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Comparison of Modules of Wild Type and Mutant Huntingtin and TP53 Protein Interaction Networks: Implications in Biological Processes and Functions

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Abstract

Disease-causing mutations usually change the interacting partners of mutant proteins. In this article, we propose that the biological consequences of mutation are directly related to the alteration of corresponding protein protein interaction networks (PPIN). Mutation of Huntingtin (HTT) which causes Huntington's disease (HD) and mutations to TP53 which is associated with different cancers are studied as two example cases. We construct the PPIN of wild type and mutant proteins separately and identify the structural modules of each of the networks. The functional role of these modules are then assessed by Gene Ontology (GO) enrichment analysis for biological processes (BPs). We find that a large number of significantly enriched ($p < 0.0001$) GO terms in mutant PPIN were absent in the wild type PPIN indicating the gain of BPs due to mutation. Similarly some of the GO terms enriched in wild type PPIN cease to exist in the modules of mutant PPIN, representing the loss. GO terms common in modules of mutant and wild type networks indicate both loss and gain of BPs. We further assign relevant biological function(s) to each module by classifying the enriched GO terms associated with it. It turns out that most of these biological functions in HTT networks are already known to be altered in HD and those of TP53 networks are altered in cancers. We argue that gain of BPs, and the corresponding biological functions, are due to new interacting partners acquired by mutant proteins. The methodology we adopt here could be applied to genetic diseases where mutations alter the ability of the protein to interact with other proteins.

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Introduction

Cellular functions are carried out by proteins interacting with other proteins and macromolecules like DNA, RNA, etc. It is believed [1] that the modular organization of cellular functions are related to the underlying modular structure of the protein protein interaction network (PPIN). Understanding PPIN would elucidate how such interactions execute basic functions in cells and may explain the abnormalities arising from mutations in genes. In particular, mutation at the binding site of a protein may lead to loss of it's ability to function together with existing interacting partner(s). On the other hand, mutation may also create regions where new protein partners can bind. Therefore, loss or gain of interaction due to mutation may contribute to causation, progression or modulation of disease. It has been reported recently [2] that out of 119 mutations in 65 distinct diseases, 95 mutations result in loss of function (LOF), 17 mutations result in gain of function (GOF) and 4 mutations changes the preferences for interaction. Based on this experimentally validated data, it has been predicted that 1428 mutations might be related to interaction defect. Using the structural information at atomic levels either through crystallography or homology modeling, it has been shown that 21,716

mutations in 624 genes either alter amino acid sequences or produce truncated proteins. Among 12,059 mutations that alter amino acid sequences, 7833 mutations are located in the interface of interaction with other proteins. Such mutations at interfaces of interactions may disrupt or enhance the interactions with the partners. This study also emphasizes the role of loss or gain of interactions of mutant proteins in human diseases. However, for such analysis, it is necessary to have structural information at atomic levels, which may be achieved if 3-dimensional structures of the proteins or their homologs are known. But, for the most of the protein protein interactions such information is not available [3]. Moreover, very little is known about the role of such altered interactions in corresponding pathological conditions. It remains a challenge to relate genetic mutation data to PPIN and to understand molecular cause of disease. In the present communication, we probe whether gain or loss of interactions of mutant Huntingtin protein (HTT) that causes Huntington's disease (HD) can explain functional abnormalities observed in HD. We have also used the same approach to find how loss or gain of interactions of mutant TP53 in cancers may result in alterations of functions.

Analysis and Results

Mutation in HTT Protein

Huntington's disease (OMIM ID: 143100) is a rare autosomal dominant progressive degenerative neurological disease caused by expansion of normally polymorphic CAG repeats beyond 36 at the exon1 of the gene Huntingtin (HTT) [4]. Over the years, various cellular processes/conditions like excitotoxicity, oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, axonal transport, ubiquitin proteasome system, autophagy, transcriptional deregulation and apoptosis have been implicated in HD pathology [5,6]. Even though GOF was inferred initially from the autosomal dominant nature of transmittance of the disease, the underlying molecular details still remain largely unknown. Inverse correlations between age at onset and number of CAG repeat beyond 36 in HTT gene, increased aggregates of mutant HTT (mHTT) and apoptosis, correlation of CAG repeat numbers in HTT gene with levels of ATP/ADP and altered expression of few genes [4,7–10] suggest toxic GOF of mutant protein that disrupts normal cellular functions and causes neuronal death. Mutant HTT preferentially interacts with DNA sequences, alters conformation of DNA facilitating binding of other transcription factors to the specific sequences and modulates transcription of genes. This result also indicates a dominant GOF of mHTT [11]. Wild type HTT (wHTT) is known to be involved in protection of apoptosis [12–15], regulation of gene expression [16,17], mitosis and neurogenesis [18], neuronal development [19] and maintenance of body weight [20]; all these processes are altered in HD [5,6]. These results indicate that loss of one of the alleles in HD could contribute to increased apoptosis and altered gene expressions observed in HD. LOF of wild type protein may thus contribute, at least partially, to HD pathology [21]. There are also several experimental evidences available against simple LOF(s) of wild type HTT [22–25].

Construction of HTT-interacting protein network. We have collected the HTT interacting proteins from published data and find that 17 proteins preferentially interact with wHTT, while 37 proteins are either identified in aggregates of mHTT only or interact preferentially with mHTT (the references for each of the observations are provided in Dataset S1 (sheet 1) and in Text S1 (Text 1)). These 17 and 37 proteins are referred to as the primary interactors of wHTT and mHTT respectively. Next, we assimilate interacting partners of these primary interactors from BioGrid (Version 3.1.88, May 2012), a public database that contains genetic and protein protein interaction data for humans and other organisms [26]. In the present study, we have considered both physical and genetic interactions (refer to the section 'Robustness analysis' for details). It turns out that there are 288 secondary interactors of wHTT (proteins which interact with the 17 primary interactors), whereas there are 1504 secondary proteins which interact with 37 primary interactors of mHTT. The PPIN of wHTT interacting proteins is then constructed by considering all these 306 proteins (wHTT+17 primary+288 secondary interactors of wHTT) as nodes of the network; two nodes are connected if corresponding pair of proteins are found to be interacting partners of each other in BioGrid. Altogether there are 1397 interactions in wHTT network which are listed in Dataset S1 (sheet 2). Similarly the PPIN of mHTT is constructed with 1542 nodes (mHTT+37 primary+1504 secondary interactors of mHTT) which has 13142 interactions from BioGrid (Dataset S1 (sheet 3)). We have used Cytoscape [27] for visual presentation of the wHTT and mHTT networks, which are shown in Fig. S1 in Text S1. Both the networks are densely interconnected and the nodes are too tangled there to find any apparent or obvious modular structures.

Characteristics of networks. A quantifiable description of these networks can be obtained by using graph theory, which provides several measures for comparison and characterization of complex networks. The most elementary characteristic of a node is its degree, k, which represents the number of other nodes (proteins) it is connected with. The degree distribution, $P(k)$, gives the probability that a randomly selected node has exactly k links. We find that both the wild and mutant PPINs follow a power law degree distribution, $P(k) \sim k^{-\gamma}$ (Fig. S3 in Text S1) with exponents $\gamma = 1.99, 1.95$ and average degrees $\langle k \rangle = 9.13, 17.05$ respectively. Another important quantity is the clustering coefficient which characterizes how connected are the neighbors of a given node. It is observed that the average clustering coefficient $C = 0.361$ for mHTT network is lower compared to $C = 0.436$ for wHTT PPIN. This indicates that, the former network is less compact and the interacting partners of the proteins are poorly connected among themselves. We have also calculated the average shortest path length L, and the network diameter D (listed in Table S2 in Text S1), which describe the structural properties of the network. The detailed definitions of C, D and L along with their evaluation procedure is illustrated in Text S1 (Text 2).

Gain and loss of interactions due to mutation. A closer look at PPINs of wHTT and mHTT reveals that among the 17 primary interactors of wHTT, 8 proteins still appear in PPIN of mHTT as secondary interactors, *i.e.* they interact with some of the primary interactors of mHTT. Again, among 288 secondary interactors of wHTT, 107 proteins are secondary interactors of mHTT, 10 proteins interact directly with mHTT and the rest 171 proteins do not take part in PPIN of mHTT (see Fig. 1(a)). Evidently, the mutant HTT network has gained several new interactions, 27 proteins as primary interactors and 1389 proteins as secondary interactors. This result is shown schematically in Fig. 1(a) and the detailed list of these proteins is given in Text 1 and Table S1 in Text S1. Since mutation of HTT has changed the PPIN substantially one expects a significant change in its functions.

Modules of wHTT and mHTT networks. There are several methods for obtaining natural modules of a network (or partitions of a graph) [28]. We adopt Newman-Girvan's modularization (NGM) algorithm [29], a commonly used method, to detect the modules of wHTT and mHTT networks. This algorithm partitions the network in a way that the intra-module connections between nodes are maximized in comparison to the inter-module connections. To find the modules, Newman and Girvan [30] proposed a score called modularity Q for every possible partition of a network; the maximum value of Q corresponds to the best partition. The details of the NGM algorithm for maximization of Q is described in Text S1 (Text 2). The NGM algorithm modularizes the PPIN of wHTT into 7 modules of sizes (18,66,79,18,82,8 and 35) (see Table S2 in Text S1), with modularity $Q = 0.415$, whereas PPIN of mHTT is partitioned into 8 modules of sizes (643,3,377,2,485,7,22 and 3) with $Q = 0.302$. Modules of wHTT and mHTT networks are denoted by W and M respectively. Figures 1(b) and (c) represent the modularized networks; all proteins belonging to a given module are shown in same color. Clearly, the mHTT network is visibly more complex than that of wHTT, which is consistent with the fact that it has a lower Q value [31].

Similarity between the modules. Once the wild type and mutant networks are modularized, it is important to ask how similar is a module of wild type network with that of mutant network, in terms of their protein constituents. Mutant and wild type HTT networks have 125 proteins common between them.
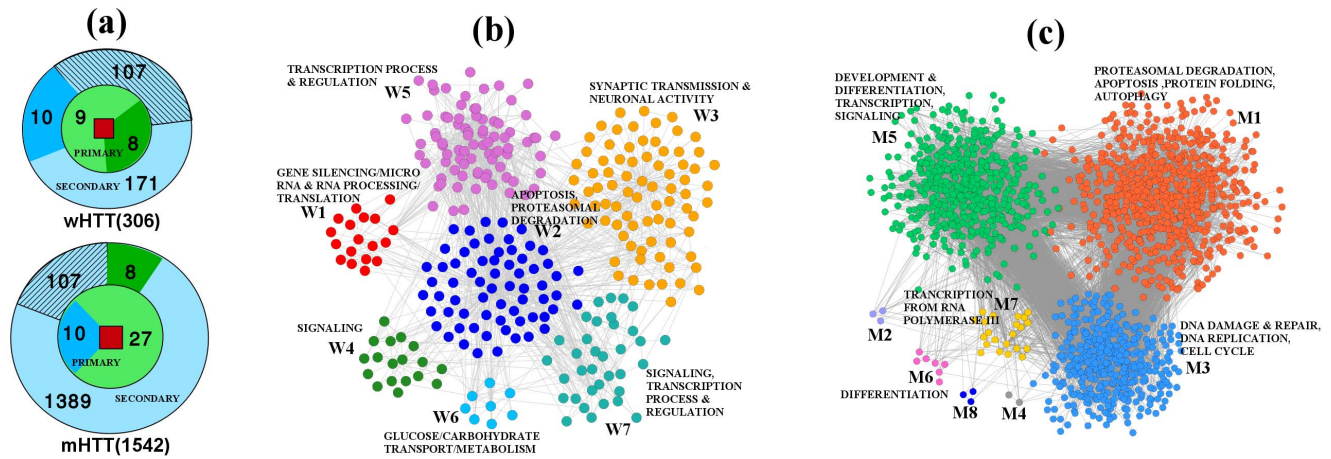
Figure 1. Construction and modularization of wild type and mutant HTT networks. (a) Proteins involved in the wHTT and mHTT networks: wHTT (mHTT) protein (red square) has 17 (37) primary and 288 (1504) secondary interactors, shown schematically as the inner and outer circles. Of the 17 primary interactors of wHTT, 8 proteins (deep green) become secondary interactors of mHTT. Among the 288 secondary interactors of wHTT, 107 (shaded) proteins remain as the secondary interactor of mHTT whereas 10 proteins (deep blue) becomes the primary interactors of mHTT. (b) and (c) Modules of the wHTT and mHTT networks from NGM algorithm, which yields 7 ($W1, W2, \ldots, W7$) and 8 modules ($M1, M2, \ldots, M8$) respectively are shown along with the relevant biological functions (obtained GO term enrichment analysis from GeneCodis3). Significant functions associated with the modules are also shown. Details of the GO terms are shown in Table S2 and Table S3 of the Supporting Text, respectively for wHTT and mHTT.
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After both the networks are modularized, these common proteins are distributed among the pair of wHTT- mHTT modules. For example, the module $M5$ (485 proteins) has 49 proteins in common with $W5$ (485 proteins), whereas it has only one common protein in $W2$ (out of 66 proteins) and two common proteins in $W1$ (18 proteins). The detailed distribution of common proteins among wild and mutant modules of HTT are shown in Fig. 2.

To calculate the similarity among modules, first we construct a unique set of proteins from combining the proteins involved in the wild and mutant networks. This set consists of 1723 proteins in

case of HTT. Now every module of wHTT and mHTT are considered as a unique 1723 dimensional vector as follows. Each protein is identified with a specific position in the vector; presence (or absence) of a specific protein in a module, say w, is mapped on to a corresponding vector $\vec{R}_w$ by inserting 1 (or 0) at respective position. A similarity measure between a pair of modules w and m is well represented by the angle $\theta(w,m)$ between the corresponding vectors $\vec{R}_w$ and $\vec{R}_m$,
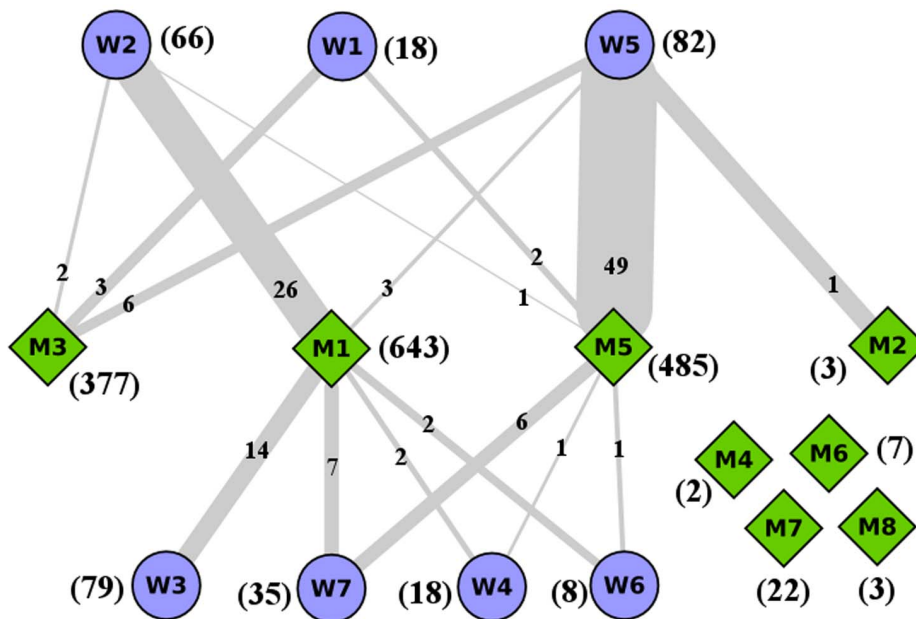
Figure 2. Similarity between modules of wHTT and mHTT networks. The figure describes pictorially the closeness between the modules of wHTT and mHTT PPIN; the modules having common protein or common GO terms are joined with edges (numerical value written on the edge as: common proteins).
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$$\theta(w,m) = \cos^{-1}\frac{\vec{R}_w.\vec{R}_m}{|\vec{R}_w||\vec{R}_m|}$$

It is rather simpler to use $\cos(\theta(w,m))$ as the similarity measure as *cosine* function is monotonic in the range $(0,\pi)$. It is easy to see that if the modules have N_w and N_m proteins individually and N_{wm} protein in common, the similarity measure is

$$\sigma(w,m) \equiv \cos(\theta(w,m)) = N_{wm}/\sqrt{N_w N_m}. \quad (1)$$

Clearly $\sigma(w,m)$ varies in the range $(0,1)$ with maximum value 1 corresponding to the fact that the modules are identical, *i.e.* they have same set of proteins.

In Fig. 2, we represent the similarity among modules of mHTT and wHTT as a bipartite network with links having thickness proportional to σ_{wm}. The thickest link between $M5$ and $W5$ indicates that these modules are significantly similar. For examples, the module $W5$ has 82 proteins and $M5$ has 485 proteins; 49 proteins are common among the proteins in these 2 modules; thus the protein similarity index for $W5$-$M5$ pair is $\sigma(W5,M5) = 0.246$. Similarly among 66 proteins in $W2$ and 643 proteins in $M1$, 26 proteins are common (corresponding $\sigma(W2,M1) = 0.126$).

Enrichment of GO terms for biological process. It has been observed that the proteins identified in a particular complex are involved in similar functions [32]. From network perspective, these complexes are represented by modules and they appear as distinct group of nodes which are highly interconnected with each other but have only a few connections with the nodes outside of the module. It is important to ask, if such a structural partition relates to any functional enrichment. Among many bioinformatics tools available for such analysis [34] we utilize GeneCodis3 [33] (explained in Text S1 (Text 3)) to obtain the possible Biological processes enriched by the proteins in a given module. Given a query set of proteins GeneCodis3 provides the enriched biological process, molecular functions, and cellular components as defined by the Gene ontology. Biological process in Gene ontology is described as a series of events carried out by one or more ordered assemblies of molecular functions [35]. The proteins in each module are used as input to GeneCodis3 [33] and significantly enriched GO terms for BPs obtained using p-values calculated through Hypergeometric analysis corrected for false discovery rate (FDR). Results of enrichment analyses for 7 modules of wHTT and 8 modules of mHTT network are shown in Datasets S2 and S3 respectively.

Since many proteins are known to be involved in a particular BP, and a given protein may also contribute to multiple BPs, it is likely that proteins in different modules in wHTT and mHTT network participate in a specific BP due to either overlap in proteins or BPs. To identify the overlaps of BPs between modules in wHTT and mHTT networks, we separately identify the common GO terms between the wHTT and the mHTT modules. It is evident from Dataset S4 (sheet 2) that 390 unique GO terms are being enriched ($p < 0.0001$) due to proteins in modules of mHTT network, while 129 GO terms are enriched with proteins in the modules of wHTT network (Dataset S2 (sheet 1)). Among the GO terms present in wHTT and mHTT network, 65 are common. As a result due to mutation, 325 GO terms are gained by mHTT and 64 GO terms are lost by wHTT. The common 65 GO terms represents both gain and loss.

For convenience, we clubbed the the GO terms in a given module to broadly assign one or more appropriate biological function(s). For example, GO:0010506 (regulation of autophagy), GO:0016559 (peroxisome fission), GO:0031929 (TOR signaling cascade), GO:0000045 (autophagic vacuole assembly), GO:0006897 (endocytosis) in module $M1$ are bought under a single biological function "Autophagy". Similarly in module $W4$ GO:0043507 (positive regulation of JUN kinase activity), GO:0072383 (plus-end-directed vesicle transport along microtubule), GO:0046330 (positive regulation of JNK cascade), GO:0046328 (regulation of JNK cascade) are clubbed under "Signaling". The assigned biological functions for modules of wHTT and mHTT are shown in Fig. 1(b) and (c) (details are given in Dataset S4).

Gain and loss of biological process in HTT networks. Comparison of enriched BPs in the modules of wHTT and mHTT reveal that the mHTT network has acquired several new BPs which were absent in wHTT, indicating gain of biological processes. Similarly enriched BPs of wHTT which are not present in mHTT are lost. Hence biological functions carried out by the BPs which are gained or lost in mHTT networks may result in functional gain or loss due to mutation in HTT.

Gain of biological process: The unique GO terms enriched in the modules of mHTT networks are listed in Dataset S4 (sheet 2) and in Text S1 (Table S3). The GO terms in module $M1$ are related to cell cycle (4 GO terms), signaling (30), transcription processes and regulation (5), apoptosis (11), DNA damage and repair (6), Immunological (7), protein folding (7), autophagy (5), translation (3), metabolism (1), development and differentiation (4), cell migration and shape (4), proteasomal degradation (14), Protein complex/membrane assembly/stabilization (9) and others (4). It is known that many of these processes are involved in HD pathogenesis [36]. In $M3$, the enriched GO terms are assigned to DNA repair (17), Transcription processes and regulation (5), DNA replication (12), cell cycle (12) and others (5). Note that, it has been shown recently that DNA repair, replication and cell cycle are involved in HD. In fact, activation of DNA synthesis and cell cycle increase apoptosis in terminally differentiated neuronal cells, instead of increasing cell division [37,38]. Besides, recent studies have explored the role of DNA repair in neurodegenerative disease [39] and show that interaction of mHTT with Ku70/XRCC6 impairs repair activity [40]. A large number of GO terms related to development and differentiation (57 GO term), transcription process and regulation (31), cell cycle (5), DNA damage and repair (4), Carbohydrate/Glucose transport/metabolism (4), Cell growth (7), signaling (31) and others (6) are enriched in module $M5$. The role of development and differentiation in HD is not clear. However recent studies in HD [19,41] indicate that neurogenesis is possibly altered and differentiation/development could be defective. Deregulation of transcription is considered to be one of the most important abnormalities in HD [42]. GO terms related to differentiation are also enriched with proteins in module $M6$, although the terms are distinct from that in module $M5$. All 4 GO terms enriched in $M7$ are related to transcription by RNA polymerase III. It is known that both tRNA and some miRNAs [43] are synthesized by RNA polymerase III, however their role in HD is unknown. Thus it is evident that the the protein interactions gained in mHTT network result in enrichment of the biological processes in its modules.

Loss of biological process: The unique GO terms enriched in the modules of wHTT which are absent in the modules of mHTT network represent the loss of functions due to mutation in HTT protein. The 5 GO terms in $W1$ include gene silencing, micro RNA processing and translational regulation. The GO terms

relating to proteasomal degradation (1 GO term), cell cycle (1), apoptosis (1) and circadian rhythm (1) are present in $W2$. Similarly, signaling (17 GO terms), synaptic transmission, neuronal activities (12) transport (ion/sugar) (5) and others (4) are associated with module $W3$; glucose/carbohydrate transport and metabolism (5), cell cycle (3) and protein/transmembrane transport (4) with $W6$. In $W7$ only one GO term describing transcription processes and regulation is enriched. The GO terms and the associated BPs that are lost due to mutation are provided in Dataset S4 (sheet 1) and in Text S1 (Table S3) respectively.

We have clubbed the relevant GO terms to represent signaling, transcription process and regulation, apoptosis, cell cycle etc. (refer to Table S3 of Text S1). For example, GO terms (GO:0000088) and (GO:0000236, GO:0000087, GO:0007091) which are enriched in $W2$ and $W6$ respectively relates to cell cycle. Similarly the 21 GO terms which are enriched in $M1(4)$, $M3(12)$, and $M5(5)$ (Dataset S4 (sheet 2)) are also associated to cell cycle. Although cell cycle is enriched in both wHTT and mHTT modules, no GO terms are common among them. Thus, the loss of interaction with wHTT may result in loss of above 4 GO terms in wild type network resulting in LOF, whereas the gain of interaction with mHTT may be associated with gain of these 21 GO terms relating to GOF of cell cycle.

It is interesting to note (from Table S3 of Text S1) that the GO terms related to DNA replication, protein folding, autophagy, cell growth are only observed in the modules of mHTT networks. So these processes are gained due to new interaction with mHTT. Similarly, GO terms related to gene silencing/microRNA processing/translation, transport (ion/protein/sugar etc) are observed in wHTT network only. Therefore, loss of interaction with wHTT may result in the loss of these BPs in HD.

Both loss and gain of biological process: Modules in wHTT and mHTT networks have several proteins or GO terms common among them, which indicate loss as well as gain of functions and support the notion that both loss and gain may occur due to mutation in HTT [21]. For example, modules ($W1,W5,W7$) and ($M3,M5,M6$) have 17 enriched GO terms related to transcription processes and regulation. Similarly, modules ($W2,W5$) and ($M1,M3,M5$) share 12 enriched common GO terms related to apoptosis and 4 common GO terms relating to cell cycle. Thus, the general function of transcription and apoptosis could arise from loss as well as gain of interactions of mHTT protein. The details of the functions associated with the 65 GO terms (common between wHTT and mHTT) are presented in Dataset S4 (sheet 3) and Table S3 of Text S1, they correspond to the gain and loss of functions in the HD.

From the above analysis we observe that most of the functions that are enriched in the modules of wHTT and mHTT networks are altered in the pathogenesis of HD. The post transcriptional regulation of genes, associated with module $W1$ of wHTT network, can be related to negative regulation of gene expression by the non-coding RNAs like micro RNAs, which are well documented [44]. Role of apoptosis [5,36], synaptic transmission [45], JNK pathway [46], transcription deregulation [42], glucose transport [47,48], estrogen [49] and various types of epigenetic changes including histone modifications in different neurological diseases [50] in HD pathogenesis have also been reported.

In summary, many new BPs (GO terms) appear in the mHTT network and some of the BPs present in wHTT network are lost; a few are found to be common between modules of wHTT and mHTT. As a result some biological functions involving the enriched GO terms are gained by mHTT and a few are lost from the modules of wHTT. This provides molecular mechanism of the gain and/or loss of functions observed in HD pathogenesis.
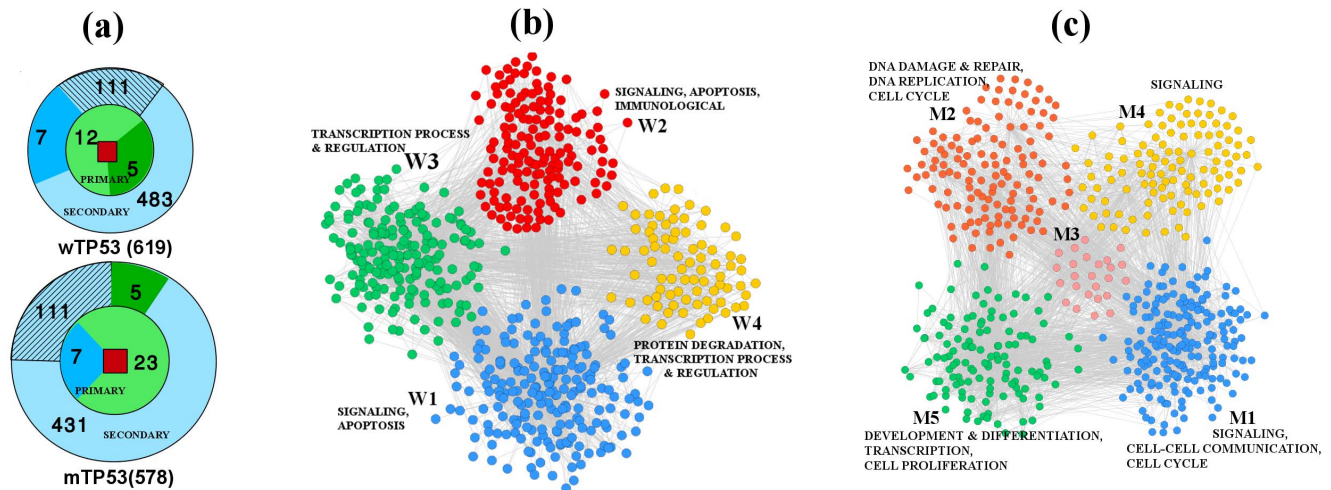
Mutation in TP53 Protein

TP53 protein, initially identified as an oncogene, is now established as a tumor suppressor gene which participates in diverse cellular functions like transcription regulation, DNA repair, apoptosis, and genome stability, and many others. Mutation to TP53 is identified in more than 50% of the tumors. It is evident from COSMIC database [51] that R175H, R273H and R248W mutations of TP53 are the most prevalent ones. Since TP53 is a tumor suppressor gene, it is expected that its mutations might result in the LOF of the wild type protein. Some mutations of TP53 are also known to attain new function(s) [52,53]. For example, exogenous expression of mutant TP53 (R273H and others) in mouse cells devoid of endogenous TP53 results in several cellular phenotypes of cancers [54–56]. To understand the underlying molecular mechanism of GOF of mutant TP53, it was recently shown [57] that nardilysin (NRD1) protein, which does not interact with wild type TP53 but interacts only with mutant TP53 (R273H), may contribute to the metastatic properties of this mutant protein.

PPIN of wTP53 and R273H mutant TP53 (mTP53). In a recent study [57], it has been shown that 17 proteins preferentially interact with the wild type TP53 (wTP53) and 30 other proteins interact exclusively with mutant TP53 (mTP53). To construct the protein interaction networks we take these primary interacting proteins of wTP53 and mTP53 and consider their interacting partners existing in BioGrid database [26]. The detailed protein interaction data are given in the Dataset S5. The PPIN is constructed separately for wTP53 and mTP53, as described for HTT. It turns out that wTP53 has 601 secondary interactors whereas mTP53 has only 547. Thus the PPIN of wTP53 and mTP53 are constructed taking 619 proteins ($\text{w}TP53 + 17\ \text{p}rimary + 601\ \text{s}econdary$) and 578 proteins ($\text{m}TP53 + 30\ \text{p}rimary + 547\ \text{s}econdary$) respectively. Both the networks (shown in Fig. S2 in Text S1) are found to be densely packed with similar structural properties. Their degree distributions are scale free ($P(k) \sim k^{-\gamma}$) with the exponents $\gamma = 2.04$ (wTP53) and 1.89 (mTP53) (Fig. S3 in Text S1) and average degree $\langle k \rangle = 12.07, 12.29$. The other network properties, like the average clustering coefficient $C = 0.452, 0.406$, the diameter of the networks $D = 4, 4$ are also comparable (listed in Table S2 in Text S1).

The change in interactions and the interacting partners due to mutation of TP53 is shown schematically in Fig. 3(a). Of 17 primary interactors of wTP53, only 5 proteins remain involved in mutant network as secondary interactors of mTP53 and the remaining 12 do not interact with mTP53. Among the 601 secondary interactors of wTP53, 111 proteins remain as a secondary interactor of mTP53 and 7 of them interact directly, *i.e.* 7 secondary interactors of wTP53 become primary interactors of mTP53. Lists of these proteins are given in Text 1 and Table S1 in Text S1.

Modules of wTP53 and mTP53 networks. In order to identify the modules of the wTP53 and mTP53 networks, we use NGM algorithm [29]. It turns out that PPIN of wTP53 is modularized into 4 modules of size 204, 151, 183 and 81, whereas mTP53 network gives 5 modules of size 193, 127, 25, 111 and 122. The corresponding modularity values are $Q = 0.331$ and 0.338. Figures 3(b) and (c) show the modules of wTP53 and mTP53 with different colours. Each module of wTP53 or mTP53 has unique set of protein. However, there is a large overlap of secondary interactors (proteins which do not interact directly with TP53) in the wTP53 and mTP53 networks, which is distributed among different modules (in total 123). We observe that among 123 common proteins, 34 belong to module $W3 - M5$, whereas

Figure 3. Construction and modularization of wild type and mutant TP53 networks. (a) Proteins in wTP53 and mTP53 networks: wTP53 (mTP53) protein (red square) has 17 (30) primary and 601 (547) secondary interactors, represented by the inner and outer circles. Only 5 (7) primary (secondary) proteins of wTP53 interact with mTP53 as secondary (primary) interactors. Again 111 secondary proteins of wTP53 remain as secondary interactors of mTP53. (b) and (c) shows the modules of wTP53 and mTP53 network along with few plausible candidate BPs. Details of the GO terms are shown for wTP53 and mTP53 respectively in Table S6 and Table S7 of Text S1.
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module pairs $M1 - W1$ (and $W4 - M2$) have 23 (and 10) common proteins. One can define a similarity measure σ_{mn} using Eq. (1) for every pair of wTP53-mTP53 modules. Taking the similarity indices σ_{wm} as weights (or thickness) of the link we have constructed a bipartite network which is shown in Fig. 4; the number of proteins is written beside each of the modules and the number of common proteins is specified along the links.

Enrichment of biological processes for the proteins present in every module of wTP53 and mTP53 PPIN using GeneCodis3 are presented in Dataset S6 and S7 respectively, where only the GO terms with $p < 0.0001$ are considered. The number of enriched GO terms in modules of wTP53 PPIN are $W1(30)$, $W2(17)$, $W3(63)$, $W4(36)$ and those for mTP53 are $M1(52)$, $M2(71)$, $M4(1)$, $M5(67)$. Note that module $M3$ has no GO terms enriched with $p < 0.0001$.

Loss and gain of biological processes in TP53 networks. Enrichment analysis of proteins in modules of wTP53 and mTP53 using GeneCodis3 reveals that respectively 127 and 172 GO terms (or biological processes) are enriched significantly ($p < 0.0001$). Among 127 GO terms of wTP53 57 GO terms do not appear in the mTP53 representing loss of the corresponding biological processes. Again the mTP53 network has 102 new GO terms (which were absent in wTP53). Besides, 70 enriched GO terms are found to be common in modules of mTP53 and wTP53 networks. We further associate each of the enriched GO terms with a relevent function. Loss and gain of these broadly classified functions are discussed below.

Gain of biological processes: The biological processes related to 102 new GO terms of mTP53 are gained due to mutation. The functions enriched in module $M1$ of mTP53 network are cell-cell communication (no of GO terms 5), signaling (13), protein complex/membrane assembly/stabilization (4), proteasomal degradation (2), cell cycle (3), DNA damage and repair (1) and others (2). GO terms related to DNA replication (11), DNA damage and repair (14), cell cycle (4), immunological functions (3), proteasomal degradation (3) and signaling (1) are enriched in $M2$. Similarly GO terms related to differentiation and development (11), signaling (7), transcription (6), cell proliferation (4), apoptosis (1), cell cycle (1) and DNA damage (2) and others (4) are enriched with

proteins in module $M5$. The extensive list of the GOF is given in Dataset S8 (sheet 2) and in Table S4 in Text S1. Thus new functions carried out by these biological processes are due to gain of interaction.

Loss of biological processes: On the other hand some of the enriched GO terms of wTP53 are absent in the mutant network. Corresponding biological processes are lost due to mutation in TP53. Altogether 57 unique GO terms are enriched with proteins in modules of wTP53 networks which are classified into broad class of functions (see Dataset S8 (sheet 1) and Table S4 in Text S1. The resulting loss of biological functions in various modules are, $W1$: signaling (8), proteasomal degradation (1), translation (1), cell migration and movement (2) and others (1); $W2$: signaling (4), apoptosis (2) and immunological (3); $W3$: cell cycle (3), signaling (1), transcription process and regulation (13), DNA replication (3), DNA damage and repair (1); $W4$: transcription process and regulation (2), proteasomal degradation (2), translation (5) and others (4); $W7$: transcription process and regulation (1).

Both loss and gain of biological processes: The 70 GO terms common between wTP53 and mTP53 networks are related to the functions, cell cycle (14 GO terms), transcription (15), DNA damage and repair (10), cell growth (2) and apoptosis (4), signaling (9), DNA replication (3), proteasomal degradation (3), immunological (2), development and differentiation (1), metabolism (2) and others (5). Thus these functions are possibly enriched due to both gain and loss of interactions (details are shown in Dataset S8 (sheet 3) and in Table S4 in Text S1.

Analysis of proteins in different modules using tool GeneDecks. Recently metastasis has been shown as the GOF as R273H cells attain metastatic property in cell model [57]. Since metastasis is not described as a "biological process" in Gene Ontology term, we have used another tool, GeneDecks [68], which provides a similarity metric by highlighting shared descriptors between genes, based on annotation within the GeneCards compendium of human genes (see Text 4 in Text S1 for details). Taking the proteins of the modules of wTP53 and mTP53 separately as a query field, we look for "metastasis" in the attribute "disorder" among many other descriptors which are enriched for different types of cancers (Dataset S9). It is observed
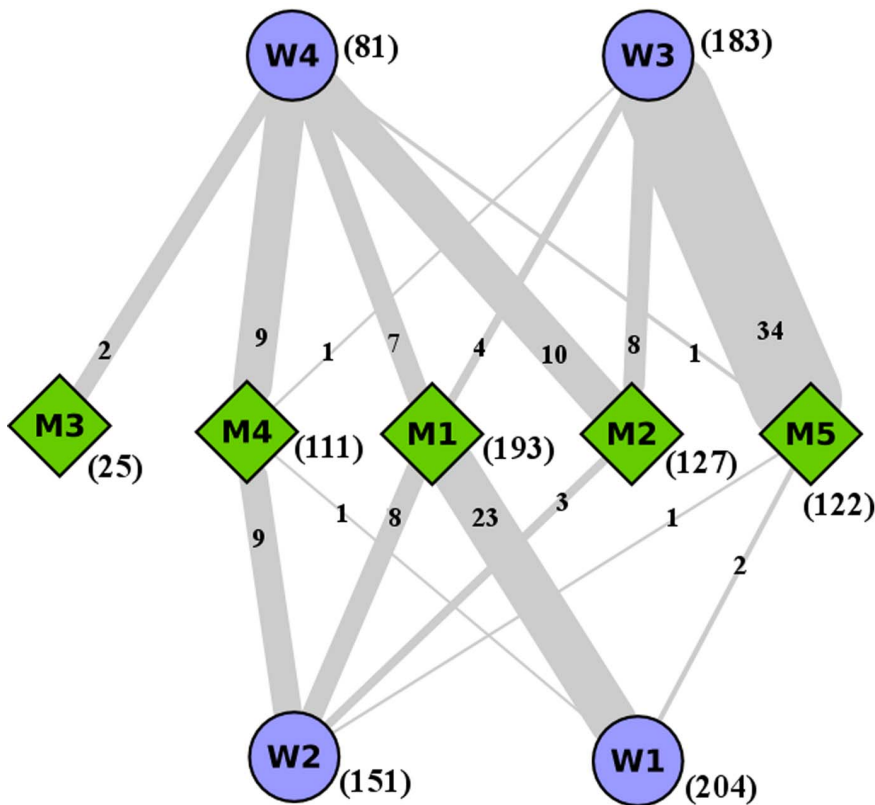
Figure 4. Similarity between modules of wTP53 and mTP53 networks. The bipartite network constructed with the modules of wTP53 and mTP53; the common proteins present between a pair of wild and mutant module is written on respective link. The number of proteins that constitute the modules are written beside it.
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that the descriptor "metastasis" is enriched with the protein modules $W1, W2, W3$ of wTP53 network and all the modules ($M1, M2, M3, M4, M5$) of mTP53 network. Thus, the loss of interactions of proteins in the modules $W1, W2, W3$ of wTP53 due to mutation may result in the LOFs related to metastasis. Similarly, the gain of interactions of proteins in all the modules of mTP53 may result in the GOFs related to metastasis.

That LOF of wTP53 and GOF of mTP53 may contribute to invasion and metastasis, is reviewed recently [69]. TP53 mutations at the DNA binding domain are common and such mutations suppress expression of target genes. It is supported by several experiments [69] that suppression of transcriptional program for genes involved in epithelial-mesenchymal transition (EMT) may contribute to induction of EMT resulting in metastasis. Further, it is ascertained that loss of functions in wTP53 lead to increased cell motility in various cell types, and increased expression of fibronectin, collagens and extracellular matrix (ECM) proteins. Enhanced expression of these proteins potentially increase the interaction between cells and ECM. LOF in wTP53 also activate Rho GTPases and modulates cell migration [69].

Role of mTP53 in metastasis has been established in many other studies. Mutant TP53 (R175H) is involved in TGF mediated invasion and metastasis in breast cancer cells through TP63 and SMAD3 [55]. Note that, in our analysis, SMAD is present in module $M5$ of mTP53 network. It is known that mutant TP53 (R175H and R273H) increases endocytic recycling of adhesion molecule integrin and EGFR promoting and metastasis [56,70]. Mutation in TP53 also activate EGFR/PI3K/AKT pathways and thereby increases invasion [71]. Various other mechanisms of

increased metastasis by the mutant TP53 have also been studied [69]. Thus the gain of biological processes obtained from the analysis of mTP53 protein networks provides an explanation of GOFs observed in cancers.

Robustness Analysis

In general, the modularization methods partition the network into communities of proteins which are densely connected. Thus in a large network it is quite expected that deletion of small fraction of links, whether selected methodically or randomly, does not alter the overall structure significantly. In fact, the degree distributions of all four networks studied here (namely PPIN of wHTT, mHTT, wTP53 and mTP53) are scale free (see Fig. S3 of Text S1), and it is known that such scale free networks are robust against random removal of nodes or links, but they could be fragile against targeted attack [72].

Again, since several databases of protein interactions largely overlap [73] in their contents, it is natural to expect that the broadly classified biological functions obtained here for HTT and TP53 networks would not differ substantially. In this study we used Biogrid [26] for creating the differential PPIN of the wild type and mutant HTT and TP53 proteins by connecting every pair of proteins which are listed in BioGrid as interacting partner of each other. This includes experimentally validated genetic and physical interactions. To check the robustness of our analysis, first let us remove all genetic interactions listed in BioGrid. This reduces the total number of protein interactions of BioGrid to 99.84%, whereas the interactions of wHTT, mHTT, wTP53 and mTP53 are reduced to 99.64%, 99.64%, 99.95% and 99.83% respectively

Table 1. Change in the total number of proteins and the interactions after excluding (a) genetic interactions and then (b) excluding interactions which are validated by only one Y2H experiment.

	Total no. of interactions	(a)Excluding genetic(%)	(b)Excluding genetic & Y2H(%)	Total no. of proteins	Excluding genetic & Y2H(%)
PPIN(human)	59027	58927(99.84%)	47244(80.04%)	12515	11630(9.29%)
wHTT	1380	1375(99.64%)	1231(89.20%)	306	292(9.54%)
mHTT	13105	13058(99.64%)	11478(87.58%)	1542	1486(9.64%)
wTP53	3718	3716(99.95%)	3205(86.20%)	619	590(9.53%)
mTP53	3521	3515(99.83%)	3136(89.07%)	578	551(9.53%)

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(see Table 1). Among the other experiments considered in BioGrid, Yeast 2 Hybrid (Y2H) assay results in larger false positives [74]. Thus we further remove all the interactions which are identified only once by Y2H. This stringent criterion consequently reduces both the number of interactions and the number of proteins by $\sim 10\%$. The total number of interactions of BioGrid is, however, reduced by 20%. Since the wild type and mutant networks are altered only a little compared to the expected value 20%, one expects that deletion of a small fraction of interactions will not change the network properties significantly.

To demonstrate this explicitly, we reconstruct the PPIN of mHTT keeping only the reduced set of interactions and then identify the protein modules using Newman Girvan algorithm. The enriched GO terms ($p < 0.0001$) from GeneCodis3 shows that every module of mHTT ($M1, M3, M5, M6$ and $M7$) has significant protein overlap with only 'one distinct module' of the reduced network, which is referred to as the 'most similar module' (MSM) henceforth. The number of overlapping proteins and GO terms between the modules of mHTT and their corresponding MSM in the reduced network are listed in Table 2. Evidently, in all cases, about 90% of the GO terms are retained. Thus, the loss, gain and loss/gain of biological processes obtained from BioGrid are quite robust.

For completeness, we also removed randomly 10% links of mHTT network and repeat the above analysis which is summarized in Table 2. Again, we find that about 90% of the GO terms enriched in this network are identical to those obtained

for mHTT. Thus, in general, the enriched biological processes obtained through this analysis are quite robust.

Discussion and Conclusion

Mutation in protein may change its preference for binding with other proteins and alter the corresponding PPIN substantially. We use a graph theory based modularization approach to identify the modules of PPINs, and provide a comparative study of these differential networks using two examples; one for HD and another for cancers. The general philosophy of this analysis is depicted schematically in Fig. 5. In this figure, the wild type protein interacts with many other proteins forming a complex interaction network. Broadly, the schematic wild type network has three subgraphs or modules (A, B and C); proteins in each module are marked there with identical colours. The mutant protein loses some proteins as interacting partners (marked as pink) and gains some new ones (marked as orange, blue and violet). The network of the mutated protein has a revised modular structure A', B' and D. Module A' and B' are re-structured and they have some proteins from other modules and some new proteins. Module D is gained by the mutation as most of proteins in this module were not present in the wild type network, and module C is lost. Correspondingly, the biological processes (GO terms) which are enriched in module D are gained and those enriched in module C are lost. We argue that this loss or gain of BPs lead to loss or gain of functions in the pathogenesis of the mutation induced disease.

Table 2. Comparison of number of proteins and GO terms in the modules of mHTT with respective of 'most similar module' of the network (a) after excluding genetic and Y2H experiments and (b) after deletion of 10% links.

	mHTT Module		(a) Excluding genetic & Y2H		(b) Random deletion of 10% links	
			MSM	Common (%)	MSM	Common (%)
M1	No. of Proteins:	643	656	542(84.29%)	612	521(81.03%)
	No. of GO terms:	161	161	147(91.30%)	163	147(91.30%)
M3	No. of Proteins:	377	287	211(55.97%)	309	209(55.44%)
	No. of GO terms:	78	95	64(82.05%)	80	59(75.64%)
M5	No. of Proteins:	485	442	423(87.22%)	424	397(81.86%)
	No. of GO terms:	198	186	173(87.37%)	174	162(81.82%)
M6	No. of Proteins:	7	5	5(71.43%)	5	5(71.43%)
	No. of GO terms:	12	16	11(91.67%)	16	11(91.67%)
M7	No. of Proteins:	22	7	7(31.82%)	612	7(31.82%)
	No. of GO terms:	5	8	5(100.0%)	7	4(80.00%)

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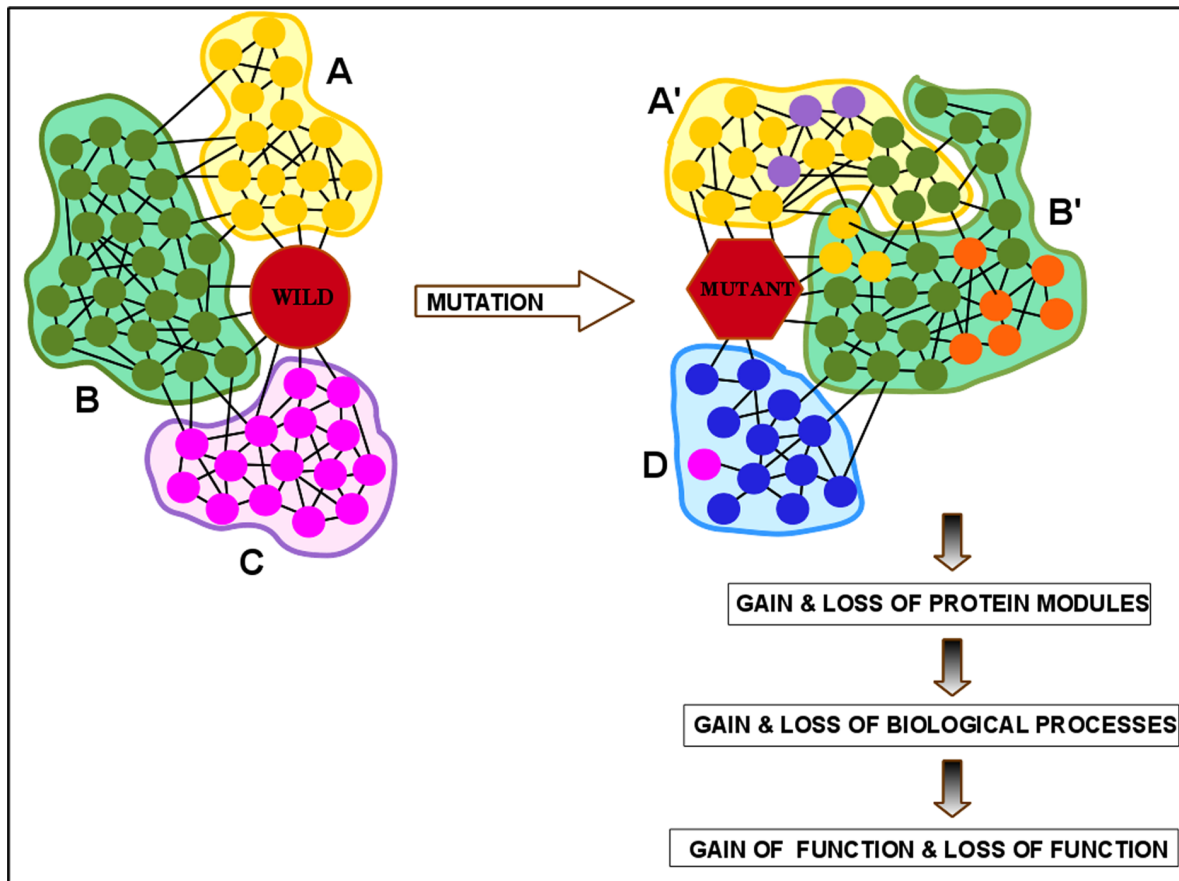
Figure 5. Loss and gain of functions from differential network studies. The general philosophy of the current work is described here for a schematic protein interaction network, where the wild type and the mutant protein have 52 and 54 interactors respectively. There are three modules in wild type network (*A*, *B*, and *C*); all proteins in a given module are marked with same colour. After mutation the protein looses some interactors (marked as pink) and gain some new ones (marked as orange, blue and violet). The PPIN of mutant protein has three modules *A′*, *B′* and *D*. Module *A′*, which primarily contains proteins of module *A*, has some proteins from module *B* (green) and some new proteins (violet). Most of the proteins in module *D* are new interactors and thus this module is gained by the mutation. Similarly proteins of module *C* have lost their interactions. Correspondingly, the BPs which are enriched in module *D* are gained and those enriched in module *C* are lost. This loss or gain of BPs lead to loss or gain of functions in the pathogenesis of the mutation causing disease.
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In this article we explained the general idea of 'obtaining the loss and gain of functions from the loss and gain of BPs enriched in protein modules' using two examples; one for HD and another for cancers. Our analysis predict a set of broadly classified biological processes (from the the GO terms enriched in the modules of HTT and TP53 networks) which could be involved in the pathogenesis of HD and cancers respectively. In HD, the broadly classified BPs, like post transcriptional regulation of genes, apoptosis, synaptic transmission, JNK pathway, transcription deregulation, glucose transport, histone modifications etc are enriched with the proteins in modules of wHTT and mHTT networks. These BPs are already known to be altered in HD pathogenesis. Similarly, the gain and loss of BPs mTP53 results in the metastatic properties, which have been observed recently.

Although, we demonstrated the plausible loss and gain of biological processes in two examples where mutation alters protein interaction networks of wild type protein, the methodology discussed here can be adopted and applied to study differential PPIN in general. In particular, knowing the changes in the protein interaction network, either due to mutations that modify the structure of the protein at the binding surface or due to the change in interaction environments, one can predict what alteration might occur in the biological processes and functions. Such analysis may

help understanding the loss or gain of biological processes/functions in genetic diseases caused by mutations. This may in future lead to better design of disease intervention through targeting the biological processes/functions of specific modules.

Supporting Information

Dataset S1 Differential interaction of the wHTT and mHTT protein.
(XLS)

Dataset S2 The proteins belonging to different modules of wHTT network and their GO term enrichment analysis.
(XLS)

Dataset S3 The proteins belonging to different modules of mHTT network and their GO term enrichment analysis.
(XLS)

Dataset S4 The list of LOF,GOF and GOF/LOF for wHTT and mHTT networks.
(XLS)

Dataset S5 Differential interaction of the wTP53 and mTP53 protein.
(XLS)

Dataset S6 The proteins belonging to different modules of wTP53 network and their GO term enrichment analysis.
(XLS)

Dataset S7 The proteins belonging to different modules of mTP53 network and their GO term enrichment analysis.
(XLS)

Dataset S8 The list of LOF,GOF and GOF/LOF for wTP53 and mTP53 networks.
(XLS)

Dataset S9 The GeneDeck analysis of the proteins in the modules of wTP53 and mTP53 networks and enrichment of metastatsis.
(XLS)

Text S1 Text 1, Differential interaction due to mutation in HTT and TP53. Text 2, Analysis of network structure. Text 3, Enriched biological processes in modules. Text 4, Enrichment of metastasis from GeneDeck.
(PDF)

Author Contributions

Conceived and designed the experiments: NPB PKM. Performed the experiments: MB NPB PKM. Analyzed the data: MB NPB PKM. Wrote the paper: MB NPB PKM.

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