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Full Length Article

In silico method for identification of novel copper and iron metabolism proteins in various neurodegenerative disorders

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ABSTRACT

Copper (Cu) and Iron (Fe) has been the subject of intensive research over several decades as numerous seminal studies robustly support the involvement of Cu and Fe metabolism dyshomeostasis as a common denominator in several neurodegenerative disorders (particularly Alzheimer's disease and Parkinson's disease); however, till date, the exact "cause-effect" association has not been elucidated. Thus, there is urgent need to look for newer association/pathways of these redox active elements in different neuropathological conditions. Therefore, in this study, we have used bioinformatics based approach to identify novel Cu and Fe metabolism proteins in neurodegenerative disorders using Cytoscape software. The network biology data demonstrated the association of secreted protein acidic and rich in cysteine (SPARC/osteonectin) protein with Alzheimer's disease, Parkinson's disease, Huntington's disease and neurodegeneration with brain iron accumulation (NBIA) disease, whereas Coagulation factor V may have a role in Brunner Syndrome, Obsessive-Compulsive Disorder, Febrile seizures and Schizophrenia diseases. Further analysis revealed Coagulation factor VII possible role in L1 Syndrome and Congenital hydrocephalus disorders. In conclusion, the present study shows the first evidence *in silico* that SPARC/osteonectin, Coagulation factor V and VII proteins may have plausible role in the pathogenesis of various neurodegenerative diseases.

1. Introduction

Copper (Cu) and Iron (Fe), essential trace elements, owing to their redox active property are imperative for several central nervous system (CNS) metalloenzymes/proteins (refer to Supplementary material 1 and 2 for list of genes/proteins involved in Cu and Fe metabolism/pathways, respectively). As these biometals are imperative for proper CNS/neuronal functioning and integrity, both excess as well as deficiency of Cu and Fe is detrimental to cell, and leads to various neuropathologies (Bulcke et al., 2017; Chen et al., 2016; Kozlowski et al., 2009; Nunez et al., 2012). In particular, excess of Cu and Fe results in production of highly damaging free hydroxyl radicals causing oxidative stress, cytotoxicity, mitochondrial dysfunction and aging. Oxidative stress results in protein oxidation, lipid peroxidation and cleavage of DNA. Due to high levels of polyunsaturated lipids in neuronal membranes, CNS is particularly susceptible to damaging effects of oxidative stress (Kardos et al., 2018). It's worth mentioning here that oxidative stress has been shown to induce cognitive deficits (Lima et al., 2008; Rahman et al., 2009).

Cu metabolism dyshomeostasis has been conclusively associated with Menke's disease, Wilson's disease, Indian childhood cirrhosis and idiopathic Cu toxicosis (Crisponi et al., 2010; Kaler, 2013; Kardos et al., 2018). On the other hand, alteration in Fe metabolism has been associated with hemochromatosis and sideropaenia (Brissot and Loreal, 2016; Guo et al., 2016; Worwood, 1999). However, various studies have also shown alteration of Cu and Fe levels as a common feature in other neurodegenerative disorders (ND) especially Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and neurodegeneration with brain iron accumulation (NBIA) (Aizenman and Mastroberardino, 2015; Comstra et al., 2017; Hayflick et al., 2018; Kardos et al., 2018; Poujois et al., 2016; Rivera-Mancia et al., 2010; Sensi et al., 2018; Uranga and Salvador, 2018; Wang and Wang, 2017); notwithstanding, a direct causative association was never established impeding the development of novel therapeutic targets/strategies.

Till date, the initiating/main cause(s) of neuronal death process in most cases of ND remains unidentified. Nonetheless, there is consistent/established observation of redox metal/s (Cu and Fe) accumulation, oxidative stress, protein misfolding/aggregation and damage to various

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biomolecules in the affected brain region(s) in different ND (Mitra et al., 2014). The clinical utility, of the established fact that there is definitive role of metal (Cu and Fe) dyshomeostasis in various ND diseases, is still not achieved primarily due to lack of mechanistic understanding of the exact role of Cu and Fe toxicity in neuronal death and ND initiation/progression (Kozlowski et al., 2009; Pal, 2014; Schneider et al., 2013). "Biometals and ND" field is also impeded by the ever expanding metalloproteome, and the extensive network of metal-metal, metal-protein, and protein-protein interactions under "normal" and "pathological" conditions. Further, these interactions are dynamic *i.e.* changing with age, disease progression and therapy. These facts support the need to investigate metal-protein and protein-protein interactions in ND (Adlard and Bush, 2018). Present study included all Cu and Fe metabolism proteins to study their inter- and intra- protein-protein interaction in ND. Also, biasness was avoided by selecting all Cu and Fe metabolism proteins, which also provides a chance to ascertain novel protein-protein interaction/association in ND using network biology approach.

Network biology is of great help in understanding gene–gene and protein–protein interactions involved in ND, and it can be exploited to identify common/novel protein(s) or gene(s) mediating the Cu and Fe mediated neuronal death (Parikshak et al., 2015). Using the *in silico* approach in a preliminary study, we have previously reported that among 8 main Cu metabolism proteins, superoxide dismutase 1 and ceruloplasmin may represent direct and indirect link with AD and PD, respectively (Pal et al., 2014). Taking the previous study to a comprehensive level, we have investigated the role all reported 204 Cu and 441 Fe metabolism associated proteins of *Homo sapiens* in ND in this study. Therefore, the present study sought to further ascertain the role of all Cu and Fe metabolism proteins in different neuropathologies. Taking into account the previously mentioned facts, we therefore asked whether network biology can answer for novel Cu and Fe metabolism proteins in various ND. In this study, we have reported the novel Cu and Fe metabolism proteins in several ND.

2. Materials and methods

Network biology studies provide a novel way to see multivariate effect of more than two factors to study old research problem. Only reviewed set of proteins (here reviewed proteins refers to experimentally validated proteins which are also confirmed/reviewed by other lab groups) annotate for Cu and Fe metabolism directly or indirectly were searched from National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) and Uniprot (https://www.uniprot.org/) databases, and extracted for further analysis. Fig. 1 shows the overall flow diagram for the present study. Subsequently, we have created a protein-protein interaction (PPI) network from Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; https://string-db.org/) database. STRING database shows functional protein-protein association networks. In the third stage, we have created a combined PPI network of Cu and Fe proteins data network, and did clustering on the basis of Molecular Complex Detection (MCODE) plugin. In the last stage, identification of hub or center protein which has maximum internode connection was done.

The clusters so formed during analysis were subjected to in built statistical analysis feature of Cytoscape for evaluating the clusters on the basis of following parameters: i) Clustering coefficient; ii) Network dimensions like radius, diameter, density, centralization and heterogeneity; and iii) Node parameters like count, isolated nodes, node pairs *etc*. The cluster formation and statistical analysis was followed by ranking of clusters in each PPI network separately. The proteins in the top ranked clusters along with the sub-network hub proteins were retrieved in a final list for pathway search using Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.genome.jp/kegg/). The associated proteins obtained were literature mined to identify the novel proteins involved in various ND.

Open source database and software like Uniprot, STRING, Cytoscape 3.0 (https://cytoscape.org/) and Reactome (https://reactome.org/) were used. Various plug in features of Cytoscape software like clustering, MCODE, merging of networks and sub-network creation were also used in this study.

3. Results

3.1. Data extraction

A dataset of only reviewed set of proteins involved in Cu and Fe metabolism in *Homo sapiens* were retrieved using NCBI and Uniprot databases. The list of Cu related proteins contained entries of 204 proteins, while there were 441 protein entries in the list of Fe related proteins (refer to Supplementary material 1 and 2 for Cu and Fe related proteins, respectively; source https://www.uniprot.org/). Thereafter, STRING database was used to generate PPI network of proteins listed in Supplementary material 1 and 2.

3.2. Networking

Cu and Fe related proteins were provided as input into Cytoscape software, and their related PPIs networks from STRING database were imported using Reactome database. The PPI networks imported using Reactome databases were visualized and analyzed using Cytoscape. The proteins in the PPI network were designated as nodes (circle), and their biological interactions with other proteins as edges (lines). The size of the node increases with the increase in the number of interacting proteins, which is known as degree of connectivity. Similarly, the increase in the thickness of the edges represents the increase in the biological interactions between interacting proteins. Table 1 shows the node and edge parameters of PPI networks imported for Cu and Fe related proteins (refer to Supplementary material 3 and 4 for Cu and Fe PPI network images using Cytoscape, respectively). We provided a list of 204 and 441 proteins as input for Cu and Fe, respectively but, surprisingly the corresponding PPI networks generated constituted 1175 and 2529 nodes for Cu and Fe, respectively (Table 1) which means that the number of proteins available for further analysis was increased exponentially upto the node count value. Hereafter, all the subsequent steps were carried out on all of these proteins, which expanded area of research under the present study significantly. The edge count also increased significantly approaching values of 1350 and 7233 for Cu and Fe, respectively (Table 1).

3.3. Network clusters

After importing of Cu and Fe related PPI networks, generation of clusters of the obtained PPI networks was performed using the MCODE plug in of Cytoscape. The clusters detect densely connected regions in this large interaction network that may be part of a protein complex or pathway. MCODE clusters were formed on basis of score of the clusters. The top five clusters of both Cu and Fe were used. MCODE detects the clusters in the interaction network and assigns them ranks on the basis of scores. The parameters and scores for top five Cu and Fe clusters are given in Tables 2 and 3, respectively. These clusters help to narrow down our search for proteins by giving us potential lead about those present in the clusters.

3.4. Network merging

PPI networks of Cu and Fe were merged to find out the proteins common to both the PPI networks. This was done in two steps sequentially: i) Network Merge tool was used to combine PPIs networks of Cu and Fe proteins by union so that we included all proteins and created a single larger PPI network containing all Cu and Fe nodes and edges, which ensures complete coverage of relevant proteins; and ii)

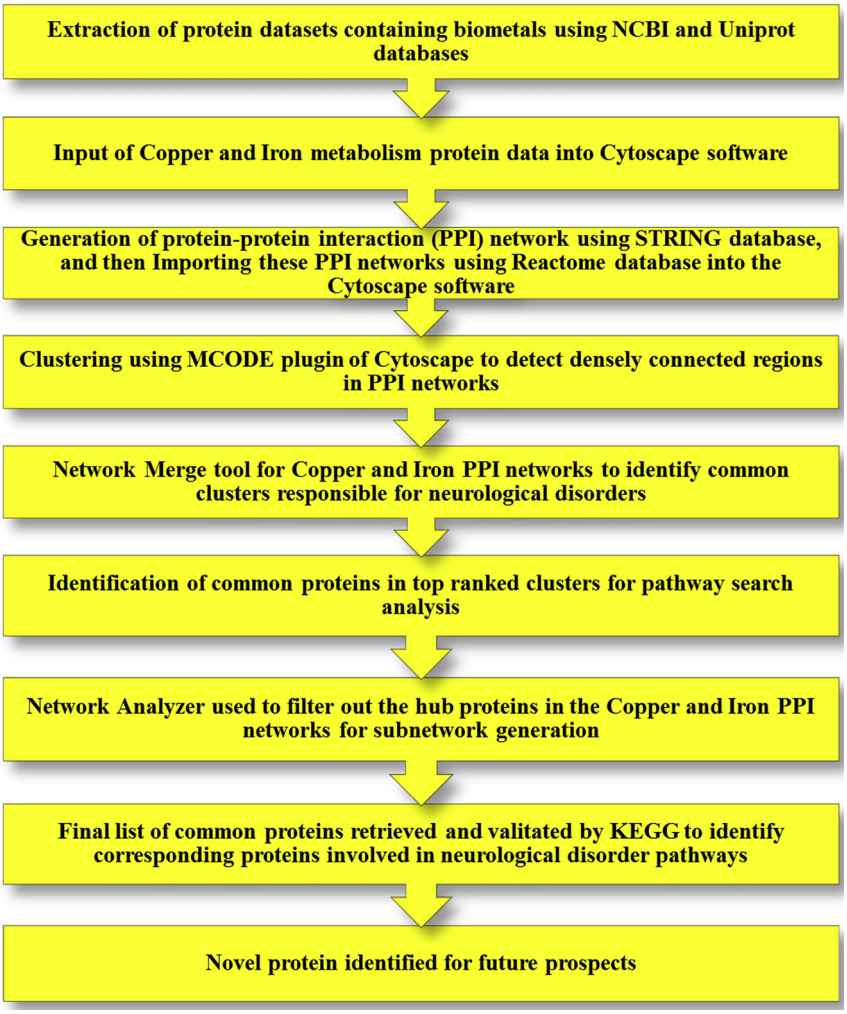

Fig. 1. A schematic flow diagram for identification of novel copper and iron metabolism proteins in neurodegenerative disorders.
KEGG: Kyoto Encyclopedia of Genes and Genomes; MCODE: Molecular Complex Detection; NCBI: National Center for Biotechnology Information; PPI: protein protein interaction; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins.

Table 1
Protein network details of Cytoscape including nodes and edges parameters for copper and iron related proteins.

Biometal Network	Nodes	Edges
Copper Network	1175	1350
Iron Network	2529	7233

Proteins are designated as node and biological interactions between the proteins are designated as edge.

Table 2
Protein network cluster details of Cytoscape including for top five copper clusters.

Cluster Rank	Nodes	Edges	Score
1	7	10	2.5
2	4	5	2
3	2	3	2
4	2	3	2
5	3	4	2

Proteins are designated as node and biological interactions between the proteins are designated as edge.

Table 3
Protein network cluster details of Cytoscape including for top five iron clusters.

Cluster Rank	Nodes	Edges	Score
1	68	325	9.42
2	26	58	4.296
3	6	15	4.286
4	69	140	4
5	182	363	3.967

Proteins are designated as node and biological interactions between the proteins are designated as edge.

Table 4
Protein network details of Cytoscape cluster for top five merged network cluster.

Cluster Rank	Nodes	Edges	Score
1	19	86	8.600
2	7	25	6.250
3	6	15	4.286
4	269	572	4.237
5	6	13	3.714

Proteins are designated as node and biological interactions between the proteins are designated as edge.

Cluster formation of three sets of PPI network were performed using MCODE plugin of Cytoscape to filter out the densely connected proteins. PPI networks of Cu and Fe proteins were individually clustered first, and same is repeated for combined or merged PPI network of Cu and Fe proteins to discover any novel association among Cu and Fe proteins. Supplementary material 5 shows the merged PPI network of Cu and Fe metabolism proteins generated using Merge tool of Cytoscape containing 2759 nodes and 8027 edges. In the merged PPI network, clusters were detected by MCODE, and assigned them rank on the basis of the scores. Table 4 demonstrates the parameters and scores for top five ranked merged PPI network clusters.

3.5. Subnetwork creation

Network analyzer tool was used to identify hub proteins within the PPI network, and create a sub-network. In addition, Workflow tool was also used to first highlight the hub proteins, which were filtered by selection on basis of Node degree attribute. The proteins that formed connections within the range of node degree attribute were identified as hubs of important pathway proteins. These proteins were then selected for sub-network creation (Supplementary material 6 and 7 shows the sub-network for the Cu and Fe network, respectively) to apply comparative analysis.

3.6. Comparative analysis

Comparative analysis was performed firstly by identifying the common proteins amongst clusters of Cu, Fe and their merged PPI network by performing merging of clusters by Intersection. Merging provided PPI networks or clusters of common proteins amongst the three clusters (Table 5). Secondly, cross comparison of the hub proteins with the cluster proteins produced a final list of common proteins (Fig. 2). Fig. 2 shows all the proteins found common between the hub proteins of either Cu or Fe Workflow or both, and the top ranked clusters of Cu, Fe or their merged PPI network. Fig. 2 is color coded after manual searching of common protein present in top 5 rank clusters in Cu, Fe and Cu + Fe PPI networks. These proteins were subjected to pathway search analysis in the decreasing order of their frequencies. In addition to that, color coding was another criterion for choosing the sequence in which proteins were subjected to pathway search analysis with yellow color being the most favorable (frequency 3) and blue/green color with frequency 2 being the next choice. Red color was least favorable with frequency 1 (Fig. 2). Notwithstanding, contrary to their favorability on the basis of frequency, their lesser favorability gives them a higher chance at novelty (As low frequency of occurrence might have resulted in them being gone unnoticed during other strategies applied to filter out the proteins involved in ND). Nonetheless, we used all the listed proteins in Fig. 2 for pathway search analysis starting with the most favorable ones.

Table 5
Protein network details of clusters with common proteins.

Clusters with common proteins	Nodes	Edges
Copper(5)-Iron(1)	1	0
Copper(5)-Merged Network(1)	1	0
Iron(1)-Merged Network(1)	5	10
Iron(3)-Merged Network(3)	6	15
Iron(4)-Merged Network(4)	59	117
Iron(5)-Merged Network(4)	36	0

The numbers in parenthesis correspond to the cluster number and the nodes correspond to the common proteins identified amongst clusters.
Proteins are designated as node and biological interactions between the proteins are designated as edge.

3.7. Novel protein identification

The common proteins so found were subjected to KEGG pathway search analysis to find out their disease association, and their role in the Cu and Fe metabolism. Finally the associated proteins were literature mined to identify the novel ones. Osteonectin [encoded by secreted protein acidic and rich in cysteine (SPARCgene)] and Coagulation Factor V & VII (encoded by F5 and F7 gene, respectively) were found to be novel proteins involved in different ND (Table 6).

4. Discussion

The present *in silico* study using the Cytoscape software and KEGG pathway search analysis database demonstrated the plausible role of: i) Osteonectin (also known as ON; SPARC; OI17; BM-40) protein in AD, PD, HD and NBIA; ii) Coagulation factor V protein in Brunner Syndrome, OCD, Febrile seizures and Schizophrenia diseases; and iii) Coagulation factor VII protein in L1 syndrome and congenital hydrocephalus diseases corroborated by role of each protein in neuronal biometal pathway as outlined in Table 6. There is growing acceptance in the scientific literature that property of failure of homeostatic mechanism of biometals is shared by various ND. In particular, Cu and Fe have received particular scrutiny due to various seminal evidences supporting the key role of these elements in neurodegeneration, and the observed accrual of Cu and Fe in the specific regions of the brain beyond the handling capacity of individual cell of brain parenchyma (astrocyte, neuron, microglia, oligodendroglia and endothelial cell). Conversely, reduction of Cu and Fe levels has also been reported in other selected regions of brain (Bagheri et al., 2018; Bulcke et al., 2017; Kozlowski et al., 2009; Nunez et al., 2012).

To solve the conundrum surrounding the role of Cu and Fe in the complex pathoetiology of various ND disorders, system biology can be of immense help initially as it facilitates the study of interaction among numerous genes and proteins, and narrow down the target biomolecule/association/pathway by quickly analyzing the large scientific data filtering out the biomolecules/associations/interactions/pathways which have been shown ineffective or rendered useless by experimental data (Barabasi and Oltvai, 2004). Cytoscape is one of the most widely used open-source software tool for the study/visual exploration of biomedical networks composed of protein-protein, gene-gene interactions, and other types of interactions. Due to its versatile and interactive visualization interface for exploring complex biological interconnections, Cytoscape has been the preferred choice in numerous seminal studies (Su et al., 2014).

The findings of the present study are of great significance because earlier SPARC/osteonectin, an acidic matricellular glycoprotein (MCP), was thought to play a key role in bone mineralization and cell-matrix interactions, and was shown to be significantly overexpressed in cancers only [particularly pancreatic cancer, gastric cancer, and breast cancer (Reviewed by Bradshaw, 2012). However, contrary to general concept, now the role of SPARC/osteonectin and other MCPs in many ND is also being recognized (discussed in next section; reviewed by Jayakumar et al., 2017). SPARC/osteonectin exerts its carcinogenic effect by increasing the production and activity of matrix metalloproteinases (which helps invading cancer cells within bone), and promoting angiogenesis, proliferation and migration. It is noteworthy here that SPARC is a source of Cu-binding peptides that stimulate angiogenesis (Lane et al., 1994).

SPARC/Osteonectin protein is encoded by SPARC gene and belongs to SPARC family of proteins. SPARC gene, having 10 exons, is present on chromosome 5 (Rosset and Bradshaw, 2016), and has been implicated in Osteogenesis imperfect type xvii, and delayed speech and language development, intra-ventricular hemorrhage, and motor delay. Each member of SPARC family possesses a characteristic conserved EC (E–F hand calcium binding) domain. Based on sequence homologies of the EC domains, the SPARC family members can be grouped into four

	S. NO.	Protein_ID	Copper	Iron	Merged	Frequency
Hub proteins of Copper network	1.	P28300	CLUSTER-1	-	-	1
	2.	P08123	CLUSTER-1	-	-	1
	3.	P09486	CLUSTER-1	-	-	1
	4.	Q08397	CLUSTER-1	-	-	1
	5.	P02452	CLUSTER-1	-	-	1
	6.	P00450	CLUSTER-2	-	-	1
	7.	P49281	CLUSTER-2	-	-	1
	8.	Q9BQS7	CLUSTER-2	-	-	1
	9.	P00451	CLUSTER-3	-	CLUSTER-2	2
	10.	P12259	CLUSTER-3	-	CLUSTER-2	2
	11.	P35052	CLUSTER-4	-	-	1
	12.	P06727	CLUSTER-4	-	-	1
	13.	P04637	-	CLUSTER-4	-	1
	14.	P05230	-	CLUSTER-5	-	1
	15.	P06493	-	-	CLUSTER-4	1
Copper and Iron	16.	P00156	CLUSTER-5	CLUSTER-1	CLUSTER-1	3
	17.	P62987	-	CLUSTER-4	CLUSTER-4	2
	18.	P62979	-	CLUSTER-4	CLUSTER-4	2
	19.	P0CG47	-	CLUSTER-5	-	1
	20.	P0CG48	-	CLUSTER-5	-	1
Hub proteins of Iron network	21.	P99999	-	CLUSTER-1	CLUSTER-1	2
	22.	P25054	-	CLUSTER-2	-	1
	23.	P35222-1	-	CLUSTER-2	-	1
	24.	P49643	-	CLUSTER-2	-	1
	25.	P14635	-	CLUSTER-4	CLUSTER-4	2
	26.	Q13177	-	CLUSTER-5	-	1
	27.	P08709	-	-	CLUSTER-2	1
	28.	P42336	-	-	CLUSTER-4	1
	29.	P27986	-	-	CLUSTER-4	1
	30.	Q13485	-	-	CLUSTER-4	1

Fig. 2. List of all common proteins found between the hub proteins of either copper or iron Workflow or both, and the top ranked clusters of copper, iron or their merged PPI network. Yellow color indicates the hub proteins common to both copper and iron PPI networks, and also to their respective clusters. Blue color indicates the hub proteins of copper PPI network common with the proteins of top ranked copper clusters. Green color indicates the hub proteins of iron PPI network common with the proteins of top ranked iron clusters. Red color is for the hub proteins present only in the clusters of merged PPI network but absent in the top ranked clusters of both copper and iron PPI networks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
KEGG Pathway search analysis for novel protein identification.

Uniprot_ID	Gene_ID	Biometal pathway	KEGG_ID	Disease Association
PO9486 (Osteonectin)	SPARC/ON	The Copper and Iron Binding Domain of SPARC Mediates Cell Survival *via* interaction with Integrin 1 and activation of Integrin-linked Kinase	hsa05016 hsa05012 hsa05010 hsa04216	HD PD AD NBIA
P12259 (Coagulation Factor V)	F5	Interacts selectively and non-covalently with Copper ions in Dopaminergic Synapse	hsa04728	Brunner Syndrome, OCD, Febrile seizures, Schizophrenia
P08709 (Coagulation Factor VII)	F7	Selective binding of Iron in axon guidance	hsa04360	L1 Syndrome, Congenital hydrocephalus

AD: Alzheimer's disease; HD: Huntington's disease; NBIA: Neurodegeneration with brain iron accumulation; OCD: Obsessive-Compulsive Disorder; ON: Osteonectin; PD: Parkinson's disease; SPARC: secreted protein acidic and rich in cysteine; KEGG: Kyoto Encyclopedia of Genes and Genomes.

distinct phylogenetic groups, (i) SPARC and hevin; (ii) SMOC 1 and 2; (iii) testicans 1–3; and (iv) and follistatin-like protein. However, it is emphasized here that several of SPARC family proteins (including some that are similar/different from that of SPARC) have been recently recognized for novel biological functions (Jayakumar et al., 2017; Lane and Sage, 1994; Rosset and Bradshaw, 2016).

4.1. SPARC/osteonectin and ND disorders

There is a differential expression pattern of SPARC in different regions of brain. SPARC is mainly expressed in microglia of the cerebellum, and cerebellar Bergmann glia. Astrocytes of cerebellum and hippocampus region of brain also express SPARC. In scientific literature, there are few reports on role of SPARC/osteonectin protein in ND;

nonetheless, its acceptance is steadily increasing (Jayakumar et al., 2017). Increased expression of SPARC gene in cerebral cortex region of brain from a Tay-Sachs and a Sandhoff disease patient has been documented (Myerowitz et al., 2002). Another study reported increased expression of SPARC gene in Charcot-Marie-Tooth disease type 2 (CMT2) patient. The authors hypothesized that the dramatic up-regulation of SPARC could be a potential biomarker for the severity of the CMT2 disease, especially if the protein enters the bloodstream (Cavalcanti et al., 2009). However, both of these studies had a major shortcoming in studying only one patient for each disease. Gillen et al. (1995), reported differential expression of SPARC gene in Wallerian degeneration (crushed rat sciatic nerve).

With reference to biometals, it has been demonstrated that augmented extracellular Cu levels produce a wide complex of alterations in the neuronal extracellular environment. In particular, Cu levels affect the secretion of molecules involved in the protection of neurons against oxidative stress, such as SPARC/Osteonectin among others (Spisni et al., 2009). It is an important finding since the most accepted theory for AD and PD is the increase in oxidative stress along with buildup of Cu, Fe and β-amyloid (in AD) or α-synculein (in PD) culminating in neuronal death. It is worth mentioning here that both β amyloid and α synculein are both Cu binding proteins (Pal et al., 2014). Cu may increase the self-aggregation of amyloid precursor proteins and β amyloid, and it also interacts with α synuclein and promotes its aggregation. PD patients exhibits increased brain Fe accrual and Fe also promotes aggregation of α-synuclein. Similarly, increased Fe accumulation has also been observed in the brain of AD patients along with evidence of Fe contributing to β-amyloid aberrant aggregation and toxicity (Chen et al., 2016).

In a significant study, it was reported that the Cu binding domain of SPARC mediates cell survival *in vitro via* interaction with integrin beta1 and activation of integrin-linked kinase. In this study, authors concluded that SPARC confers protection to cells from stress-induced apoptosis *in vitro via* an interaction with integrin beta1 heterodimers that enhances integrin-linked kinase activation and pro-survival activity (Weaver et al., 2008). One landmark study have shown that repeated administration of morphine causes increase in locomotor activity of mice, and that morphine-induced increase in SPARC protein levels in the basolateral amygdala resulted in locomotor sensitization (Ikemoto et al., 2000). Another seminal study also showed upregulation of SPARC inhibited DNA synthesis in human microvascular endothelial cells (Kupprion et al., 1998).

In a proof of concept study, SPARC null-mice demonstrated increased microgliosis in and around the lesion site of the cerebral cortex on induction of ischemic injury. Furthermore, same study exhibited that in an ischemic stroke model, SPARC expression at the lesion site was downregulated by reactive microglia (Lloyd-Burton et al., 2013). These findings indicate that SPARC is also an important regulator of microglial proliferation (Lloyd-Burton et al., 2013). Parallel observations were reported by Baumann et al. (2009), in which significant reduction in SPARC levels in brain blood vessels was observed upon transient global brain ischemia (Baumann et al., 2009; Jayakumar et al., 2017). A biphasic induction of a SPARC-related protein mRNA in cortical neurons along with a prolonged induction of SPARC in astrocytes was observed adjoining the lesion area following a localized injury to the adult rat forebrain (Mendis et al., 2000). It is noteworthy here to mention that there are also reports of role of hevin (SPARC like protein 1), a secreted protein with high structural similarity to SPARC/osteonectin, in AD (Medway et al., 2010; Seddighi et al., 2018; Vafadar-Isfahani et al., 2012).

4.2. Coagulation factor V & VII, and ND disorders

In our study, we have also found association of coagulation factor V with Brunner Syndrome, OCD, Febrile seizures, Schizophrenia diseases whereas coagulation factor VII have shown link with L1 Syndrome, Congenital hydrocephalus (Table 6). In scientific literature there are very few studies showing link of coagulation factors with outlined ND (Hoirisch-Clapauch et al., 2014, 2016). One case reports demonstrated the association of Factor V Leiden mutation with hemiconvulsion, hemiplegia, and Epilepsy syndrome (Scantlebury et al., 2002). English et al. (2018) have shown that dyshomeostasis of complement and coagulation system (including factor V among other factors) occurs in the blood during childhood before the development of the psychotic diseases including Schizophrenia. There was also one report linking factor VII with Congenital hydrocephalus (Oldenburg et al., 2000).

Interestingly, there are reports linking F5 gene single nucleotide polymorphism (SNP) with AD. Genome-wide association studies have shown significant association of hippocampal volume with 24 SNPs spanning the proximal portion of F5 gene in AD patients, and most significant SNP reported was rs6703865 in F5 gene (Melville et al., 2012). In addition, F5 SNP rs2213865 was demonstrated to be significantly associated with rate of cognitive decline among 331 AD patients, which further supports the role of F5 gene SNPs in AD (Sherva and Farrer, 2011). There are also reports linking other coagulation factor (XIIIa) with AD. It has been shown that coagulation factor XIIIa crosslinks amyloid β into dimers and oligomers, and to blood proteins (Hur et al., 2019). In addition, coagulation factor XIIIa forms unique complexes with β-amyloid, and colocalizes with deposited β-amyloid in cerebral amyloid angiopathy (de Jager et al., 2016).

F5 gene is present on chromosome 1, and consists of 25 exons, and encodes coagulation factor V (proaccelerin, labile factor). F5 gene encodes a crucial cofactor which functions to allow Factor Xa to activate thrombin. Defects in F5 gene cause either an autosomal recessive hemorrhagic diathesis or an autosomal dominant form of thrombophilia. Additionally, Budd-Chiari syndrome (deficiency of factor V), thrombophilia type 2-F5-related, and stroke are also caused by defects in F5 gene. F7 gene, present on chromosome 13, having 11 exons encodes coagulation factor VII which is a vitamin K-dependent factor essential for hemostasis and activation of the coagulation cascade. Defects in this gene can cause coagulopathy. Factor VII deficiency ensues autosomal recessive bleeding disorder (Hypoproconvertinemia) showing variable severity (James et al., 2014; Palta et al., 2014).

Based on very recent findings like potential role of F5 SNPs in AD and its large gene size having 25 exons; it is tempting to speculate that there might be other unrecognized SNP in F5 gene in different races/population which might have role in initiation/progression of other ND. Another approach is to stratify diseases like AD and PD into different subgroups, and identification of percentage of individuals who are more susceptible to these diseases upon imbalance in Cu and/or Fe homeostasis (For Cu subgroup of AD hypothesis refer to comprehensive review elsewhere by Pal et al., 2015).

5. Summary and future directions

Taken together, the findings herein illustrate the first system biology based evidence of possible association of SPARC protein in AD, PD, HD and NBIA disorders using Cytoscape software. In addition, Coagulation Factor V protein might have a role in Brunner Syndrome, OCD, Febrile seizures and Schizophrenia diseases whereas Coagulation Factor VII protein demonstrated link with L1 syndrome and congenital hydrocephalus. It is emphasized here that these novel identified proteins in various ND need to be experimentally proved before making a concluding remark.

The systems biology approach could be used for the construction of sub-networks of ND, and merged with the Cu and Fe metabolism gene networks to study co-expression, co-localization and physical interaction between the genes of multiple ND and biometal metabolism. Future prospective studies can immensely help in understanding the multifactorial pathoetiology of various ND disorders along with recognizing novel therapeutic targets. Further, network biology based approach for identifying novel genes/proteins/pathways for possible

role of Cu and Fe in several ND can provide platform for future prospective studies for the wide-spread screening, allowing early detection of ND in asymptomatic individuals, prognosis, precise evaluation and management of neuronal death process at earlier/different stages (Pal, 2014; Pal et al., 2014).

Conflict of interest statement

Author declares no competing financial interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neuro.2019.02.020.

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