAs, were identified. In order to identify the hub regulators in the original network constructed, based on degree and betweenness analysis, 16 genes (VEGFA, KLF2, PNPLA3, GRK2, HMOX1, EDN1, IL6, TGFB1, NOS3, TNF, SERPINA1, SPP1, AGTR1, ADRB3), 3 miRNAs(has-miR-335-5p, has-miR-124-3p, has-miR-181a-5p), 4TFs(NF_KB, FOXO1, RELA, SP1) are over-represented in the following significantly enriched functional pathway groups such as AGE-RAGE signaling pathway, Renin-Angiotensin System, HIF-1 signaling pathway. The majority of the regulatory relationships from published literature studies demonstrated the reliability and validity of these miRNA and TF mediated regulatory network. Hence, our study has aided in deciphering the complex regulatory mechanisms involved in MetS and will provide putative therapeutic targets by further validation of these pathways by biological experiments.

Keywords: Metabolic Syndrome; regulatory network; transcription factor; miRNA; pathway.

1. Introduction

Metabolic Syndrome (MetS) is a growing public health and clinical problem in sedentary lifestyles worldwide. It is characterized as a cluster of metabolic abnormalities associated with visceral adiposity, particularly insulin resistance, hypertension, dyslipidemia, and central obesity [1]. MetS syndrome is diagnosed with the occurrence of three metabolic disorders of the five previously described. Such irregularities significantly increase the risk of

cardiovascular disease (CVD) and Type2 (T2DM), one of the major causes of death for people with MetS [2, 3]. To develop therapeutic strategies, it is important to understand the possible mechanisms common to MetS and its associated diseases. The primary causes of morbidity and mortality are Met-related disorders. The study focuses on finding the root causes of MetS [4]. Knowing key metabolism control pathways, goals, and risk factors leading to MetS may result in the creation of pharmaceutical interventions [5]. The entire gene expression and protein formation processes of living cells are modulated by gene regulatory networks and thus determine the fate of cells. The major regulators of these networks are MicroRNAs (miRNAs) and transcription factors (TFs) [6]. MicroRNA is a type of short non-encoding RNA involved in gene regulation that can operate either directly on the target genes or indirectly by initially regulating TFs regulated by gene expression. As a result, miRNA and TF do not really function independently in many diseases, such as cancer [7]. In that context, MetS, obésity, hypertension, CVD, and T2D M have been used to classify genetic variants and their regulators. In order to detect possible TF-miRNA mediated regulatory pathway, the BFS (breadth-first-search) approach was used [8] with a basic network structure and interpretation that could be validated further by biological experiments. Knowing the regulatory network of MetS regulated by miRNA and TF would thus shed light on the pathogenesis mechanisms of the network [9].

2. Materials and Methods

2.1. Collection of genes related to MetS & its associated components.

The DisGeNET discovery tool was used for the purpose of accessing a catalog of genes associated with their respective diseases: "metabolic syndrome," "type 2 diabetes mellitus," "cardiovascular disease," "hypertension" and "obesity." In this study, 601 genes, 1507 genes for T2DM, 934 genes for CVD, 1962 genes for obesity, and 78 hypertension genes were extracted from the DisGeNet database [10] for the process of determining particular genetic signatures among these diseases with JVenn, an intergraphical tool for comparing lists with Venn graphics [11]. This analysis provided a collection of genetic diagrams.

2.2. Identification of gene-miRNA/TF, TF-miRNA regulatory relationship.

Regulatory relationships of Gene-miRNA with the specific genes selected have been evaluated using experimentally checked targets. The TarBase v8 [12], miRTarBase v7 [13] database, has been experimentally tested, and only goals in the two datasets have been maintained in this analysis, to improve the reliability of the tests. In the Transfac [14] databases, TRRUST v2 [15] incorporated gene-TF regulatory relationships, using experimentally verifiable and expected gene targeting. RegNetwork Regulatory Network Repository of Transcription Factor and Mediated Gene microRNA Regulations [16] were then built into regulatory relationships with TF-miRNA.

2.3. Construction of gene-TF-miRNA mediated regulatory network.

As individual networks, we have established gene-miRNA regulating relationships, gene-TF regulatory relationships, and TF-miRNA regulatory relationships. Later, we merged these networks with the tool "merge" of Cytoscape (an open-source software framework for visualizing expressive profiles and other state data) [17]. We achieved a potential active sub-

network based on the grade cutoff of 2 (node score of 0.200, k-core) by using a cytoscapape plug-in MCODE, a novel clustering algorithm that recognizes substructures in broad networks.

2.4. Identification of potential active regulatory cascades.

Throughout this analysis, we focused on regulatory routes that were the routes connected in the TF-miRNA-curated regulatory network to multiple TFs, miRNAs, and target genes. We have established all guided acyclic paths from 0 in-degree nodes to 0-degree nodes from the possibly active TF-miRNA regulatory sub-network. A guided diagram was used for the future active subnetwork. The first thing we used is to cross all vertical pictures of the BFS (Breadth-First Search) algorithm [18]. Second, the backtracking procedure was used to remove all paths from the 0-indegree node to the 0-outdegree node based on the results of graph crossing. The directed acyclic paths with more than two nodes have been considered as potential active regulatory paths in this study.

2.5. Identification of hub nodes in the network.

We have used Cytoscape plug-in cytohubba v1 [19] to explore the main hub nodes in the developed gene-miRNA-TF regulatory network. It provides 11 topological methods of analysis, including the degree, edge percolated component, maximum component neighborhood, density for maximum neighborhood components, maximum central cliques, and six centralities (Eccentricity, Nearness, Radiality, Betweenness, and Stress) based on the shorter distances. This plug-in was used for rating the nodes in the network with its network features.

2.6. Functional enrichment analysis of common genes.

The Kyoto Genes and Genomes Encyclopedia (KEGG) has indeed been developed as a database for the mapping of linked genes on their own pathways [20]. Clue GO, a Cytoscape plug-in for gene ontology and pathway notation systems, can be decrypted functionally with a hypergeometric check, the kappa coefficiency of pathways can be calculated [21], and functional pathway correlations can be examined [22]. In this study, a ClueGO method for the study of the functions of specific genes was used in KEGG pathway enrichment research. A P value < .05 was regarded as a threshold value, a kappa coefficient of 0.4. They were identified and noted as the most important pathways linked to the syndrome.

3. Results and Discussion

3.1. Identification of common genes related to MetS & its associated components.

We have put together a list of GAD diseases using the DisGeNET v4.0 database, which were correlated by the Venn diagram (Figure 1), with a view to identifying the specific genes in MetS with cardio-vascular, obese, hypertensive, and type 2 melites. We focused on 27 specific genes between metabolism, obesity, CVD, and T2DM.

3.2. Identification of gene-miRNA/TF and TF-miRNA regulatory relationship.

There have been a total of 631 miRNA genes and 216 TF gene regulatory links. This collection of miRNA-gene, TF-gene, and TF-miRNA rules were imported into the cytoscape as a network file for the separate TF-miRNA, miRNA-gene, and TF-gene networks [23]. The

561 nodes and 691 edges miRNA-gene regulatory network (Figure 2), the 150 nodes and 265 edges regulatory network TF-gene (Figure 3), and the 189 and 289 edges regulatory system TF-miRNA (Figure 4).

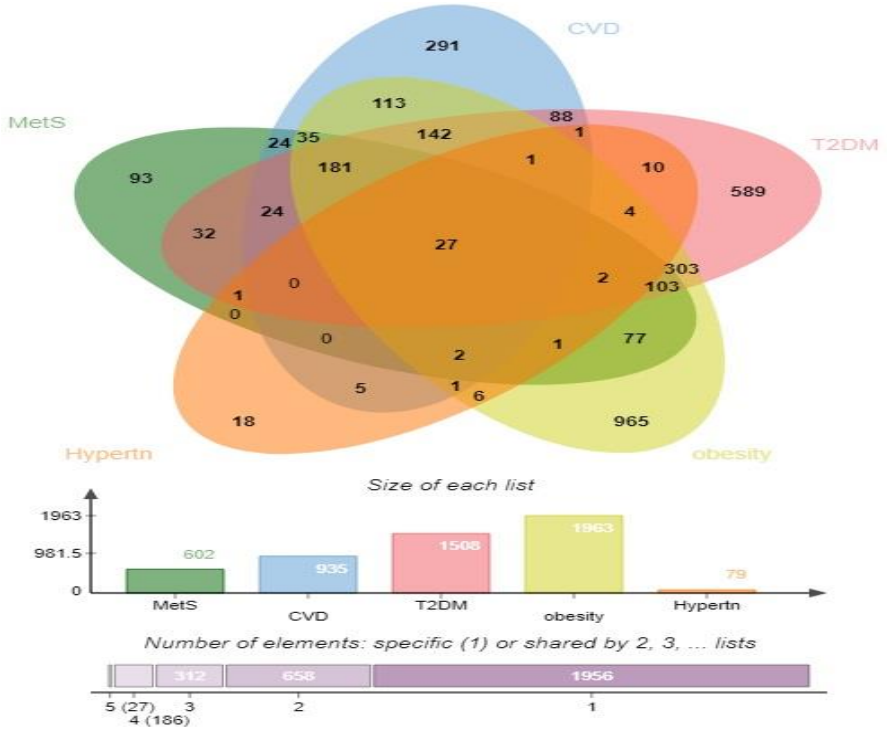

Figure 1. Venn diagram showing common genes associated with MetS-T2DMCVD-Obesity-hypertension.

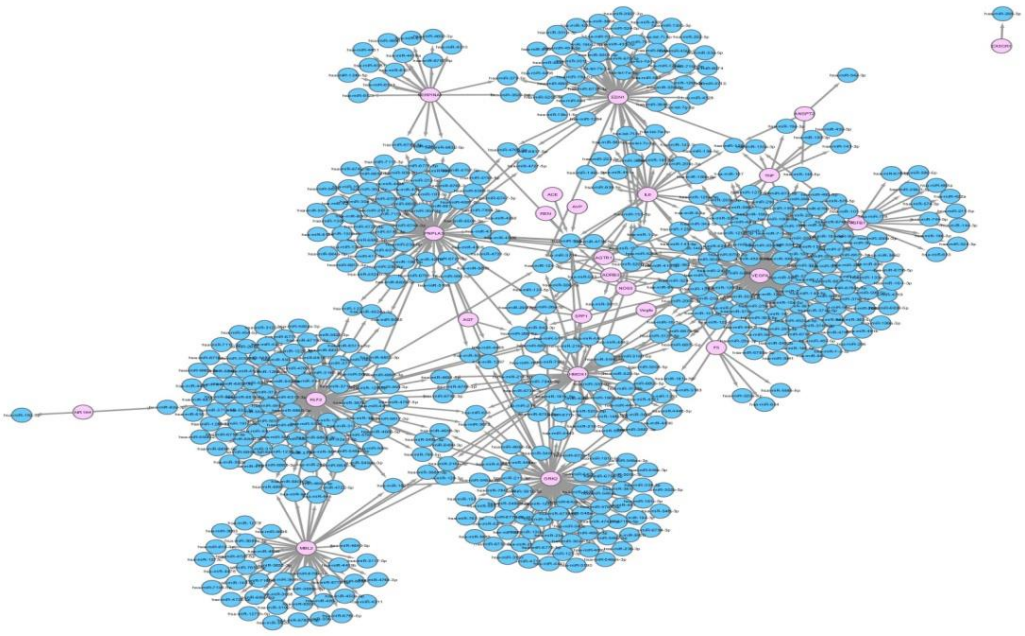

Figure 2. Gene-miRNA regulatory network [pink-gene, blue-miRNA].

3.3. Construction & analysis of gene-TF-miRNA mediated regulatory network common for MetS and its associations.

By combining the 3 networks built in the above parts, we built a common regulatory network for MetS, CVD, T2DM, Obesity, and Hypertension with miRNA & TF. List of gene-TF-miRNA regulatory pathways common for Mets and its components is listed in Table 1.

There are 810 nodes and 1111 edges [26 genes, 143 TFs, and 644 miRNAs] in the network. On the basis of the topological analysis, the global properties of this network were evaluated.

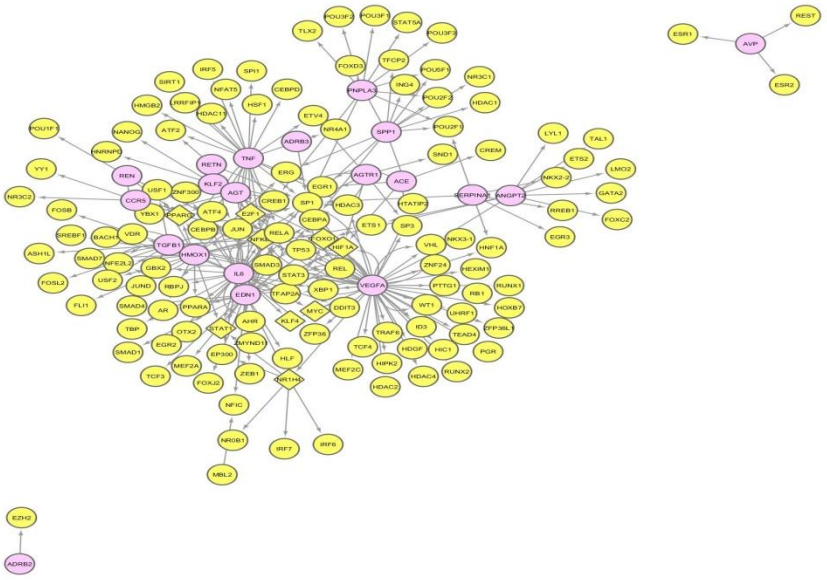

Figure 3. Gene-TF regulatory network [pink-gene, yellow-TF].

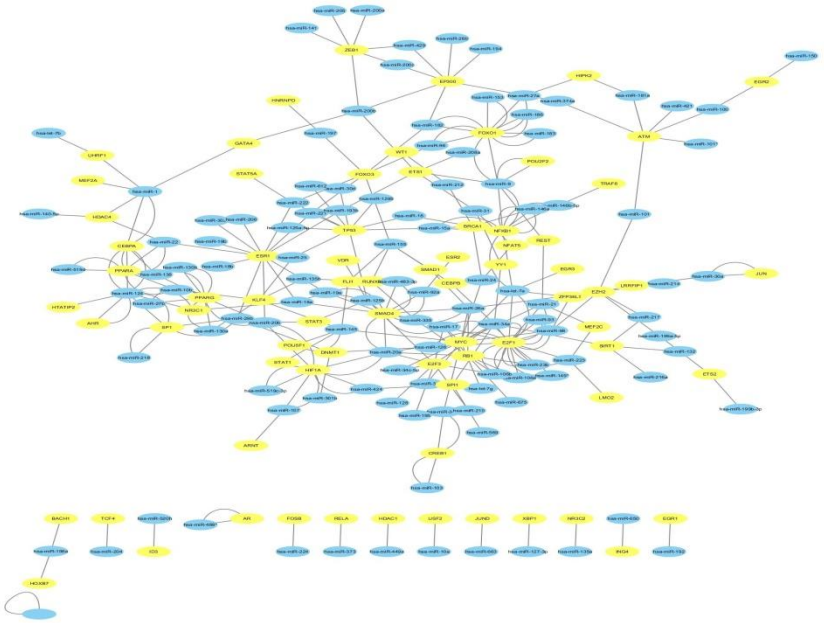

Figure 4. TF-miRNA regulatory network [yellow-TF, blue-miRNA].

The rating of the most nodes was high, as seen in Figure 5, and a fairly small number of nodes were communicating with other nodes. The possible active subset (Figure 5 and Figure 6) obtained has been exploited for further analysis, based on this study.

Table 1. Gene-TF-miRNA regulatory pathways common for Mets and its components

No of pathways	List of gene-TF-miRNA regulatory pathways in MetS	No of elements
1	AGT->NFKB1->hsa-let-7a	3
2	AGT->NFKB1->hsa-miR-148b-5p	3
3	AGT->NFKB1->hsa-miR-146a	3
4	AGT->NFKB1->hsa-miR-9	3
5	AGT->NFKB1->hsa-miR-15a	3
6	IL6->NFKB1->hsa-let-7a	3
7	IL6->NFKB1->hsa-miR-148b-5p	3
8	IL6->NFKB1->hsa-miR-146a	3
9	IL6->NFKB1->hsa-miR-9	3
10	IL6->NFKB1->hsa-miR-15a	3

No of pathways	List of gene-TF-miRNA regulatory pathways in MetS	No of elements
11	IL6->STAT1->hsa-miR-145	3
12	IL6->KLF4->hsa-miR-145	3
13	IL6->MYC->has-miR-20b	3
14	IL6->MYC->has-let-7a	3
15	IL6->FOXO1->has-miR-9	3
16	CCR5->NFKB1->hsa-let-7a	3
17	CCR5->NFKB1->hsa-miR-148b-5p	3
18	CCR5->NFKB1->hsa-miR-146a	3
19	CCR5->NFKB1->hsa-miR-9	3
20	CCR5->NFKB1->hsa-miR-15a	3
21	EDN1->NFKB1->hsa-let-7a	3
22	EDN1->NFKB1->hsa-miR-148b-5p	3
23	EDN1->NFKB1->hsa-miR-146a	3
24	EDN1->NFKB1->hsa-miR-9	3
25	EDN1->NFKB1->hsa-miR-15a	3
26	EDN1->STAT1->hsa-miR-20a	3
27	EDN1->NR1H4->hsa-miR-192-3p	3
28	EDN1->NR1H4->hsa-92a-3p	3
29	EDN1->FOXO1->has-miR-9	3
30	EDN1->HIF1A->has-miR-519c-3p	3
31	EDN1->HIF1A->has-miR-107	3
32	EDN1->HIF1A->has-miR-20b	3
33	EDN1->HIF1A->has-miR-424	3
34	EDN1->E2F1->has-let-7a	3
35	EDN1->PPARG->has-miR-20b	3
36	NR1H4->STAT1->has-miR-145	3
37	ACE->HIF1A->has-miR-519c-3p	3
38	ACE->HIF1A->has-miR-107	3
39	ACE->HIF1A->has-miR-20b	3
40	ACE->HIF1A->has-miR-424	3
41	AGTR1->HIF1A->has-miR-519c-3p	3
42	AGTR1->HIF1A->has-miR-107	3
43	AGTR1->HIF1A->has-miR-20b	3
44	AGTR1->HIF1A->has-miR-424	3
45	VEGFA->NR1H4->has-miR-192-3p	3
46	VEGFA->NR1H4->has-miR-92a-3p	3

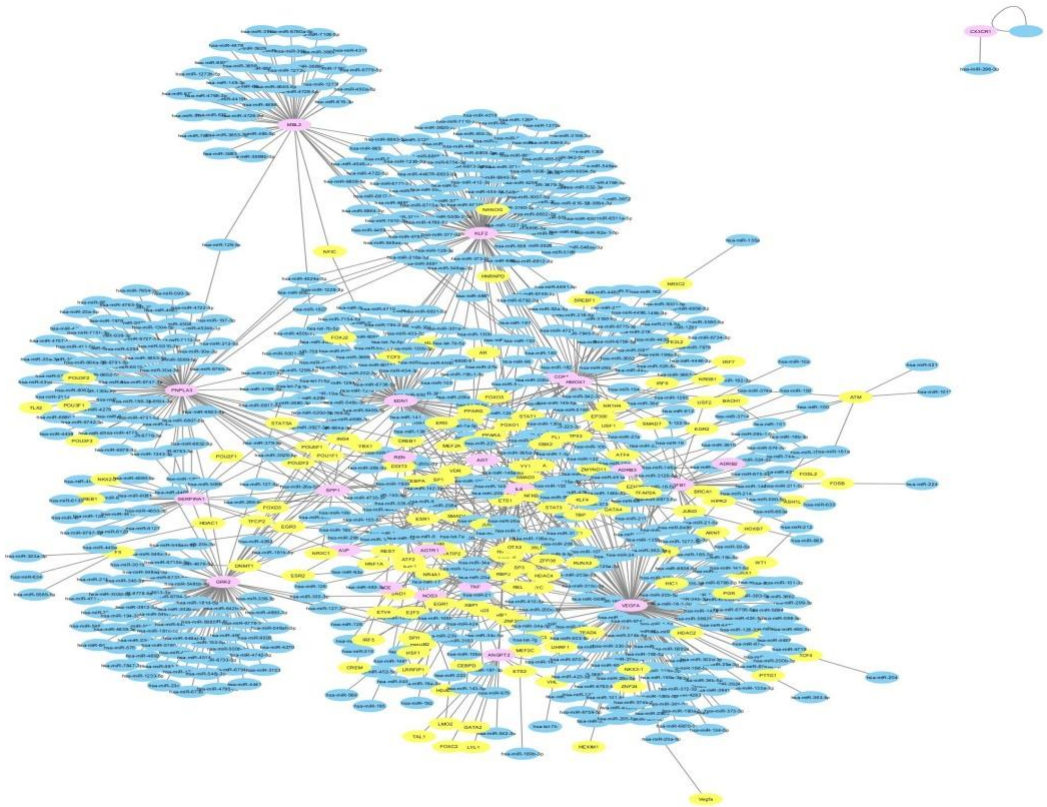

Figure 5. Gene-TF-miRNA regulatory network [pink-gene; blue-miRNA; yellow-Transcriptional Factor].

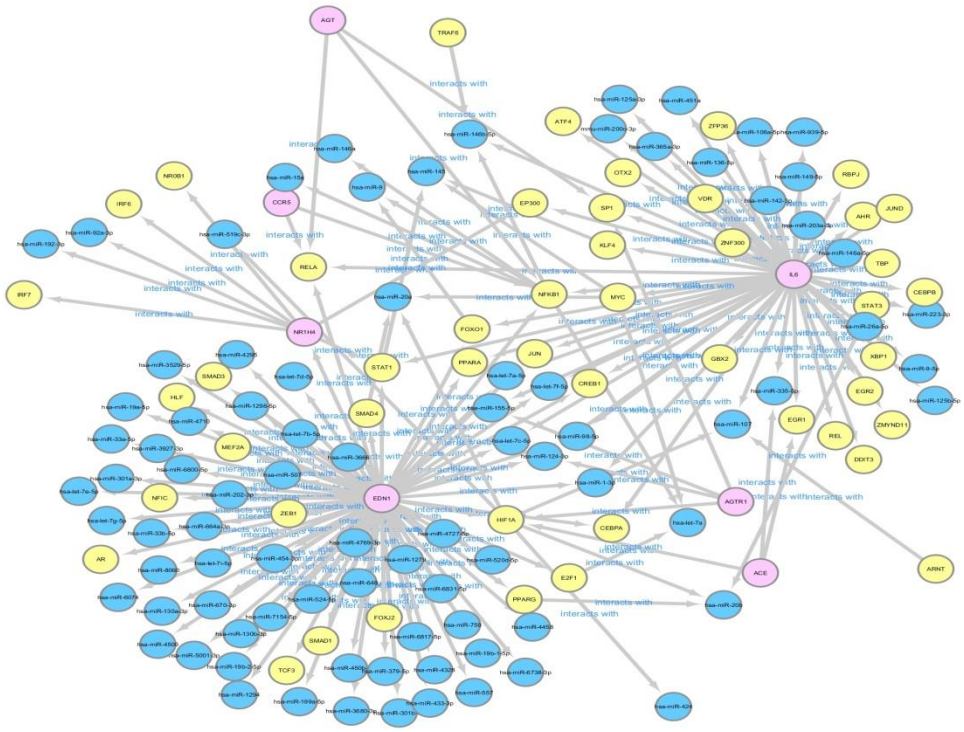

Figure 6. Degree analysis of the regulatory network [pink-gene; blue-miRNA; yellow-Transcriptional factor].

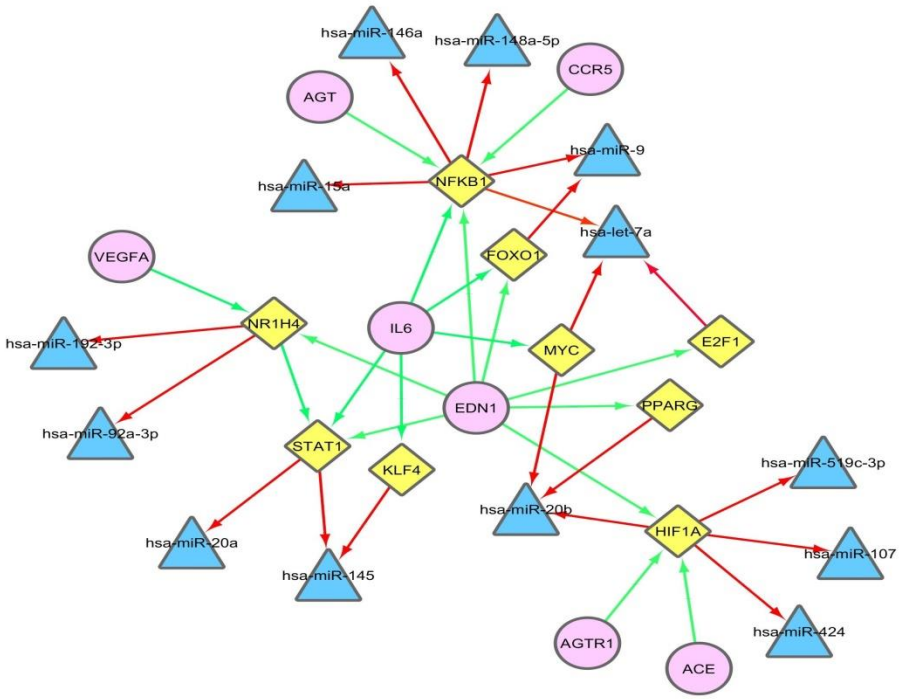

Figure 7. Re-constructed regulatory network common for MetS and its components [pink-gene; blue-miRNA; yellow-Transcriptional Factor].

3.4. Identification of potential active regulatory cascades.

In this analysis, we focused on regulatory paths that were linked in the curated TF-miRNA regulatory network to several TFs, miRNAs, and target genes in order to determine active regulatory trajectories of MetS, the molecular mechanisms of MetS are identified not only by uncovered transcription or post transcription regulatory cascades. We've established all the directed acyclic paths of 0 in-degree nodes to 0 out-degree nodes using BFS algorithms

using a possible active TF-miRNA regulatory sub-network. Certain regulators cannot regulate the 0 in degree gene / miRNA, which means it is upstream of the regulatory pathway. The gene / miRNA at 0 out-degree still does not regulate other genes / miRNA because it is downstream from the regulatory pathway. The upstream / miRNAs are important because their activation may result in a cascading effect that changes downstream gene / miRNA expression and leads to MetS. Therefore, we may find key upstream genes / miRNAs on the regulatory pathways by searching for all pathways between 0 in-degree genes / miRNAs and 0 out-degrees genes/ miRNAs. We found out that 46 regulatory cascades are listed in Table 2, and it was reassembled in the regulatory network module, as shown in Figure 7.

3.5. Identification of hub nodes in the network.

Nodes that have high centrality and are strongly connected (hub) have been analyzed, and these together show that the nodes play a major role in maintaining the overall network connectivity [24]. The regulatory network built was upgraded to the top 20 percent of the network on the basis of the central (BC) and node degree parameters. The number of the shortest routes passing one node is indicated by BC. The class represents one node's number of interactions. Nodes with BC>0.05 levels of thresholds and above each network's average value were called hub genes. The top 20% of the network constructed based on betweenness and degree in Cytohubba is shown in Figure 8 and Figure 9.

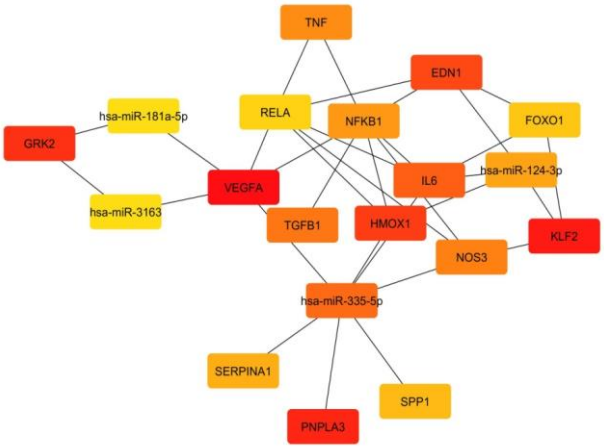

Figure 8. Betweenness analysis of genes and regulator.

In the case of the regulatory network's betweenness-analysis, 13 genes (VEGFA, KLF2, PNPLA3, GRK2, HMOXI, EDN1, MBL2, IL6, TGFB1, NOS3, TNF, SERPINA 1, SPP1) have been identified as nodes of a hub in the top 20 percent of the network.

In the case of the degree analysis (DA) for the regulatory network, the Top 20 percent of TF [NFKB1, SP1] and 1 miRNA [HAS-MIR-335-5p] have been identified as core nodes in 17 genes [VEGFA, KLF2, PNPLA3, GRK2, HMOX1, EDN1, IL6, MBL2, TGFB1, T NF, SPP1, NOS3, AGPT2, AGRT1, NR1H4, ADRB3] (Figure 9).

3.6. Pathway enrichment analysis.

In ClueGO, Cytoscape plug-in using the KEGG database, the functional annotation of the common genes found among MetS, CVD, T2DM, hypertension, obesity was carried out. Three key pathways, for example, the HIF-1 signaling pathway, the AGE-RAGE signal signaling route, and the Renin Angiotensin network, were significantly enriched (Figure 10) (Table 2).

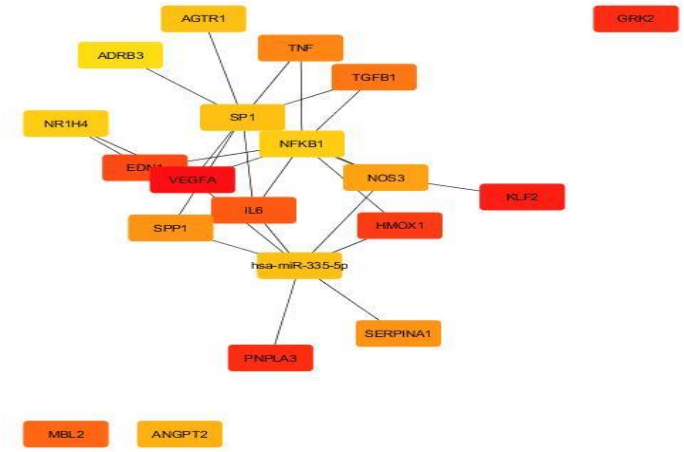

Figure 9. Degree analysis of genes and regulators.

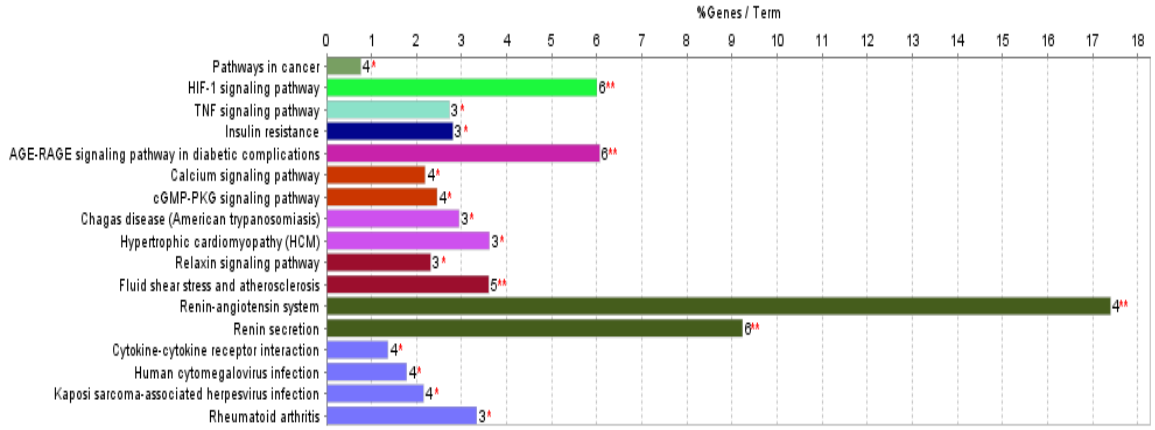

Figure 10. Pathway enrichment analysis of MetS and associated disorders.

Table 2. Most significant pathways involved in the syndrome.

KEGG ID	**Pathway**	**P-value**	**No. of genes**	**Genes**
KEGG:04022	cGMP-PKG signaling pathway	0.005945544	4	ADRB2, ADRB3, AGTR1, NOS3
KEGG:04668	TNF signaling pathway	0.00300178	3	EDN1, IL6, TNF
KEGG:05323	Rheumatoid arthritis	0.00349747	3	IL6,TNF,VEGFA
KEGG:04614	Renin-angiotensin system	5.98E-08	4	ACE,AGT, AGTR1, REN
KEGG:04924	Renin secretion	5.98E-08	6	ACE,ADRB2,ADRB3,AGT, AGTR1, REN
KEGG:04926	Relaxin signaling pathway	3.52E-04	3	EDN1, NOS3, VEGFA
KEGG:05200	Pathways in cancer	0.048666807	4	AGTR1,HMOX1,IL6, VEGFA
KEGG:05167	Kaposi sarcoma-associated herpesvirus infection	0.00349747	4	ANGPT2,CCR5,IL6, VEGFA
KEGG:04931	Insulin resistance	0.002774931	3	IL6, NOS3, TNF
KEGG:05410	Hypertrophic cardiomyopathy (HCM)	0.01161327	3	ACE, IL6, TNF
KEGG:05163	Human cytomegalovirus infection	0.00349747	4	CCR5, IL6, TNF, VEGFA
KEGG:04066	HIF-1 signaling pathway	1.71E-07	6	ANGPT2, EDN1, HMOX1, IL6, NOS3, VEGFA
KEGG:05418	Fluid shear stress and atherosclerosis	3.52E-04	5	EDN1,HMOX1,NOS3, TNF, VEGFA
KEGG:04060	Cytokine-cytokine receptor interaction	0.00349747	4	CCR5,CX3CR1, IL6, TNF
KEGG:05142	Chagas disease (American trypanosomiasis)	0.01161327	3	ACE, IL6, TNF
KEGG:04020	Calcium signaling pathway	0.005945544	4	ADRB2, ADRB3, AGTR1, NOS3
KEGG:04933	AGE-RAGE signaling pathway in diabetic complications	1.61E-07	6	AGTR1,EDN1, IL6, NOS3, TNF, VEGFA

The RAS pathway offers a possible causal link between MetS 'risk factors. Disruption of the RAS activity by any drug or genetic factor can promote weight gain, contributing to insulin resistance and the relief of hypertension [25, 26]. Increased AGE, RAGE, NF-KB, and RAS mediators are closely linked to blood pressure and vascular wave reflection and connection RAGE gene polymorphism and insulin resistance, which is subsequent [27]. Hypoxia-inducing factor-a loss / gain-of-function in animal models highlights the identification of hypoxia reaction in the pathogenesis of obesity and insulin resistance [28].

4. Conclusions

The majority of the regulatory associations are verified by literature studies published showing the efficiency and validity of the regulatory network regulated by MIRNA and TF. Our study has, therefore, been used to decipher the complex regulatory mechanisms in MetS and, through further validation of those pathways, will provide therapeutic targets.

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Conflicts of Interest

The authors declare no conflict of interest.

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