Network, Structure and Functional Characteristic Features of the Human proteins targeted by both HIV and MTB proteins

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Research Article

Keywords: HIV-MTB coinfection, PPIN, Commonly targeted human proteins

Posted Date: April 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1552871/v1

Abstract

What are the network, structural and functional characteristics of the human proteins targeted by both HIV and MTB proteins in the human protein-protein interaction network (HPPIN)? To answer this question, we identified the human proteins targeted by both HIV and MTB proteins (CHPs) by integrating the experimentally determined HIV-human as well as MTB-human protein-protein interactions and analysed the network characteristic features of CHPs in the human protein-protein interaction network. Our analysis reveals that CHPs are associated with highest centrality values in the human protein-protein interaction network and are endowed with such structural and functional attributes that are distinct from the proteins targeted by either of the two pathogens or none of them. We also found that some of the interacting partners of CHPs from HIV as well as MTB are the targets of some of the drugs used for treating AIDS and TB and this finding suggests that these drugs could be used judiciously in combinations to treat patients with HIV-MTB co-infection.

Introduction

Coinfection by HIV and MTB is known to be more pathogenic than either of the two mono infections and is the leading cause of death worldwide [1, 2]. Pathogenesis of HIV-MTB coinfection is bidirectional and both the pathogens derive certain benefits from each other [3-6]. HIV infection in TB patients promotes active TB progression or reverts MTB infection from the latent stage by various mechanisms [7, 8]. For example, HIV depletes the MTB specific T cells [9-11]; inhibits the phagocytosis of MTB in macrophages [12]; interferes with the MTB antigen-presenting process [13, 14] and also disrupts the stable structure of granuloma resulting in the dissemination of MTB [15]. MTB infection helps in increasing the HIV viral load by means of increasing the density of the viral-entry receptor leading to an increased viral susceptibility of the host cells [16, 17]. MTB also activates pro-inflammatory cytokines response which exacerbates HIV replication in bystander cells [18-20]. Furthermore, MTB also increases HIV transmission via tunnelling nanotube between macrophages. [21]

Anti-retroviral therapy (ART) is used for treating HIV patients associated with TB within 2-8 weeks of starting antituberculosis treatment [22, 23]. TB in HIV-infected patients is resistant to multiple drugs leading to disease severity and deaths [24-26]. The molecular diagnostic tests used for detection of TB in HIV infected patients are either expensive or time-consuming or not sensitive [27, 28].

Although the extensive study has been done to understand the mechanism of HIV and MTB mono-infections in terms of the protein-protein interactions, to the best of our knowledge, there is no report yet available where the MTB-human and HIV-human protein-protein interactions are integrated to study the tripartite network characteristics that represent HIV-MTB coinfection status of the humans. In this study, we have therefore integrated the available experimentally derived protein-protein interactions of MTB-human and HIV-human infections and identified the human proteins commonly targeted by both the pathogens (such human proteins henceforth referred to as CHPs). Our investigations reveal that the CHPs correspond to those with the highest centrality values in the human network and are also endowed with

some structural and functional attributes distinct from the proteins targeted by either of the two or none of the two pathogens. Our study, therefore, gives a network perspective of the grave morbidity caused by the coinfection as compared with the mono-infections and hence is helpful in designing further studies toward development of effective therapeutic strategies against the HIV-MTB co-infection.

Materials And Methods

1.1 HIV-Human and MTB-Human protein-protein interaction data

HIV-Human PPIs were extracted from HPIDB [29], PHISTO [30], Virus mint [31], BioGrid [32], virus Mentha [33] and were also curated from literature [34]. Most of the MTB-Human PPI data was obtained from the literature [35-37] in addition to sourcing from the databases HPIDB [29], PATRIC [38], PHISTO [30], MorCVD [39] and Reactome Pathway Database [40]. It is interesting to note that data for MTB-human interactions are scantly available in the publicly available databases and therefore majority of the interactions were curated from literature.

All the interactions that were derived from different sources are experimentally verified physical interactions. The interactions were further curated by removing duplicates and obsolete entries from the data. Later, we used BLAST [41] to remove ambiguous human protein identifiers. The Final dataset comprises of 5053 unique interactions between HIV proteins and human proteins and 367 unique interactions between MTB and Human proteins.

1.2 Human protein-protein interaction data

Human PPIs were obtained from the HIPPIE database, which contains experimentally verified protein interactions. We considered only those interactions associated with a confidence score of more than 0.63. Furthermore, obsolete entries, self-interactions and duplicates were removed from the dataset. The pairs of human proteins having > 98% similarity scores and associated with a P-value < 10e-10 were considered as the same proteins and therefore one of it was retained by removing the other for the sake of reducing redundancy [42].

We used the igraph program of the R package [43] to calculate degree centrality(k), betweenness centrality (BC), closeness centrality (CC), and eigenvector centrality (EC) values for the proteins in a given network. We also carried out functional enrichment analysis for the human proteins using the FunRich standalone tool (V3.1.3) [44]. Any function associated with a with a P-value < 0.05 (Benjamini - Hochberg corrected) was considered a significant functional enrichment.

Results And Discussion

The final dataset of human protein interactions comprises of 311035 interactions among 16806 human proteins. After integrating the MTB-human PPIs and HIV-human PPI with the human PPIs four groups of human proteins were identified and they are as follows: (a) The human proteins that interact with HIV as

well as MTB proteins (denoted as **CHPs; 103 human proteins**), (b) The human proteins that only interact with MTB proteins (denoted as **MHPs; 167 human proteins**), (c) The human proteins that interact only with the HIV proteins (denoted as **HHPs; 1744 human proteins**) and (d) The human proteins not interacting with any of the two pathogens were considered as untargeted Human proteins (denoted as **NtHPs; 14827 human proteins**). All the four protein sets were subjected to network, sequence, and structural analysis and the results are given below.

The human proteins targeted by both the pathogens have the highest centrality values than the proteins targeted by either one or none of the two pathogens

The degree, betweenness, eigenvector, and closeness centrality value distributions for CHPs, MHPs, HHPs, and the NtHPs, are shown in **Fig.1**. As can be seen from the figure, the CHPs are associated with the highest centrality values among the four groups ($P << 10^{-16}$). This indicates that the proteins commonly interacted by MTB and HIV proteins are perhaps the essential proteins. In order to confirm this, we calculated the percentage of essential proteins among these four categories. In general, we found that the proportion of essential proteins correlates with the centrality values in all the four categories. The CHPs with the highest centrality values are mostly the essential proteins (88.34%) followed by HHPs (72%) and MHPs (67%) and NtHPs (47%) (**Table S1**).

Proteins with high centrality values are mostly conserved and also the targets of pathogens. We calculated the evolutionary rates (dN/dS) for the four categories of proteins using Mouse and Chimpanzee orthologues and compared them with the other another categories. We found that CHPs, the highly central proteins, are associated with slow evolutionary rates than other human proteins. ($P << 10^{-16}$, **Fig 2**) in other words, they are constrained to evolve slowly.

Commonly targeted proteins are the products of the abundant and widely expressed genes

Expression breadth is simply the number of tissues in which a protein is expressed. Proteins that express in almost every tissue are considered housekeeping proteins. We used gene expression profiles for 44 normal human tissues and found that CHPs are expressed across many tissues as compared with MHPs, HHPs, and NtHPs. ($P << 10^{-16,}$ **Fig 3-A**)

Protein abundance is defined as the number of protein copies in a cell and is correlated to the expression level of the corresponding gene. We found that the CHPs express abundantly as compared to other human proteins. ($P << 10^{-16,}$ **Fig 3-B**) Above results indicate that common human targets of HIV and MTB are housekeeping proteins that are abundantly expressed.

Commonly targeted proteins are the components of the innate immune response against the HIV and MTB

GO functional enrichment analysis that includes Cellular components (CC), Molecular function (MF), and Biological process (BP) was carried out for the human proteins using the Funrich Tool [44]. CHPs are

enriched with proteins that are found in most of the extracellular and intracellular components that form the first line defence as components of the innate immune response against the two pathogens. (**Fig 4, Fig S1, S2, S3).**

Commonly targeted proteins are conformationally versatile.

A node in the human protein-protein interaction network is an ensemble of all possible splice variants corresponding to a protein. It has been shown that the hub proteins are enriched with splice variants than non-hubs [45]. We used Biomart [46] to get the splice variant counts for the human proteins and this exercise revealed that CHPs are associated with a higher number of splice variants than other human protein categories ($P << 10^{-16}$) (**Fig 5-A**)

Intrinsically disordered regions (IDRs) in proteins lack stable 3D structures but adopt transiently formed multiple conformations and this conformational versatility potentiates the proteins harbouring them to interact with multiple partners and hence such proteins mostly form hubs in protein-protein interaction networks [47]. We, therefore, analysed the % of disordered residues in the four categories of human proteins. The disorder was predicted using the IUpred2A tool [48]. We found that highly connected CHPs are significantly more disordered than the other categories (P = 0.0197, **Fig 5-B**).

We also calculated the number of binding sites in the disordered region by using Anchor [48]. And we found that binding sites in the disordered regions of the CHPs are presumably promiscuous. (P = 0.003483, **Fig 5-C**), enabling CHPs to interact with more proteins. Splice variants, disorderness, and the number of binding sites all together represent conformational variability of proteins, and hence their ability to bind to multiple partners. Here CHPs show the highest propensity to bind multiple partners, hence making them more prone to get targeted by pathogen proteins.

HIV and MTB drug targets and their CHP interacting partners

We further investigated the number of AIDS and tuberculosis drug targets among the HIV and MTB proteins using the data available in the DrugBank [49]. We found 157 drugs against 8 HIV proteins and 136 drugs against 81 MTB proteins (**Table 1**). All the drug targets of HIV interact with 70 CHPs. For MTB, of the 81 drug targets, three interact with 3 CHPs (**Fig. 6, Table S2**). Taking this information together it is likely that the 157 drugs against HIV and 4 drugs against MTB that are linked to CHPs could be useful toward development of a cocktail of drugs for treatment of coinfection.

Discussion

Our study reveals that the human proteins commonly targeted by both HIV and MTB have the highest centrality values as compared with the human proteins targeted by either of the two pathogens. Since coinfection is more pathogenic than the mono infections, the present network analysis further reinforces the lethality-centrality principle in the context of viral and bacterial infections. Furthermore, the CHPs are abundantly expressed across multiple tissues, indicating their housekeeping nature. CHPs are associated

with conformational flexibility, constrained evolution, and involvement in various pathways. They are found in most the cellular components and are the components of innate immune response against the two infections. Our study has also identified the CHPs that interact with the drug targets of the two pathogens. We find that 157 drugs and 4 drugs target HIV and MTB proteins respectively. These subset of HIV and MTB proteins interact with CHPs. This information we believe helps in the development of a treatment regime comprising of a judicious cocktail of drugs to treat patients with co-infection.

Abbreviations

HIV
Human immunodeficiency virus
TB
Tuberculosis
MTB
Mycobacterium tuberculosis
AIDS
acquired immune deficiency syndrome
CHPs
Commonly targeted Human Proteins
HHPs
HIV targeted Human Proteins
MHPs
MTB targeted Human Proteins
NtHPs
Not targeted Human Proteins

Declarations

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

A. G, a registered PhD student at University of Hyderabad, gratefully acknowledges the Boarding cum Lodging (BBL) fellowship from University of Hyderabad, and Lab members Mr. Guruprasad, Mrs. Nithya and Ms. Rachana for discussion. Prof. HAN gratefully acknowledge the core grant support from the University of Hyderabad. The Bioinformatics Infrastructure Facility (BIF) at the School of Life Sciences is also gratefully acknowledged.

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Table

Table 1: Details of HIV and MTB drug targets and CHPs.

Pathogen	Number of drugs	The number of pathogen drug targets (A) and the number of CHPs, HHPs and MHPs interacting with those drug		The number of drugs targeting pathogen proteins that interact with CHPs
		(A)	No. of drug targets interact with CHPs and No. of CHPs	
HIV	157	8	8; 70	157
MTB	136	81	3; 3	4

Figures

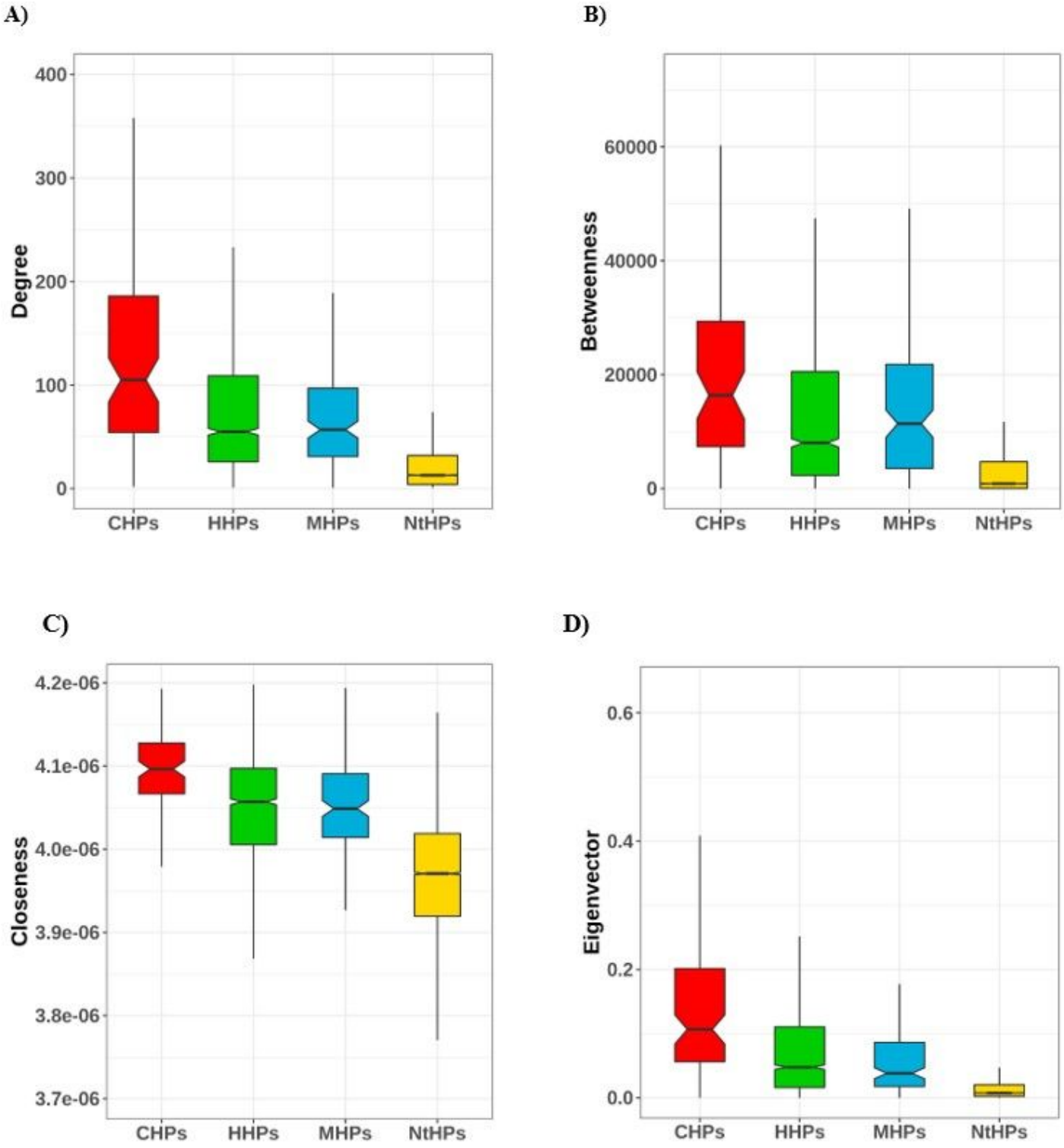

Figure 1

Topological properties of CHPs and other category (CHPs (Red), HHPs (Green), MHPs (Blue) and NtHPs (Yellow)). (**A**) Degree centrality of different categories of Human proteins. (HHPs-MHPs pair P value > 0.05, Other pair's P values $<<10^{-16}$, Kruskal-Wallis's test). (**B**) Betweenness centrality of different categories of Human proteins. (All pair's P value $<<10^{-16}$, Kruskal-Wallis's test). (**C**) Closeness centrality of different categories of Human proteins. (HHPs-MHPs pair P value > 0.05, other pair's P value $<<10^{-16}$,

Kruskal-Wallis's test). (**D**) Eigenvector centrality of different categories of Human proteins. (HHPs-MHPs pair P value > 0.05, other pair's P value $<<10^{-16}$, Kruskal-Wallis's test). CHPs having higher centrality values than the other groups of proteins. Outliers have been masked for quality purpose.

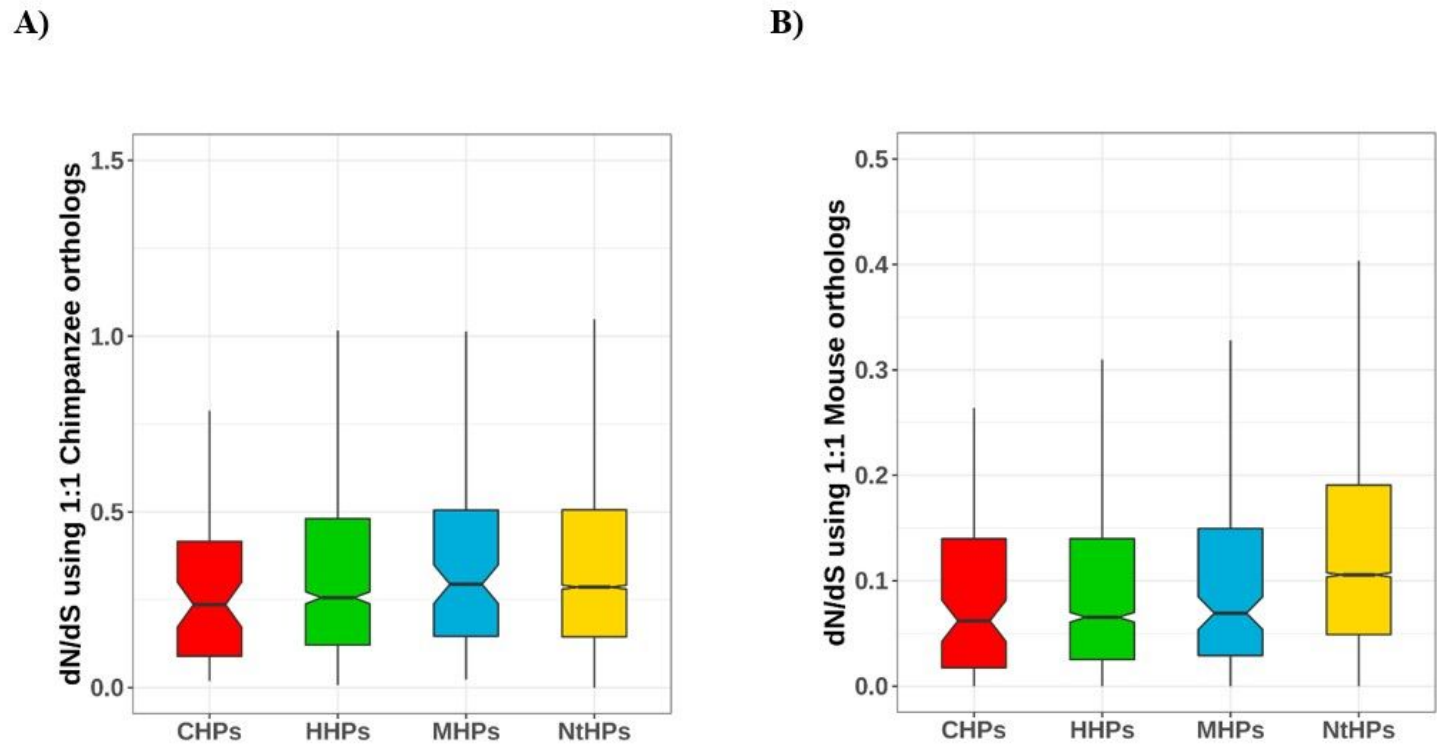

Figure 2

Evolutionary rates (dN/dS ratio) of the four categories of Human Proteins (CHPs (Red), HHPs (Green), MHPs (Blue) and NtHPs (Yellow)). The nonsynonymous substitutions (dN) and synonymous substitutions (dS) were collected from the BioMart of Ensembl database [46]. (**A**) Evolutionary rate (dN/dS ratio) by using Chimpanzee orthologs; CHPs evolve at slower rates than another human category (CHPs-HHPs, & CHPs-MHPs pairs P value > 0.05, other pair's P value $<<10^{-16}$, Kruskal-Walli's test). (**B**) Evolutionary rate (dN/dS ratio) by using Mouse orthologs; CHPs are evolving slowly than other human categories (CHPs-HHPs, CHPs-MHPs & HHPs-MHPs pairs P value > 0.05, other pair's P value $<<10^{-16}$, Kruskal-Wallis's test). Outliers have been masked for quality purpose.

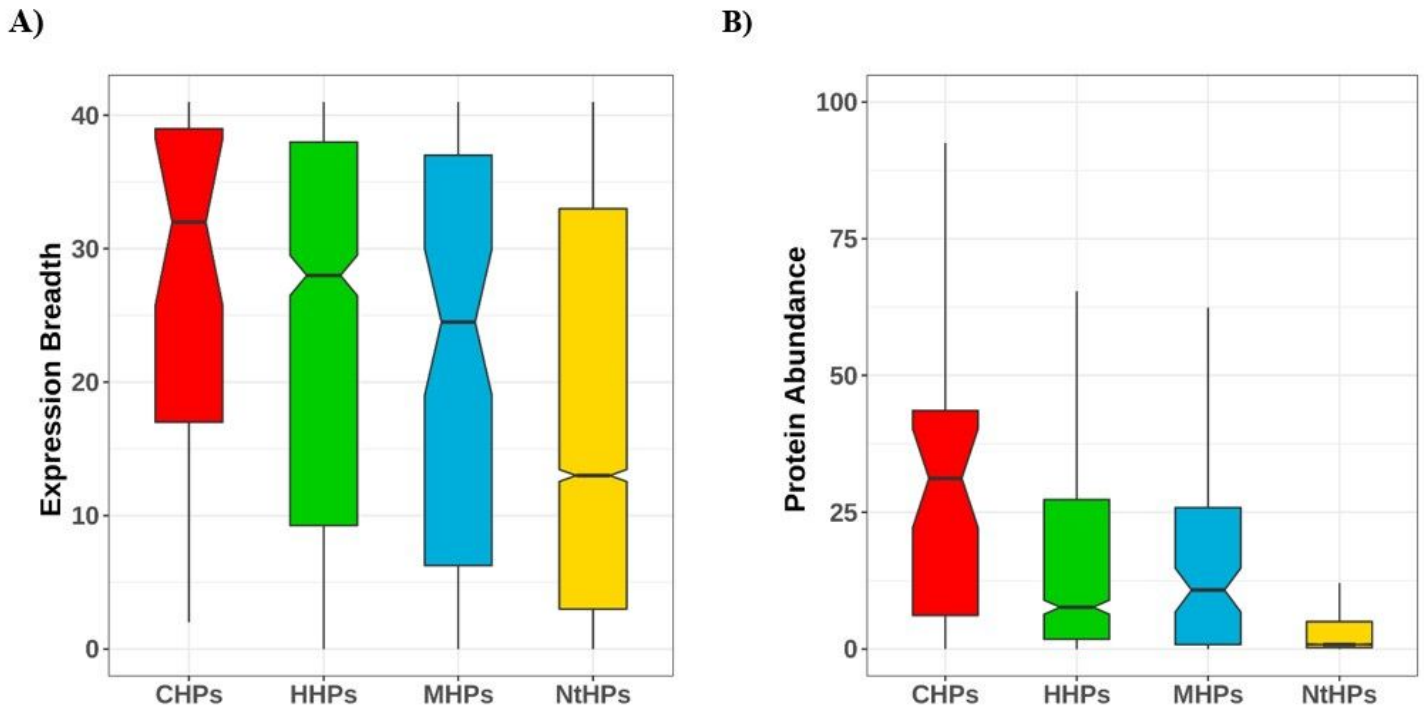

Figure 3

Expression breath and protein abundance of the Human proteins (CHPs (Red), HHPs (Green), MHPs (Blue) and NtHPs (Yellow)). (**A**) **Expression breadth**: Gene expression data were obtained from the Human Protein Atlas (HPA) database [50]. CHPs are expressed in multiple tissues when compared with other human proteins. (HHPs-MHPs pair P value > 0.05, other pair's P value $<<10^{-16}$, Kruskal-Walli's test) (**B**) **Protein abundance:** Protein abundance information was collected from the PaxDb [51] database which provides the integrated knowledge of absolute protein abundance in ppm. CHPs are more abundantly expressed than other categories of the human proteins. (HHPs-MHPs pair P value > 0.05, other pair's P value $<<10^{-16}$, Kruskal-Walli's test) Outliers have been masked for the sake of clarity.

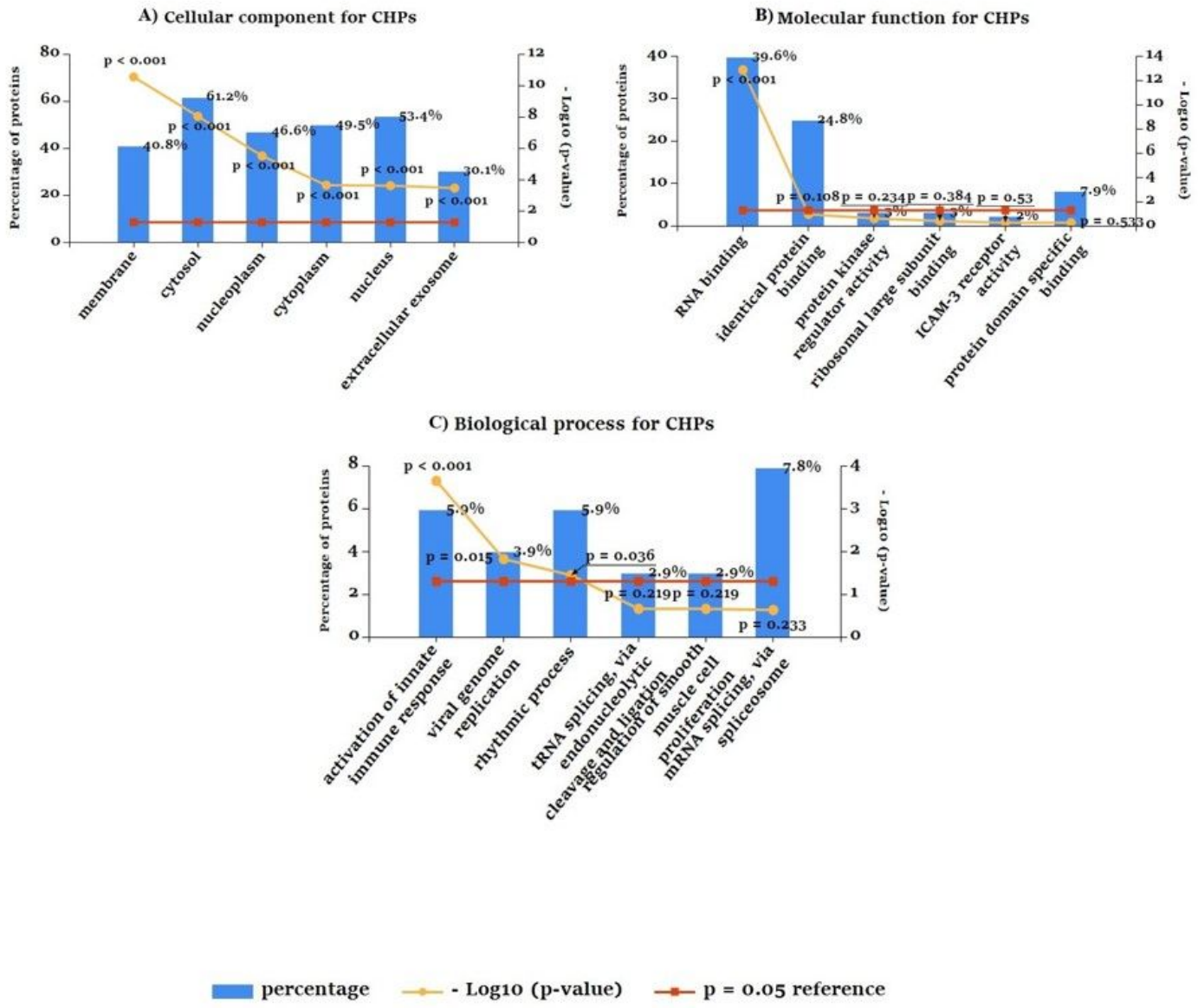

Figure 4

Functional Enrichment Analysis for CHPs: (A) CHPs are mostly found in cytosol, extracellular exosome. (**B**) CHPs are mostly involved in RNA binding molecular process. (**C**) CHPs are enriched with immunological, rhythmic and core cellular process (The blue bar shows the percentage of the genes, the red line, and yellow line stands for the reference p-Value and relative p-value respectively)

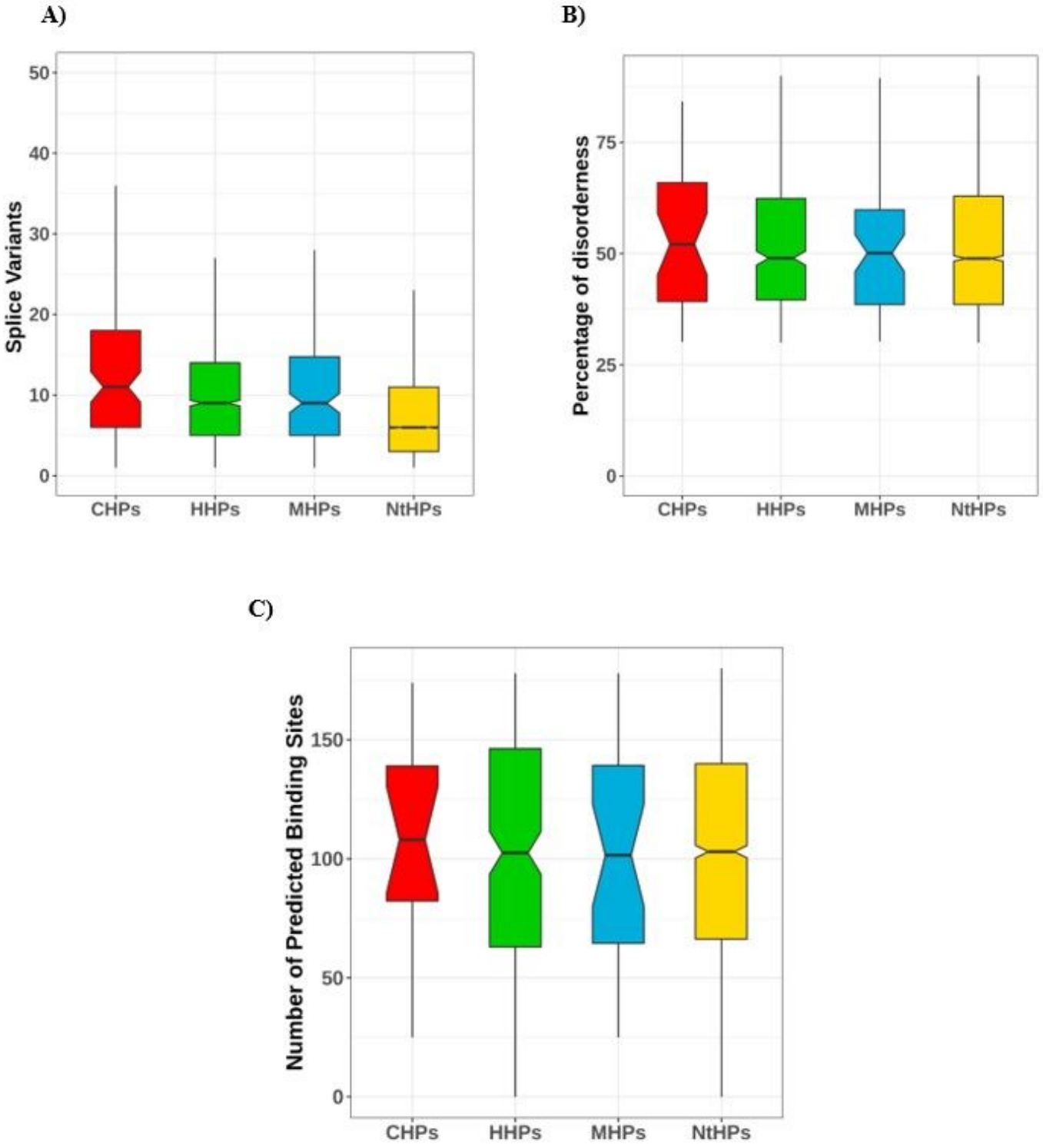

Figure 5

Intrinsic disorder and Binding interface of Human protein (CHPs (Red), HHPs (Green), MHPs (Blue) and NtHPs (Yellow)). (**A**) **Splice Variants:** Splice variant information obtained from BioMart. [46] CHPs have higher number of splice variants than the other categories of human protein (HHPs-MHPs pair P value > 0.05, other pair's P value $<<10^{-16}$, Kruskal-Wallis's test). (**B**) Percentage of disorderness (**C**) Predicted number of binding interfaces in disordered region. CHPs are mostly disordered (CHPs-NtHPs, HHPs-

NtHPs and CHPs-MHPs pair's *P value* < 0.05, Kruskal-Wallis's test) and have a greater number of binding sites. (Overall *P* = 0.003483, Kruskal-Wallis's test).

Figure 6

Schematic diagram illustrating a network of AIDS and tuberculosis drugs, their HIV and MTB drug targets and their interacting human proteins (CHPs)

Supplementary Files

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- SupplementaryInformation.docx