Original Article

Uncovering Systemic Lupus Erythematosus Candidate Proteomic Biomarkers: A Bioinformatics Approach

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Abstract

Background: Molecular analysis of different types of disease could be helpful to understand the mechanisms of the related abnormal functions at that level of disorder. Lupus is not an exception; by using protein-protein interaction network analysis, it is possible to investigate the molecular basis and malfunctions in this kind of disease.

Materials and Methods: Thirteen proteins were explored for interaction purposes, and some 12 central proteins were obtained via Cytoscape analysis.

Results: Among these proteins, three proteins were from the differentially expressed proteins or reported biomarkers of lupus. These proteins include Haptoglobin (Hp), Apolipoprotein C-III (APOC3), and Apolipoprotein A-I (APOA1).

Conclusion: It can be concluded that the biological processes of the central proteins could be part of Lupus's underlying mechanisms. Finally, validation studies are proposed by this current evaluation of the introduced panel of hub biomarkers.

Keywords: Systemic lupus erythematosus, Protein-protein interaction network analysis, Biological process

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Introduction

Worldly autoimmune heterogeneous disease, systemic lupus erythematosus (SLE) accounts for the most frequent type of lupus (1). In addition, many complications in human organs are associated with this disease (2). The most important ones are nephritis (3), atherosclerotic heart disease, osteoporosis, and the less frequent ones are malignancies (4). The first mentioned associated complication disease with lupus (Lupus nephritis (LN)) is very lethal; therefore, it requires substantial targeting (5). Early detection is a key challenging goal for treating lupus as a complex disease (6). In the light of hardship in SLE clinical management, identifying abnormal proteins as potential biomarkers in the disease mechanisms could optimize therapeutic approaches (7). Through the

spectrum of molecular studies, many biomarkers for SLE are searched and introduced. Some of these dysregulated biomarkers are cytokines such as interleukin-6 (IL-6), interleukin-10 (IL-10) (8), complement component-3 (C3), and complement component-4 (C4) as complement proteins (9), and microRNAs including miR-21 and miR-16 (10, 11). Proteomics is one of the outstanding molecular tactics that can facilitate deciphering the nature of SLE and its related complications (12). From the trigger of a disease to its development and treatments, proteomics can be applied to monitor molecular alteration from different resources including plasma, salivary, and urinary (13-15).

What is more, protein-protein interactions (PPIs) analysis could provide a principal outlook for recognition of the most leading biomarkers in biological processes and mechanisms of a corresponding disease (16, 17). In this way, molecules that depict significant interactions in a network scale of physical relations may be designated as more promising biomarkers of underlying mechanisms of disease (16). Central elements in network stability via topological analysis could be recognized and identified (18). Centrality parameters are frequently degree and betweenness values that biomarkers with the highest amount of these features are central in a network function (19). For the future direction, linked biomarkers with centrality features in network stability are essential to be explored to reveal the etiopathogenesis of SLE better. In this sense, a protein-protein interaction network analysis of important SLE biomarkers from proteomics data sets is designated in this study. It is aimed that limited numbers of possible biomarkers be introduced as an outcome of this investigation which can be considered as drug targets or diagnostic elements for lupus disease.

Methods

In the present study, possible protein biomarkers of SLE are investigated via bioinformatics methods, and data are extracted from the literature.

Source of data: The proteins from the proteomics review study based on mass spectrometry identification were examined until July 2015 from the databases of MEDLINE and EMBASE (13). This study was handled in 2017, a systematic review of different studies was carried out, and 25 types of research were chosen. Sources of the differentially expressed proteins (DEPs) were different biological samples, but serum was the main source. Since the presented data was a suitable collection for network analysis to find the new concept about SLE, it was a candidate to be assessed via the bioinformatics method.

Network construction: Next, thirteen significant candidate dysregulated proteins were assigned for network mapping assessment. Cytoscape version of 3.8.2 (https://cytoscape.org/) was used to evaluate protein-protein interaction network analysis of the biomarkers designated by different studies. Cytoscape software is used commonly to integrate and visualize the networks to find the properties of the nodes in relationship with the other elements of the network (20, 21). At first, these proteins were queried by the STRING database available through Cytoscape application (20). PubMed, protein query, disease, and Stitch compound query were the sources available from the STRING database (https://string-db.org/) (22). A protein query was applied for the three types of network search. The first network was the construction of searched proteins with a confidence score of 0.4 as the default option of software and the second query was with a score of 0.5. The final network was with the addition of 50 surrounding proteins (as the first neighbors) with a score of 0.5. In the network query, proteins are shown as nodes and the linkage between them denote physical interactions (23).

Network analysis: Centrality analysis was performed for the final network in terms of three different topological features, including degree (K), betweenness centrality (BC), and stress centrality (SC) via Network Analyzer that is well integrated into Cytoscape (24). The first two centrality parameters are the most important ones, and with the highest amounts are the indicators of hubs and bottlenecks, respectively. Additionally, biomarkers with these features are the most central ones in a network connection (25). This evaluation introduces 20% of top valued proteins in degree as hub nodes.

Gene ontology: Moreover, for the gene ontology (GO) analysis, biological process (BP) was determined via ClueGO2.5.7+CluePedia v1.5.7 plug-ins (26, 27). Statistical criteria for this analysis are: GO network connectivity (Kappa Score = 0.5) which is the moderate connectivity. A minimum number of proteins per term: 2 and minimum percentage of proteins in terms: 3. Furthermore, the corrected P-value method applied in this evaluation was Bonferroni step down ≤ 0.01.

Results

Systemic lupus erythematosus biomarkers detection is important through bioinformatics evaluations of proteomics data set. The most-reported proteins in this regard are gathered based on the study by Orthodoxia Nicolaou et, al. in 2017 (13). The evaluation of 241 proteins reported for SLE was narrowed down to 13 based on their mostly reported associations with the disease (see table 1).

Network construction of 13 proteins without the addition of neighbour nodes and default confidence score was performed by the platform of Cytoscape (see figure 1). This figure shows that the STRING database recognizes 12 individuals among the 13 queried proteins.

As indicated in figure1, the edge between all the proteins is present, and none remained as an individual one in this experiment. This pattern shows acceptable connectivity and linkage between identified biomarkers.

As shown in figure 2, in the next step, the 13 query biomarkers (or 12 recognized individuals); are interacted as same as figure 1; however, a higher confidence score of 0.5 is applied.

The figure 2 data depict that while raising the confidence score cut off, there is still a satisfactory linkage between biomarkers in this study.

In the final step of network construction, neighbour addition is performed better to understand the biomarker role in a whole interacting network (see figure 3).

"NetworkAnalyzer" plug-in detected high degree and betweenness centrality nodes known as hubs and bottlenecks, respectively for the differentially expressed proteins (see table 2).

Albumin (AL) and amyloid precursor protein (APP) have the highest degree and betweenness centrality scores. APOA1, Hp, and APOC3 are the query DEPs among the hubs based on the computation of "NetworkAnalyzer". While these proteins are important in degree values, there is no betweenness centrality score except for APOA1 which is 0.02.

Visualization of biological process (BP) of lupus was through ClueGO+CluePedia. In figure 4, the grouping is based on the percentage of proteins contributing to the related group. Each group consists of associated terms with a leading one chosen for naming that group. The two-star indicates that group's statistical significance, which is p≤0.01.

Table 1: The list of differentially expressed proteins in Lupus disease reported by different studies from the systematic review.

Row	Protein Name	Gene Name	Uniprot Code
1	Annexin A2	ANXA2	P07355
2	Annexin A5	ANXA5	P08758
3	Alpha-1-antitrypsin	Serpina1c	Q00896
4	Serotransferrin	TF	P02787
5	Ezrin	EZR	P15311
6	Elongation factor 1-alpha 1	Eef1a1	P68104
7	Glyceraldehyde-3-phosphate dehydrogenase (G3PD)	G3PD	P48812
8	Alpha-enolase	ENO1	P06733
9	Haptoglobin	Hp	P00738
10	Transthyretin	TTR	P02766
11	Apolipoprotein A-I	APOA1	P02647
12	Apolipoprotein CIII	APOC3	P02656
13	Apolipoprotein C-I	APOC1	P02654

Table 2: The list of hub proteins ranked based on the degree values, the gene name, centrality properties (Degree=k, Betweenness centrality= BC Stress=S) is included.

Row	Display name	Query term	K	S	BC	Query term
1	ALB	APOA1	51	1908	0.05	APOA1
2	APP		51	1646	0.04	
3	APOB		48	1188	0.02	
4	APOA1		47	978	0.02	
5	APOA2		45	824	0.01	
6	APOE		43	880	0.01	
7	FN1		41	1462	0.04	
8	HP	Hp	40	564	0	Hp
9	APOH		40	712	0.02	
10	IL6		40	998	0.02	
11	APOC3	APOC3	38	488	0	APOC3
12	APOA5		38	516	0	

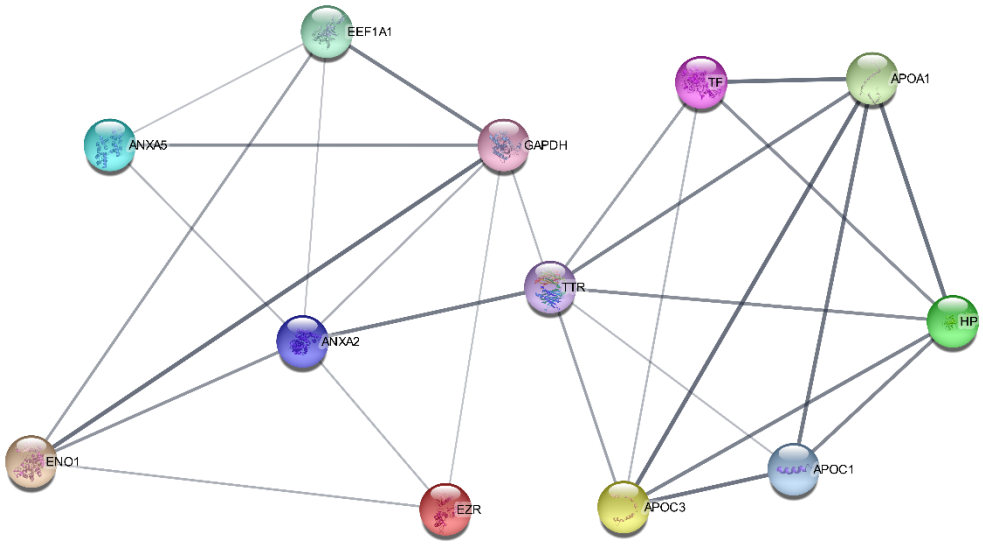

Figure 1. The first network of query nodes with a confidence score cut of 0.4 consisted of 12 nodes connected via 28 links.

In figure 4, six groups of biological processes are present. The most significant one is Plasma lipoprotein particle remodelling. This group occupies almost 50% of all the terms related to the hubs. The next important group is an acute inflammatory response, about 17% of the whole biological process group associated with these central proteins. The rest of the groups are negative regulation of blood coagulation, lipoprotein lipase activator activity, lipid storage, and positive regulation of amyloid fibril formation. These latest three indicate the same portion of the associations with central proteins. Moreover, almost all the hubs belong to the first group of terms. In addition, all the apolipoprotein (APO) families are connected to the first highlighted group.

Another presentation of hub annotations regarding BPs is analyzed via ClueGO+ CluePedia as shown in figure 5.

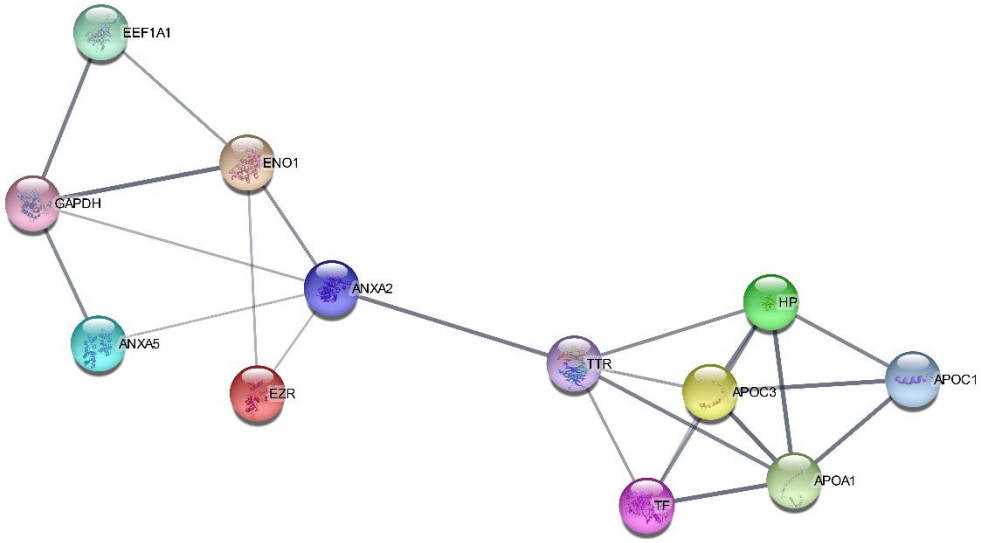

Figure 2. The second network of query nodes with a confidence score cut of 0.5 including 12 nodes and 23 links.

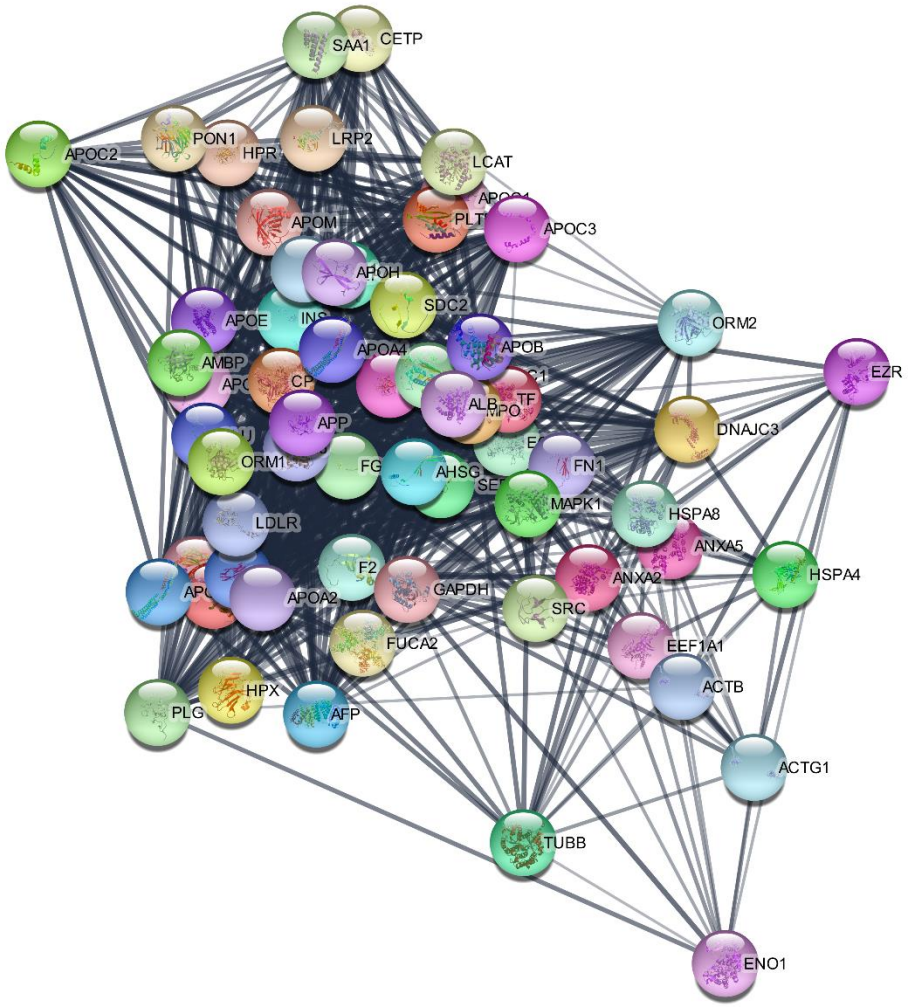

Figure 3. A network of query nodes with the addition of neighbor proteins, numbers of nodes: 62 and connections: 910.

Discussion

Molecular analysis is beneficial in different aspects of a disease. One of the important ones to be studied is lupus as an autoimmune disease (15). A better understating of the disease origin could be retrieved by applying a protein-protein interaction network. More central proteins could be essential to the strength of that network and consequently the disease onset and developments (28). Thirteen proteins were chosen as biomarkers of Lupus from a meta-analysis of proteomics studies (13). These biomarkers were then subjected to conducting protein-protein interaction network analysis via Cytoscape. The query shows that the first and second networks are in condensed interactions without considering additional first neighbor proteins. However, the second network as designating a higher cut-off, a lower interaction would be achieved. The final network consists of main query proteins and the First neighbor proteins that they could also be vital in the network stability. Investigations are showed that adding first neighbors to the queried proteins provides critical information about molecular mechanism of the studied subjects (29).

A centrality analysis of the final network showed that 12 proteins are in high values in degree. Among them, three proteins are from the biomarkers significantly reported by previous studies. These proteins (APOA1, Hp, and APOC3) are more noteworthy and could be considered as the most important biomarkers.

Moreover, the biological process analysis of the central nodes indicates highlighted groups of terms about the central nodes. These biological processes could be key in the disease mechanisms and worth complementary study. Furthermore, other central proteins are also valuable for further study to reveal their part in Lupus mechanisms. What is more, a literature review of differentially expressed hubs, provides further knowledge of their significance in Lupus disease.

APOA1 (Apolipoprotein A1) is an antioxidant and anti-inflammatory protein (30), and its down-regulation has been reported for lupus (31). One of the main parts of high-density lipoprotein (HDL) is this protein that displays protective properties against atherosclerosis (32). Furthermore, the anti-apolipoprotein A1 signature in patients with Lupus is active, correlated with heart disease complications risk (33).

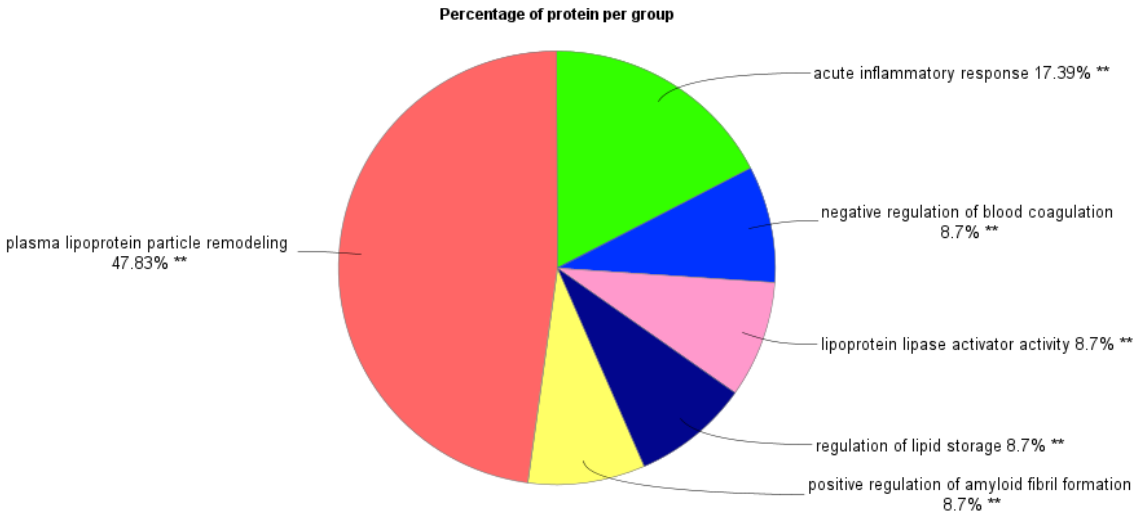

Figure 4. The pie chart view of biological processes groups correlated to the top hubs of the lupus network.

According to some studies, haptoglobin as an acute-phase protein plays a fundamental role in Lupus, which shows dysregulation in serum and urine (34, 35). In addition, there is a linkage between Hp and the disease severity and complications such as cardiovascular disease (13, 36, 37).

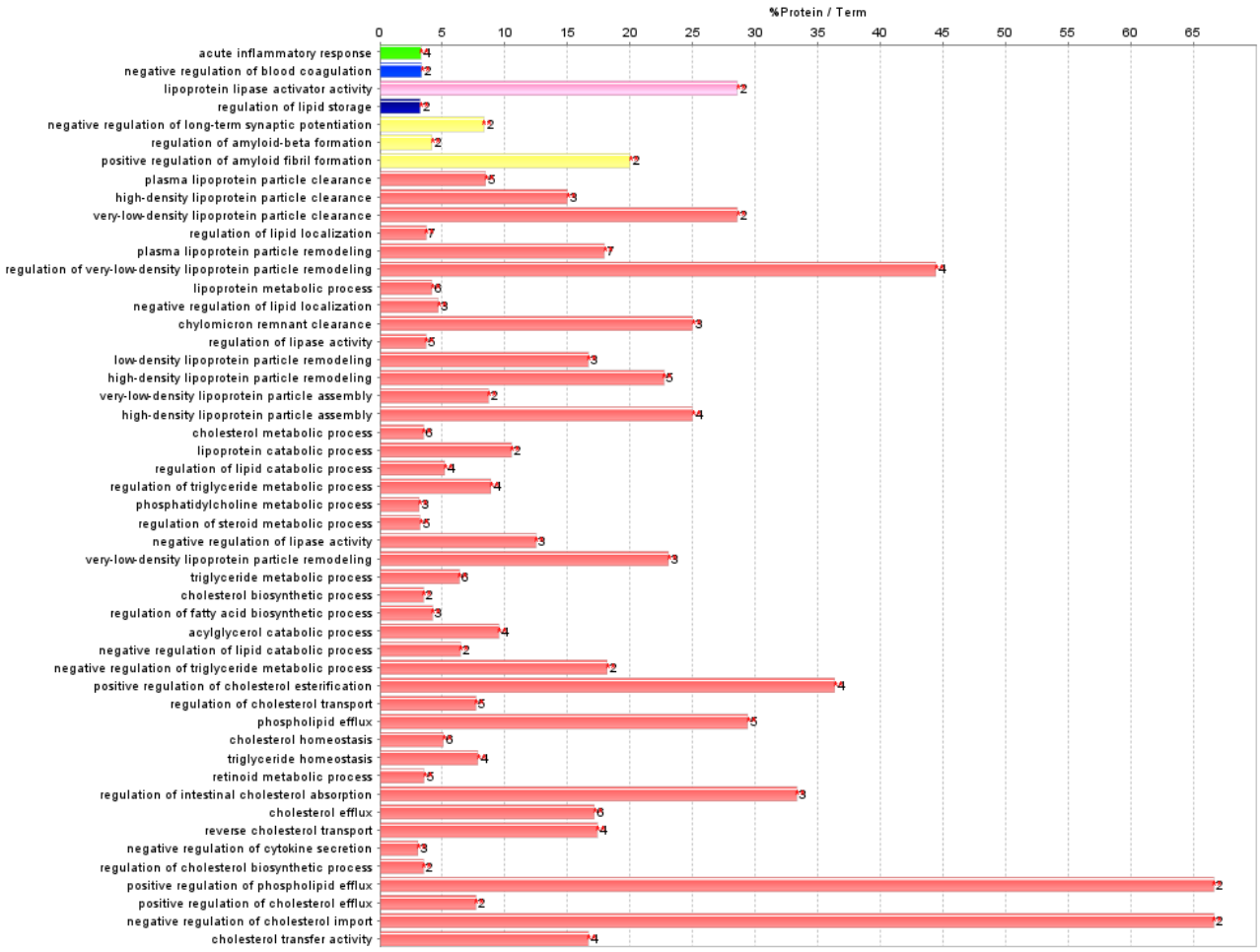

Figure 5. Bar chart of biological processes terms represented as a bar chart. Each group contains different processes and is highlighted with different colors.

The last DEP is apolipoprotein CIII (APOC3), the third important hub from queried proteins, indicating some relations with atherosclerosis, especially Nephritis lupus. In a way, in patients with the manifestation of renal complications, APOC3 has a higher level of expression in serum (38).

As depicted in figures 4 and 5 the main class of biological terms is “plasma lipoprotein particle remodeling, " characterized by 47.83% of the biological terms. VA Kudinov et al indicates that HDL remodeling protects from atherosclerosis (39). It was pointed out that atherosclerosis is the main complication of lupus (40). Since APOC3 is the main related protein to the discussed biological term, it seems that regulation of APOC3 can improve SLE or prevent its development, however, more investigations are suggested.

Conclusion

In summary, lupus is a complex disease, and many molecular elements contribute to its development. After validation studies, this study's protein-protein interaction network analysis showed that Hp, APOC3, and APOA1 could be the novelist biomarkers of Lupus.

Acknowledgment

None.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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