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MAPPING PROTEIN INTERACTIONS BETWEEN RABIES VIRUS AND ITS HUMAN HOST

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Keywords:

Rabies virus, Modeller, Enrichr, Human Protein Reference Database (HPRD), Protein Structure Validation Server (PSVS) and STRING

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ABSTRACT: Rabies is a fatal viral disease which is caused by negative sense, single-stranded RNA virus belonging to Rhabdoviridae family of genus Lyssavirus, Rabies Virus (RABV). It is transmitted by the bite of a rabid animal and directly affects the CNS of the host (neurotropic). Sources of human rabies deaths are dogs (99%), cats, bats, etc. Rabies holds the seventh position in the world for causing the most severe infections that result in the death of an organism. India lies on the top in the number of affected individuals with rabies. Our aim in this study is to analyze the interactions between the RABV and its human host and to develop novel therapeutic strategies to combat this deadly disease. Further analysis of the predicted protein-protein interactions can help us design strategies against clinical manifestations of the disease in humans. An in-silico approach has been adopted because computational approaches play a vital role in providing an important tool in further investigation of host-pathogen systems.

INTRODUCTION: Rabies is caused by a negative sense, single-stranded RNA and belongs to the genus Lyssavirus and Rhabdoviridae family. It is transmitted through saliva *via* a bite or scratch of a rabid dog, cat, wild animals as fox, wolves, raccoons, jackal, skunk, coyote, bats, *via* aerosol and transplantation of organ or cornea of infected person [1]. Rabies is fatal because it directly affects the CNS *i.e.* it's a neurotropic virus that completes its pathogenesis by crossing the blood-brain barrier and then it becomes impossible to treat [2]. The RABV genome is approximately 12 kb (11615-11996 nt) in size and has mainly five proteins, namely Nucleoprotein (N), Glycoprotein (G), Matrix-protein (M), Phosphoprotein (P), and RNA-dependent RNA polymerase (L) [3].

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The P, N and L proteins together form ribonucleo-protein complex, which aids in the replication of the RABV inside the host cell's cytoplasm. Major aspects of host cell infection are controlled by G protein, such as binding, antigenicity, and host adaptation [5]. The G protein alone is responsible for the pathogenicity of the virus as it is present on the viral surface. Moreover, it also induces protective immunity against RABV [3, 4]. Different RABV strains influence the homeostasis of infected neurons through variations in the sequence of the glycoprotein (G protein).

Pathogenic RABV strains support neuron survival preserving the cell integrity and in this manner advancing viral dissemination. Non-pathogenic (weakened) strain leads to neuron apoptosis. The death/survival parity of infected neuroblastoma is dependent on a single mutation situated in the C-terminal cytoplasmic end of G protein (Cyto-G) [6]. The fabrication and budding of bullet-shaped particles depend on M protein, and it also interacts with a transmembrane projection of G protein [7];

however, the precise mechanism by which M mediates these processes remains unclear [8]. The multifunctional RABV P-protein has fundamental roles in genome replication and structures interactions with cell Signal Transducer and Activator Transcription (STAT) proteins that are basal to viral enmity of interferon-dependent immunity [9].

Just like other viruses, RABV cannot survive outside the host and can easily be heat-killed or inactivated, or killed under Ultraviolet light [10]. Rabies holds 7th position across the globe and causes a severe infection that results in host's death [11, 12]. According to The Association of Prevention and Control of Rabies in India (APCRI), rabid dogs cause infections which results in 20,000 deaths in India annually [13]. India lies on the top when it comes to the number of individuals with rabies [11, 14]. On the other hand, approximately 40,000-70,000 mortality rate is reported by World Health Organization (WHO) globally [15, 16].

All mammals are at risk of RABV infection, but carnivorous mammals are the primary reservoirs of rabies virus [17] whereas primarily dog accounts for 10% of the RABV infections [18]. Wild animals, including raccoons and bats, are more susceptible to RABV infection, and a huge number of cases of rabies have been reported every year from them [19, 20]. Saudi Arabia, Yemen, Israel, and Iran are the countries where wildlife rabies is a serious threat to human life [21].

The first symptoms of the deadly disease are pain and itching while in the later stage the patient starts suffering from an intense headache, depression, high fevers, irritation, and intense itching at the site of bite wound [22]. Rabies is 100% fatal with no cure [23] in spite of modern and advanced therapeutic approaches [24]. But the disease is completely preventable via prompt administration of human post exposure prophylaxis (PEP) and vaccination of animal reservoirs [25]. At present scenario, due to the wide communication gap between the veterinary and public health officials, control and prevention of RABV is limited in developing countries [26].

Previously, rabies modelling has concentrated on dynamics and control of disease inside earthbound mammals like raccoons and foxes [27]. New techniques to genetically manipulate the RABV genome encouraged another period in the study of the RABV life cycle, pathogenicity and therapeutics and vaccine development [28]. Nucleotide sequence analysis allows us to determine the type of virus and aids in understanding the transmission of RABV from reservoirs to hosts [29].

Computational approaches help in vast studies of host-pathogen systems. Predicting host-pathogen protein-protein interactions provides insight into pathogenesis and recognizing specific targets for further experimental work, like visualizing systemic behaviour, and discovering potential therapeutics [30] by knowing which protein interactions allow a pathogen to infect its host [31]. Thus, the mechanism of viral action may be better illustrated by an elucidative structure depending on entire biological pathways and networks instead of only on individual genes. Interaction maps already exist for few organisms, and efforts have been made for viral interactions maps to assemble from public databases, even then, at present, comprehensive and detailed interaction maps among viral and host proteins are not provided publicly by any source, except the HIV-1 Human Protein Interactions Database [32, 33].

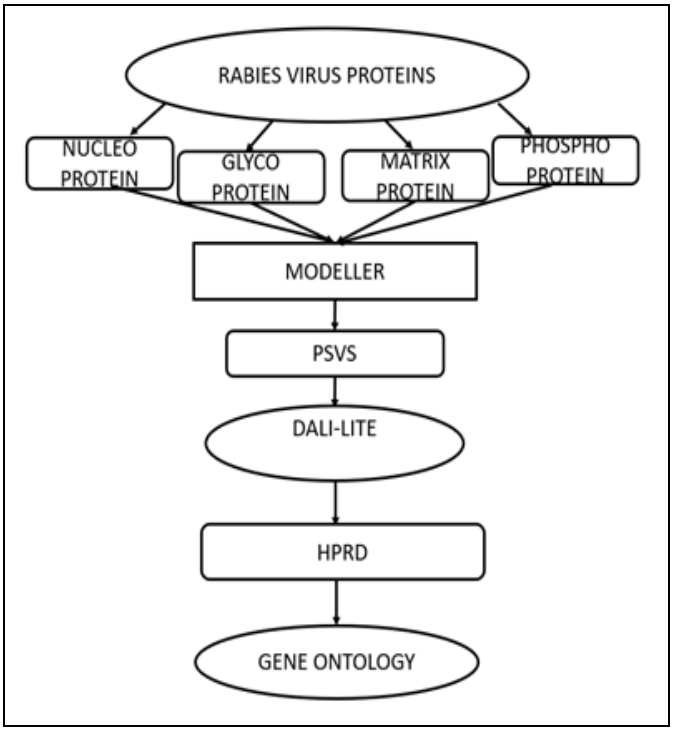


FIG. 1: EXPERIMENTAL DESIGN

In this study, we predict interactions between RABV proteins and human proteins. The predictions depend on structural similarity, initially

determining similarities among RABV and human proteins utilizing an entrenched methodology to differentiate 3-dimensional structures. We allude to host proteins that have high structural similarity to a RABV protein as ‘‘hRABV’’. Then we distinguish familiar intra-species interactions for hRABV, and allude to host proteins that hRABV interact with as ‘‘targets.’’ These targets can help in developing modern scientific approaches or strategies for the development of advanced novel therapies, drugs or vaccines to treat rabies through the in-silico approach. Workflow indicating the experimental design **Fig. 1**.

Literature Review: Rabies' history returns to ancient times. It is possibly the oldest recorded infection of mankind. In 2300 BCE, the first rabies cases were reported where Aristotle mentioned that dog saliva is venomous [32]. Rabies is a multi-host zoonotic disease [34, 35].

Hippocrates, Aristotle, and a considerable number of physicians and other authors of ancient times provided texts that improved and developed a better insight on rabies. Some gave an early depiction of palliative care in rabies patients –give accurate and detailed descriptions of symptoms, regardless of whether in dogs or in humans. On the other, Galen noticed the absence of symptoms in bite victims before the onset of rabies. A latency period was reported by Dioscorides and Philomenos, of different duration between 6 weeks to several years after an infective bite. They additionally described in “Emergency Formulas to Keep up One’s Sleeves” of the Jin Dynasty prolonged incubation periods in humans [34].

Incredible early insights into RABV pathogenesis were provided by the writings of Fracastoro(1546) and Morgagni (1769). Eusebio Giacinto discovered a treatment for the prevention of rabies, successfully attenuating the RABV by inoculating it with frog’s gastric juice [36].

Galtier and Pasteur experimented on rabbits in their rabies experiment. Pasteur immunized the first patients in 1885 by developing a vaccine by partial inactivation after periods of desiccation by inoculating infected spinal cord fluids from rabbits. Neurons from brain tissue of rabid host were reported for the presence of eosinophilic inclusion bodies by Negri (1903). Fluorescent antibody staining became a significant diagnostic test for rabies in 1958 and also useful in early pathogenesis studies. The molecular era in rabies diagnosis started with the fist clone of RABV genes in 1983 and was used for epidemiological and pathogenesis studies [37]. Purified Chick Embryo Cell Vaccine (PCECV) came into practice in the United States (1997). It was prepared from rabies virus strain Flury LEP and was grown in primary cultures of chicken fibroblasts [38].

The WHO recommends post-exposure prophylaxis treatment to patients who are affected by rabies virus (III category) by wound cleaning followed by administration of antibodies (RIG) together with complete course of vaccination. Equine Rabies Immunoglobulin (ERIG) and Human Rabies Immunoglobulin (HRIG) have great clinical applications nowadays. These plasma-derived, polyclonal products are derived from rabies vaccinated horses or humans [39].

Medical care of rabies patients is challenging, especially in areas where the perception of pathogenesis or intricate treatment issues are less understood [40]. Efforts to avert lethal outcomes have been unsuccessful, and no recovery has been observed [41]. In humans, it advances to death in 5-7 days after the beginning of side effects. Medicinal administration may delay death by 133 days [42]. However, the treatment seems to be incapable once the infection manifests its way to CNS. A palliative methodology ought to incorporate liberal utilization of sedatives and analgesics as needed to achieve comfort. Unfortunately, no effective treatment for rabies is available even after the advancement of medicine. Clinical administration of rabies has incorporated a combination of treatments, rabies immunization, immunotherapies, and ketamine [40]. To develop a better therapeutic intervention for rabies, a better understanding of the pathogenesis of the disease is required [43].

MATERIALS AND METHODS:

Data Sources: Integrative approaches form an important and essential understanding of the protein interactions between a virus and its host. Different data types to predict these interactions that came from various biological dimensions such as MODELLER, PSVS, Dali-Lite, HPRD, Gene

Ontology terms, ENRICHR, and STRING. UniProt and Genbank offer a variety of nucleotide and protein sequences from viruses and their host organisms.

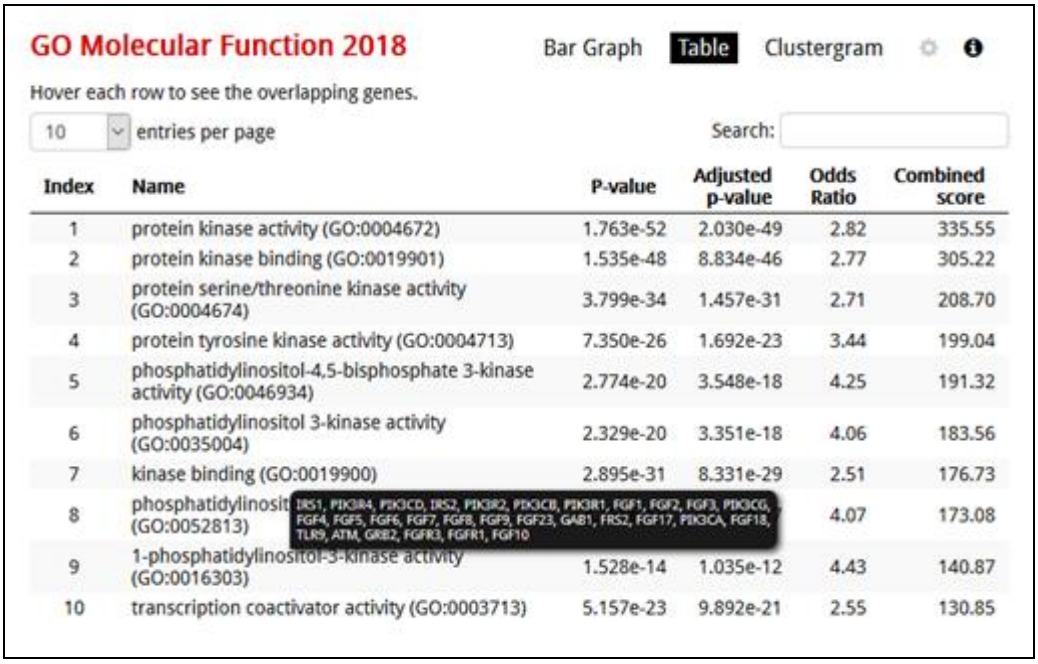
The protein sequences used were UniProt IDs A3RM19, A3RM20, A3RM21, and A3RM22 for N, P, M, and G protein, respectively. The structures of RABV proteins were not completely annotated, and hence it was not feasible to work with them; therefore MODELLER was used for homology modelling, i.e., to produce models to produce protein tertiary and quaternary structures. PSVS was used to analyze the stability of structures based on statistics on the Protein database validation, goodness of fit between structures and experimental data, and knowledge-based structure quality scores in a standardized format suitable for database integration. The lesser value of the disallowed region signifies the higher stability of structures. Stable structure of each protein is taken forward to the Dali-lite v. 3 webservers for further analysis and we took RABV similar to human proteins as hRABV [44, 45]. HPRD 1 Release 7 was used to acquire recognized human protein interactions with hRABV. Network diagrams predicting the viral and human protein interactions were created using STRING [46].

Determining Structural Similarity Between Rabv and Host Proteins: To determine the protein structural similarities between RABV and the Human host, we use the DALI web server. The DALI or distance alignment matrix method measures structural similarities by dividing the input structural sequences into hexapeptide fragments and calculates a distance matrix by evaluating the contact patterns between successive fragments using the sum-of-pairs method. The 3-dimensional structural coordinates of these proteins are compared by alignment of alpha carbon distance matrices. When distance matrices of two proteins share the same or similar features in approximately the same positions, they are said to have similar folds with similar-length loops connecting their secondary structure elements. Dali-lite compares the three-dimensional coordinates of two PDB entries, and therefore we used it to determine the protein mimicry using structural similarity score [44, 45, 47].

All four protein structures of RABV were put in Dali-lite against the entire database for proteins with similar structure with a "Z-score" of more than 2. We have taken only the Homo sapiens PDB IDs from these results for further study. We refer to these structurally similar human proteins as hRABV.

Predicting hRABV – Host Protein Interactions: To find out the interactors of hRABV proteins, The Human Protein Reference Database (HPRD) contains datasets that provide all interactions among human proteins established via in vitro and/or in vivo methods [48]. To predict the human host proteins that interact with RABV proteins, sorting of target human proteins which interact with hRABV was done, and the interactors of hRABV proteins with those of human target proteins were found. For each hRABV protein, the target proteins which interact with that hRABV protein might also interact with that RABV protein. Entries in the HPRD require gene symbols, and so PDB IDs were converted to gene symbols.

Go Term Enrichment: The Gene Ontology (GO) is a hierarchically organized system that describes and annotates gene products [49]. It was performed using ENRICHR, which is a web-based integrative software application that contains new gene-set libraries to rank enriched terms and many interactive visualization approaches to display results, Data-Driven Documents (D3) [56] **Fig. 6, 7, 8, 9, 10, 11**. In Gene Ontology, the distance from the roots increases when the terms become more specific. Gene Ontology has two main domains, viz. Molecular function and biological process and analysis of results were done on the basis of these domains. The graphs were obtained from ENRICHR only by using the combined score of both p-value and z-score. To make the study distinct and revealing, GO level 4 terms were used. Once impartial lists of genes or proteins are generated, they are used as input for computing enrichment with existing lists created from existing gene-set libraries. This software was used for the gene set enrichment and network analysis formed using STRING to visualize the interaction between each RABV protein and host genes by obtaining an interaction list, particularly for each RABV protein using HPRD.



GO Molecular Function 2018 Bar Graph Table Clustergram

Hover each row to see the overlapping genes.

10 entries per page Search:

Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	protein kinase activity (GO:0004672)	1.763e-52	2.030e-49	2.82	335.55
2	protein kinase binding (GO:0019901)	1.535e-48	8.834e-46	2.77	305.22
3	protein serine/threonine kinase activity (GO:0004674)	3.799e-34	1.457e-31	2.71	208.70
4	protein tyrosine kinase activity (GO:0004713)	7.350e-26	1.692e-23	3.44	199.04
5	phosphatidylinositol-4,5-bisphosphate 3-kinase activity (GO:0046934)	2.774e-20	3.548e-18	4.25	191.32
6	phosphatidylinositol 3-kinase activity (GO:0035004)	2.329e-20	3.351e-18	4.06	183.56
7	kinase binding (GO:0019900)	2.895e-31	8.331e-29	2.51	176.73
8	phosphatidylinositol (GO:0052813)	IRS1, PIK3R4, PIK3CD, IRS2, PIK3R2, PIK3CB, PIK3R1, FGF1, FGF2, FGF3, PIK3CG, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF23, GAB1, FRS2, FGF17, PIK3CA, FGF18, TLR9, ATM, GRB2, FGFR3, FGFR1, FGF10		4.07	173.08
9	1-phosphatidylinositol-3-kinase activity (GO:0016303)	1.528e-14	1.035e-12	4.43	140.87
10	transcription coactivator activity (GO:0003713)	5.157e-23	9.892e-21	2.55	130.85

FIG. 2: ENRICHR: RESULT PAGE SHOWING EACH GENE IN SPECIFIC MOLECULAR FUNCTION. Each gene can be visualized in the form of bar graph or cluster gram and specific values can be seen regarding each molecular function and biological process.

RESULTS:

Structure Formation of Each Protein of Rabv: We have selected the top two models for each protein which were created using MODELLER on the basis of the DOPE Score. Out of the two, we have taken the best structure in terms of stability (less disallowed region). Disallowed region of structures is found with the help of the PSVS tool **Fig. 3**.

Proteins	Models	Most favoured regions (%)	Additionally allowed regions (%)	Generously allowed regions (%)	Disallowed regions (%)
Nucleoprotein (A3RM19)	Model 1* (2j42)	79.7	15.9	3.3	1.0
	Model 2 (2gtt)	80.0	15.2	3.5	1.3
Glycoprotein (A3RM22)	Model 1 (5a22)	80.5	14.9	2.4	2.2
	Model 2* (4d6w)	79.2	15.7	3.5	1.6
Matrix-protein (A3RM21)	Model 1 (2ygt)	89.3	6.8	2.3	1.7
	Model 2* (2w2s)	89.8	8.5	1.1	0.6
Phosphoprotein (A3RM20)	Model 1.1* (2wzl)	94.3	5.7	0.0	0.0
	Model 1.2 (2wzl)	91.6	7.6	0.8	0.0
	Model 2.1 (3oa1)	95.0	4.6	0.0	0.4
	Model 2.2 (3oa1)	94.3	5.3	0.4	0.0

FIG. 3: PSVS VALIDATION RESULTS

Determining the RABV-Similar Host Proteins: Dali-Lite server was used to compare RABV structures against every structure in the protein database to help identifying RABV similar host proteins [44, 45, 50]. Dali-lite processing is shown in the **Fig. 3**. Only conspicuous structural matches with proteins from RABV's hosts *i.e.* humans were

considered. 1040 human proteins similar to RABV protein (hRABV) were extracted **Fig. 4.**

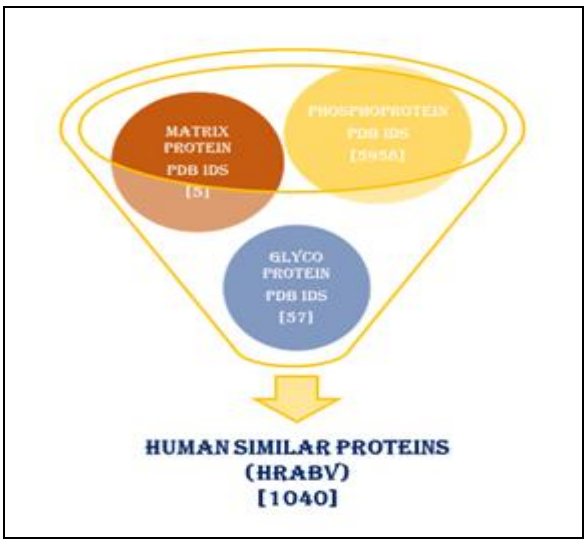


FIG. 4: HUMAN SIMILAR PROTEINS (hRABV)

Prediction of RABV-Host Interactions: Entries in the HPRD should be either in the protein name or gene symbol (gene name). Therefore, we converted all the PDB IDs into gene names with ENSEMBL. After that, 1040 possible human proteins were fed into the HPRD server and a total of 4778 interacting proteins were found, 4058 interacting with those proteins similar to Phosphoprotein, 699 interacting with those similar to Glycoprotein, and 20 interacting with those similar to matrix protein of RABV. Out of these proteins, unique interactors were filtered out on the basis of occurrence of those proteins in humans. Upon filtering, a total of 3628 proteins were observed including 3046 interacting with those proteins similar to Phosphoprotein, 564 interacting with those similar to Glycoprotein and 18 interacting with those similar to matrix protein of RABV. There were no interactions found for Nucleoprotein.

TABLE 1: PDB IDS & GENE NAMES IN EACH PROTEIN FOR EACH STEP

RABV Proteins	DALI-LITE	HPRD (Human Protein Reference Database)	Gene Ontology
N	0	0	0
G	22	699	113
M	1	20	8
P	1017	4058	552

Go Term Enrichment: Apoptosis, regulation of transcription and translation, transport, and defense mechanism of neurons obtained from ENRICHR were most significant GO terms enriched among these proteins and are analogous with RABV infection. Following ontology, the phosphoproteins were mainly found to be involved in signaling cascades activation, the glycoproteins were observed to be associated with axonal transport, and negative regulation of immune responses and matrix protein was associated with localization binding and pathogenic symptoms like salivary production and spasms.

The results we found are in consonance with results from previous studies, mentioned in **Fig. 7**, of genes that are expressed in response to RABV infection [54, 55].

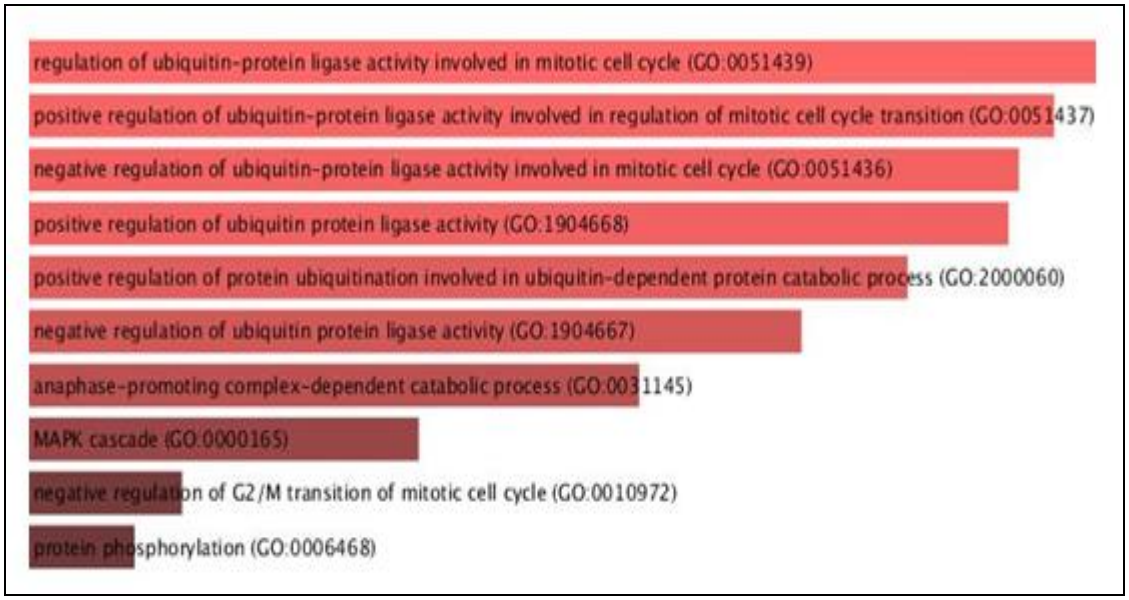


FIG. 5: BIOLOGICAL PROCESSES OF HRABV (PHOSPHOPROTEIN) OBTAINED FROM ENRICHR

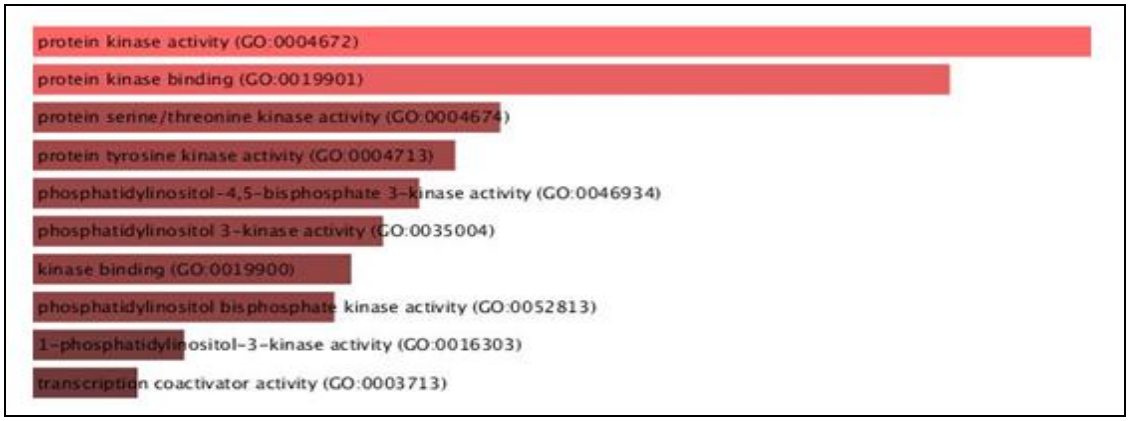


FIG. 6: MOLECULAR FUNCTION FOR hRABV (PHOSPHOPROTEIN) OBTAINED FROM ENRICHR

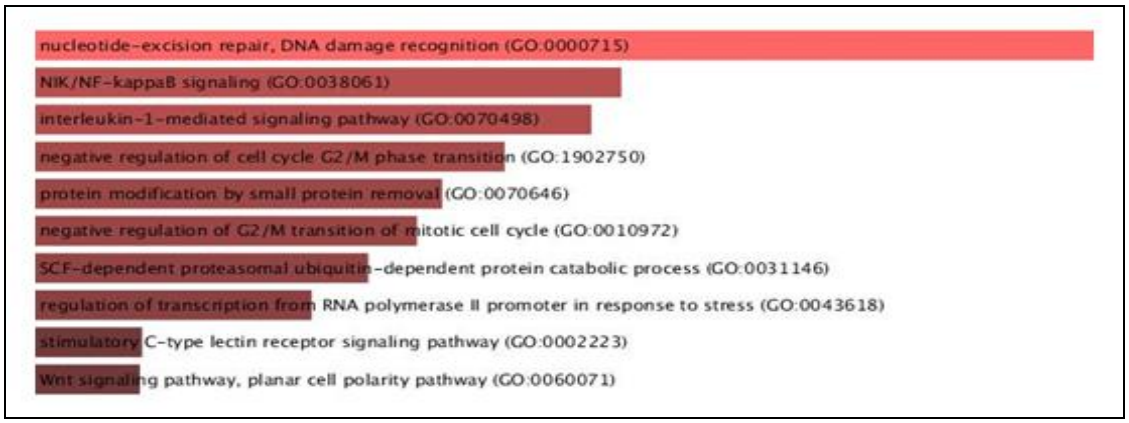


FIG. 7: BIOLOGICAL PROCESSES OF hRABV (GLYCOPROTEIN) OBTAINED FROM ENRICHR

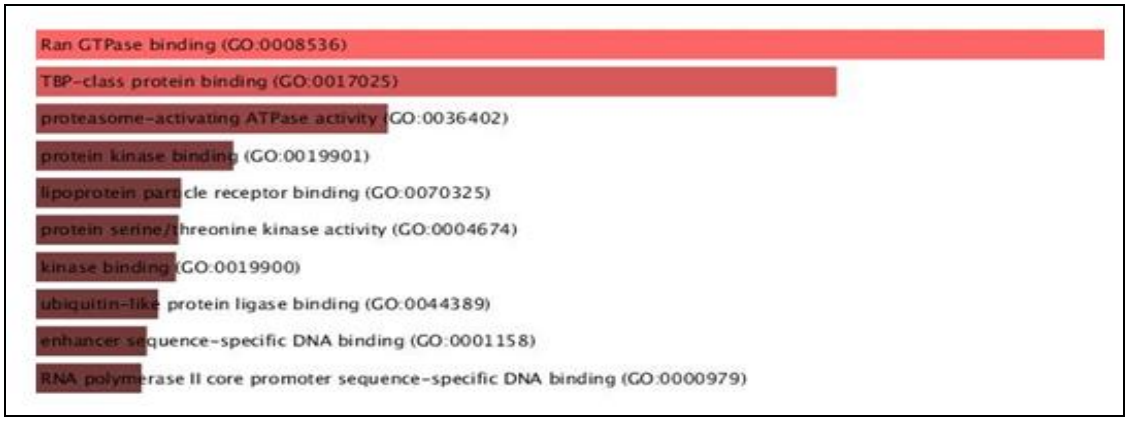


FIG. 8: MOLECULAR FUNCTION FOR hRABV (GLYCOPROTEIN) OBTAINED FROM ENRICHR

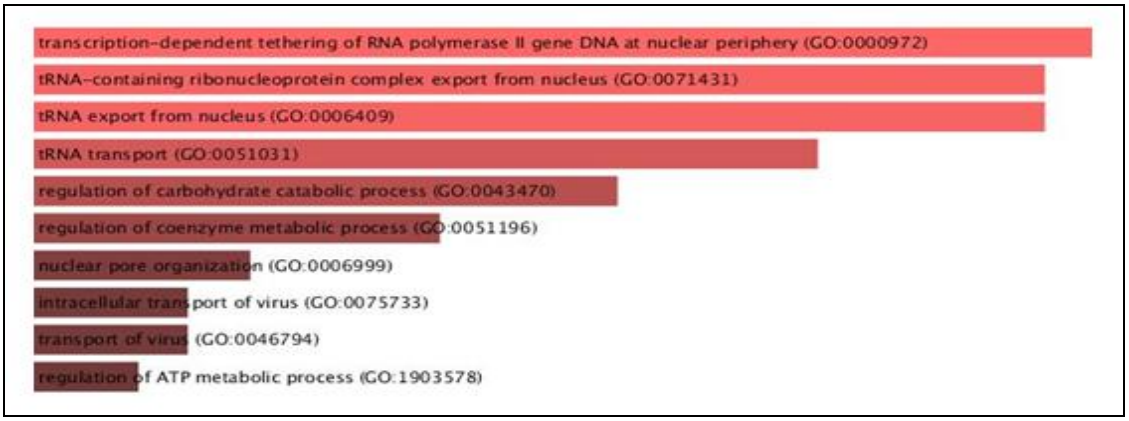


FIG. 9: BIOLOGICAL PROCESSES OF hRABV (MATRIXPROTEIN) OBTAINED FROM ENRICHR

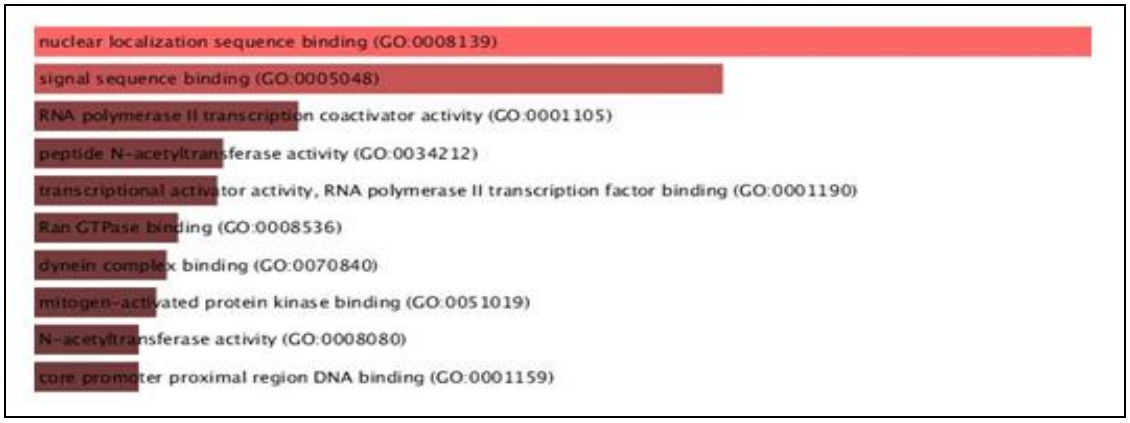


FIG. 10: MOLECULAR FUNCTION FOR hRABV (MATRIX PROTEIN) OBTAINED FROM ENRICHR

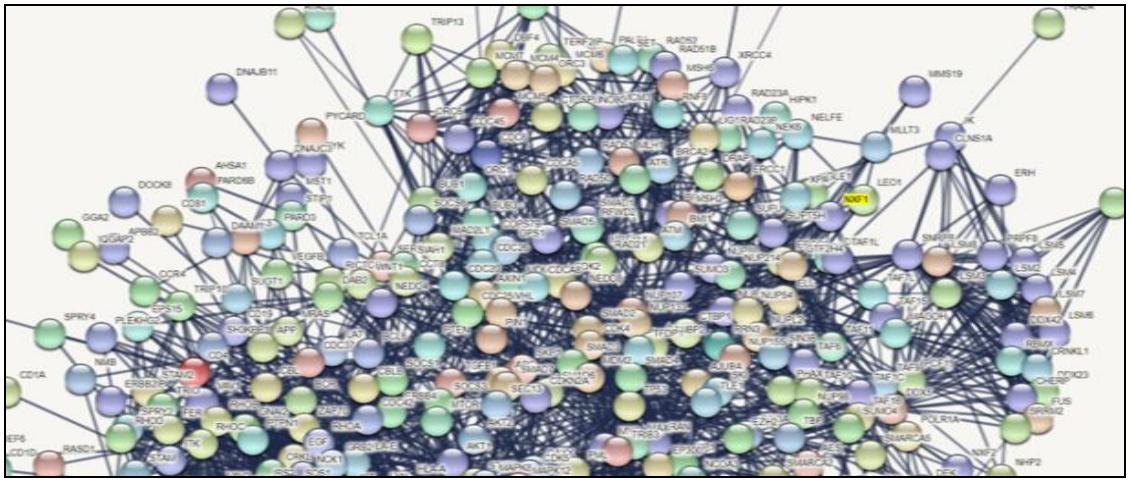


FIG. 11: NETWORK OF PUTATIVE RABV-HUMAN HOST PROTEIN INTERACTIONS STRING

The different coloured circles represent RABV proteins interacting with each other. The Nucleoprotein (N) of RABV had no structurally similar human protein and therefore, we didn't have any results for N protein. Black lines represent the interactions.

TABLE 2: KNOWN RABIES – HUMAN INTERACTIONS WHERE PROTEINS IN BOLD ARE FOUND IN OUR RESULT

Rabies Proteins	Human Proteins	References
PHOSPHOPROTEIN	ITK	
PHOSPHOPROTEIN	RAB27A	
PHOSPHOPROTEIN	RGS2	
PHOSPHOPROTEIN	EPHA3	
PHOSPHOPROTEIN	NFKB1	
PHOSPHOPROTEIN	TAF9	
PHOSPHOPROTEIN	EPHA4*	61, 62
PHOSPHOPROTEIN	TNNI3	
PHOSPHOPROTEIN	TGFB1*	
PHOSPHOPROTEIN	NXF2	
PHOSPHOPROTEIN	TAF15	
MATRIXPROTEIN	NXF1*	60
GLYCOPROTEIN	FMR1*	63
GLYCOPROTEIN	FXR1*	63
GLYCOPROTEIN	FXR2*	63
PHOSPHOPROTEIN	DAB*	60

Literature Filtering to Obtain Known Interactions Between Rabv and Host Proteins:

NXF1- NUCLEAR RNA EXPORT FACTOR 1: NXF1 majorly directs the interaction between the export mRNP (messenger Ribonucleoprotein) and the NPC (nuclear pore complex) [51, 52]. NXF1 is a conserverd protein that is an export receptor for cellular mRNAs. Proteins of this family can act on nuclear and cytoplasmic mRNA transport [53]. The site of expression of NXF1 gene is placenta, skeletal muscle, leukocyte, and saliva. RABV site of replication is striated muscles, and also it causes excess saliva production, probably due to the influence of the virus on the CNS. From this crucial information, we can predict and target the salivary gland receptors because if the swallowing of saliva is easy, then the risk of spreading of the virus is reduced. Rabies causes spasms of the muscles, which are very painful.

We can reduce the impact of partial paralysis of the body by targeting the skeletal muscle receptor of an individual [54, 55].

NGF- Nerve Growth Factor: NGF binds p75NTR (A protein receptor found on the tips of peripheral neurons); both are allowed to enter the neuron and form vesicles that move towards the neuronal cell body. RABV mimics the NGF and binds to p75NTR; therefore viral particle is internalized, and found in acidic vesicles moving towards the neuronal cell body [56]. Researchers had shown that when the RABV transmission from one neuron to another was extremely slowed down when the cell had no p75NTR receptor.

TGFB1- Transforming Growth Factor Beta 1: The growth factor required for glandular tissue morphogenesis TGFB1 is also present in GO enriched list as the virus stimulates the glands to produce more saliva. RABV uses facial nerve's mandibular branch to travels down the ganglions of the mandibular gland, therefore, reaching the acinar epithelium *via* the salivary gland myoepithelium after proliferating in the brain. Also, the the ductal system does not have nerve endings passing through the myoepithelium which suggested that viral proliferation and cytotoxicity could not occur there, thus ensuring that viral secretions are secreted in the oral cavity [56]. In some infections where viruses attack glial cells, self damage by the immune system is prevented by TGF-βs [58, 59].

DAB- DISABLED 1 and EPHA4- EPHRIN A4: DAB1 regulates a function/process that happens in cerebral cortex regulates a function/process that happens in CNS neuron is involved in the positive regulation of phosphorylation. This could be how RABV might be replicating inside neurons and making phosphoprotein P. DAB is also known to be used in rapid diagnosis immunoeassay due to its interaction with RABV proteins [60]. EPHA4 regulates a function/process that happens in hippocampal neuron regulates also invloved in positive regulation phosphorylation [61, 62].

FXR1, FXR2 and FMR1- Fragile (X) Mental Retardation Protein 1 & 2: The axon and its branches have very little ability to synthesize macromolecules and therefore, most macro-molecules required for axon or terminal function are synthesized in the cell body and have to pass along the axon to reach their sites of action. RABV also uses the same retrograde axonal transport. The genes FXR1, FXR2 and FMR1 are known to be involved in axonal transport and interaction of these proteins with RABV Glycoprotein has been studied by Guo *et al*., 2015 [63].

Predicted Interactions: As we finished ontology, each graph gave us the proteins found in specific biological processes and their molecular functions. Upon filtering that data and searching literature, we came upon those proteins which are already proven to interact with RABV proteins as described in 4.4. We also got a large list of proteins that can be possible interactors with RABV proteins and most of them are kinases as the readers will realize while going through the data. Describing that large list will be too difficult for a single research article, therefore we took the most probable proteins on the basis of the combined score of the graphs.

IFNB1- Interferon Beta 1: RABV might be using motor proteins for entry in the axons of the nerve cell, or it might be using the cell signaling pathway. IFN-stimulated genes & IFN-β gene (*Ifn*-β) in myocytes expression is suppressed by phosphoprotein. It suggests that RABV phosphoprotein aids in replication inside myocytes by impeding the host IFN system and, thus, resulting in intense infection of peripheral nerves.

Axon Receptor: Axon receptor – (stress-activated MAPK cascade). The process in which an axon recognizes and binds to the set of cells with which viral protein may form a stable connection. This shows that the virus might be using the GPCR (G-Protein Coupled Receptor) to attach to the cell using this pathway [65].

CDKS- Cyclin-Dependent Kinase: CDKS gene with acetylcholine receptor regulator activity shows that the virus might be using this path for traveling up the nerve via neuromuscular junction. A study has shown that RABV glycoprotein leads to increased expression of cyclin CLB2, and depending on kinase in yeast *pistia pastoralis* therefore, it might also interact with the cyclin dependant kinases [66].

MAPK14: MAPK14 involved in erythrocyte differentiation shows that somehow the virus might induce positive regulation of erythrocyte differentiation as the virus is shown to aggregate erythrocytes. This is done by Hemaglutinin, a part of viral surface antigen. RABV is known to sometimes stay in the myocytes and multiplies

inside the cells. Altogether, many deductable hypotheses can be derived from the results for future experimental validation to increase our understanding of RABV biology and suggest interactions that can be aimed for developing therapeutics.

DISCUSSION: Consequential challenges worsen the experiments to study viral-host interactions of RABV, which encouraged the implementation of an *in-silico* approach based on the structure and sequence similarity to propose possible host protein interactors. RABV influences and exploits key cellular pathways of the human through their interactions with the component proteins. In our research work, we employed an *in-silico* approach based on structures to divulge several host protein interactors, which might be involved replication and pathogenesis of RABV inside the human host. We analyzed that these protein interactors are majorly involved in RNA binding, signal transduction, and protein kinase binding. Together with the available knowledge about RABV, we realized that the rabies virus might be seizing these pathways in order to destabilize the transcription machinery in its favour. The complete results of gene ontology are available on ENRICHR database linking it with HPRD results and each RABV protein interaction.

CONCLUSION: We have found the genes which are interacting with the rabies virus protein and have to demonstrate which target or gene is feasible for drug development. As of now, no drug has been developed so far for the treatment of this deadly disease. In this research paper, we tried to find out the interactions of human genes with that of rabies virus (RABV), which could help to find the effective treatment of rabies and also to design specific drugs. The result indicates that all the three proteins of RABV interacting with the human host i.e., NXF1, NGF, TGFB1, RGS2, TNNI3, and various other proteins that are involved in its journey to infecting the brain and manifesting in various other tissues in the human body. Also, we predicted proteins that might actually play a role in the pathogenesis of RABV and understand how it exploits the key cellular pathways of the human through their interactions with the host.

FUTURE ASPECTS: The viral proteins might imitate the structurally similar host protein and encroach the pathways, or they might interact with a host protein involved in those pathways. This invasion results in manipulation of the host by (i) bypassing the cellular machinery components or fully seizing the cell to abet in successful replication for viral progenies and/or (ii) modifying expression levels of genes. Genes obtained after performing gene ontology were analyzed by forming a network between them. We will try to identify key players of that network that can be used in developing therapeutic strategies by seeing the interactions between the genes. Proteins of the viruses that prove to be take up similar pathways as that by human proteins will help in drug design by understanding the pathways followed by a virus.

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CONFLICTS OF INTEREST: Our organization maintains high standards of integrity and commitment. All the authors in this research hereby, declare that they have no conflict of interest.

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