Computational Studies on Molecular Evolution of Tau Phosphorylation and Its Correlation With Alzheimer's Disease

Dissertation report submitted in partial fulfilment of the requirement for the degree of

Master of Science in Biotechnology

Submitted By

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Under the Guidance of

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Declaration

I hereby declare that the work presented in this report, titled "Computational Studies on Molecular Evolution of Tau Phosphorylation and Its Correlation With Alzheimer's Disease'' submitted for the award of the Master of Science in Biotechnology degree, is an authentic record of my research conducted from July 2024 to May 2025. This work was carried out under the guidance of Dr. Tiratha Raj Singh (Professor, Department of Biotechnology and Bioinformatics) at the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat. I further confirm that this project was completed under the proficiency stream, and the content of this report has not been submitted for the award of any other degree or diploma.

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Supervisor's Certificate

This is to certify that the work presented in the M.Sc. dissertation titled"Computational Studies on Molecular Evolution of Tau Phosphorylation and Its Correlation With Alzheimer's Disease'' submitted by Divisha Sen (235111018)at Jaypee University of Information Technology, Waknaghat, India, is a genuine record of her original research conducted under my supervision. This work is not to be found elsewhere.

Signature Of The Supervisor

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Full Form
AD	Alzheimer's Disease
NFT	Nuerofiberly Tangles
PHF	Paired Helical Filament
GO	Gene Ontology
PONDR	Predictor Of Natural Disorder Regions
MF	Molecular Function
BP	Biological Process
CC	Cellular Component
KEGG	Kyoto Encyclopedia of Genes and Genomes
PTM	Post Trancriptional Modification

Abstract

Tau is abnormally hyperphosphorylated and forms neurofibrillary tangles in Alzheimer's disease (AD) and other tauopathies. It is something that is without cure.The mechanisms behind this hyperphosphorylation are not fully understood. This study shows that tau in the human brain is modified by O-GlcNAcylation, a process where a glucose derivative attaches to tau at specific serine/threonine sites. O-GlcNAcylation regulates tau phosphorylation, usually reducing it. In a mouse model of low glucose metabolism, similar to AD, reduced O-GlcNAcylation led to increased tau hyperphosphorylation AD brain extracts also showed decreased O-GlcNAcylation compared to controls. These findings suggest that decreased O-GlcNAcylation, possibly due to impaired glucose metabolism, contributes to tau hyperphosphorylation in AD. The sequence of various species was taken and through string database interaction partners of MaPT, we analyzed, further clustered, and various parameters were analyzed. GO terms were analyzed. Modeling of protein was performed phosphorylation sites were analyzed and disorder was predicted via algorithm. Tau is a neuronal protein that regulates microtubule assembly but also interacts with various partners due to its intrinsically disordered regions (IDRs). This study used bioinformatics to explore how tau's sequence evolved in vertebrates, leading to new interactions and changes in its phosphorylation pattern, which may contribute to tauopathiesIt was discovered that the amino-terminal region of tau, which is crucial in membrane organization and apoptosis, had greater protein disorder, allowing for new interactions. Phosphorylation sites changed during evolution, with some regions gaining more sites, while disease-specific hyperphosphorylation sites remained conserved. These findings suggest that new tau interactions, not related to microtubules, emerged during evolution, potentially contributing to tau pathology. Additionally, phosphorylation sites in specific regions, such as the alternatively spliced exon 2, evolved, highlighting the potential use of less conserved disease-related phosphosites as biomarkers. Since Alzheimer's disease is related to hyperphosphorylation, Pondr's DEPP and NetPhos are used for phosphorylation site analysis (Ser, Thr, and Tyr). One of the hallmarks of Alzheimer's is hyperphosphorylation, so the sequence of tau on the amino acid level becomes largely disordered. To analyze the quantum of disorder in 15 homologous sequences of tau. Amino acid analysis in the sequence was done by ClustalW. As disorder in protein regions is related to hydropathy or hydrophobicity so, average hydrophobicity was analyzed via Pondr's VL XT, VLA2, and DEPP was used to get phosphorylation sites in disordered regions to find Alzheimer's-susceptible regions. IuPred- 2a was used to get a disorder score with a particular

threshold. G profiler was used for GO enrichment and was annotated, and the structure related to human tau was visualized.

Keywords- Alzhimer's, Hyperphosphorylation, Taupathies, GO Terms, Disorder

Chapter -1

Introduction

The Central Nervous System-The central nervous system (CNS), which is composed of a vast mass of nerve cells that are shielded by the skull, is led by the brain. It is composed of three main parts: the brainstem, the cerebellum, and the cerebrum. It regulates bodily intellectual functions, such as the processing, integration, and coordination of sensory organ information. At around 1.4 kg, it is a jelly-like mass of tissue that is home to 86 billion nerve cells. The brainstem, which links to the spinal cord at the opposite end, is attached to the cerebrum. The midbrain, pons, and medulla oblongata are the three components that make up the brainstem. The thalamus, pineal gland, hypothalamus, pituitary gland, amygdala, and hippocampus are among the brain regions that lie under the cerebral cortex. Each cerebral hemisphere's cross-section reveals a ventricular cavity, which is the site of the production and circulation of cerebrospinal fluid. The membrane known as the septum pellucidum, which divides the lateral ventricles, is located underneath the corpus callosum. The human brain's biggest region is the cerebrum. Two-thirds of the brain's weight is distributed throughout its two cerebral hemispheres. The functional dominance of one hemisphere governs speech and language. Visual and spatial information is interpreted by the other hemisphere. A network of nerve fibers known as the corpus callosum connects the left and right hemispheres of the human brain. The four lobes that make up each hemisphere are the frontal, temporal, parietal, and occipital lobes. The frontal lobe regulates voluntary movements and cognitive processes, including language, judgment, emotional expression, problem-solving, memory, and sexual behavior. The primary auditory cortex, located in the temporal lobe, receives sensory data from the ears and secondary areas and processes it into meaningful terms that are expressed verbally. It also regulates primary auditory perceptions like hearing. Temperature, taste, touch, and movement information are all processed by the parietal lobe.[1].The primary organinvolved in seeing is the occipital lobe. The supporting non-neuron cells are known as glial cells,[48] whereas the brain cells themselves are known as neurons.

1. **Nerve Cells:** Neurons are the brain cells, whereas glial cells are the supporting non-neuron cells. About 86 billion neurons make up the typical adult human brain. Numerous studies have proposed that for the brain to operate properly, both neurons and glial cells are required. Electrical and metabolic signals are sent and received by neurons in the brain. The three

fundamental components of a neuron are the axon, branching dendrites, and the cell body, or soma. They serve as the brain's fundamental units, sending signals to other neurons, muscles, and other tissues. Our ability to move, think, feel, and understand the world around us is aided by neurons. Additionally, glial cells are crucial nervous system cells. The Latin word for "glue," "glia," describes the name. Glial cells play an active role in brain signaling and are essential for neurons to operate properly. The brain contains glial cells of several kinds. Oligodendrocytes are one of the three major kinds of glial cells; they shield the axons and enable electrical signals to travel great distances at lightning-fast speeds.

1. **Microglia:** sometimes referred to as CNS immune cells, these cells travel throughout the brain and are in continual communication with other glial cells. The BBB helps to heal neural tissue, supply nutrients to neurons, and promote neurotransmission.
2. **Blood Brain Barrier:** The brain barrier of blood (BBB)
   The body's blood arteries play a vital role in providing oxygen and nourishment to every tissue and organ. The CNS's blood vessel system is distinct; it makes up the blood-brain barrier (BBB), which enables the vessels to carefully control the flow of ions, chemicals, and cells between the blood and the brain.
   The blood-brain barrier (BBB) serves to shield the brain from circulating toxins and infections while simultaneously facilitating the delivery of essential nutrients. Traverse the materials through BBB efficiently.[1]
3. **Brain Disorders:** The entire body is impacted by any abnormalities, illnesses, and dysfunctions in the brain. The brain is prone to tissue infections and neurological diseases. Trauma (psychiatric illness) or a stroke (accidental or environmental circumstances) can both result in damage.
   Brain cell degeneration happens when there is brain damage. Numerous internal and external variables influence it. Whereas neurotoxicity refers to chemically generated neuronal damage, trauma-related brain damage is caused by human circumstances or psychologically unstable settings.

   In general, neuropsychiatric disorders and neurodegenerative illnesses are the two groups into which human brain problems fall-

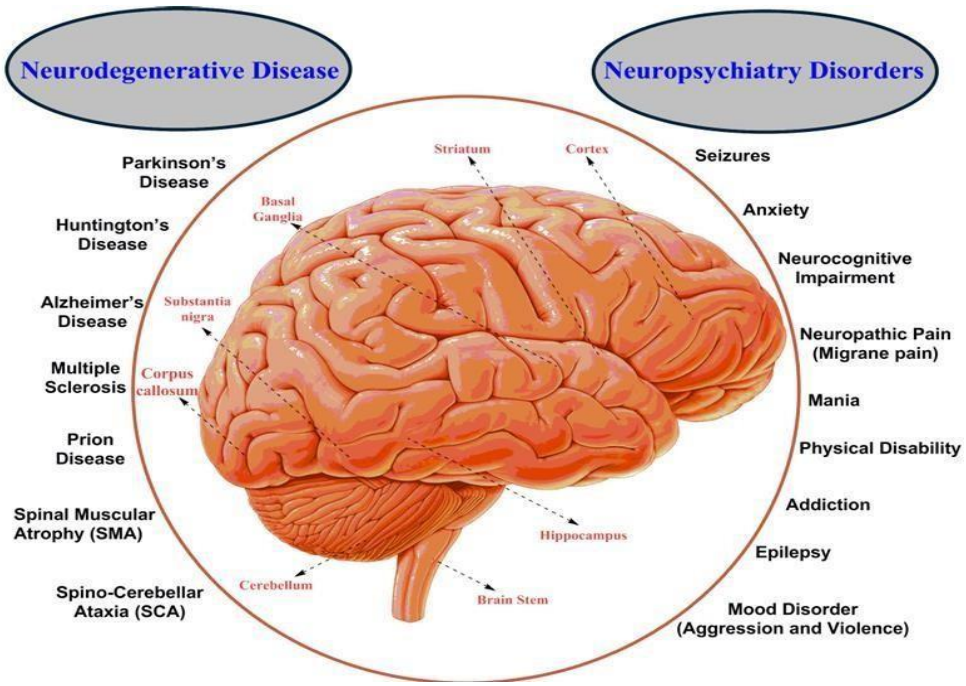

Figure 1-Figure Describing Different Types of Brain Disorders[1]

Alzheimer's disorder affects a substantial amount of the senior population. Scientists from all over the world are concentrating on the severity of this condition to understand the source and strive to resolve it with a good solution because it is a hot subject that has to be resolved and is now trending worldwide. Numerous investigations were being conducted to identify the cause; under that heading, scientists were paying close attention to neuroinflammation because it is a significant activity that is proven to be involved at the cellular level and affects metabolism in AD patients.

Problem- The pinpoint cause of the disease is unknown, the research is solely bound to the hypothesis, the disease is uncurable and the evolution of causative protein is not fully decoded As vertebrates evolved, tau protein's proline-rich region (PRR) [26]and amino-terminal region (NTR) became increasingly disorganized. This alteration suggests novel relationships that have developed throughout time and are connected to the organization of membranes and the death of cells. Tau protein acquires an excessive number of phosphate groups in AD. As a result, it gathers into paired helical filaments (PHFs), which in turn create neurofibrillary tangles. These tangles cause neurons to become unstable, which ultimately results in their death. There is a pattern to the transmission of protein in the disease. It affects the entire outer layer of the brain, beginning in the medial temporal regions and progressing to the limbic regions. AD is linked to a class of brain disorders called taupathies. NFTs have been discovered by researchers in over 20 taupathesis, mostly there are transient tau conformations. Tau-containing neurofibrillar

protein clumps are the primary hallmarks of the disease. The pathways for intracellular traffic, axonal microtubules, are stabilized by tau in healthy cells.[23] Tau clumps into paired helical filaments, gets aberrantly phosphorylated and loses its capacity to keep microtubule tracks in place in AD. Recently found mutations in the gene encoding tau have reignited interest in tau as a cause of neurological illness. This article explains how tau protein alterations may cause neuronal processes to retract, which would result in cell death. It makes the case that tau pathology, not β-amyloid,[40] may be the most accurate indicator. Research on the role of Aß in AD has advanced more rapidly than that on tau for several reasons. Research on Aß was centered on the development of the "amyloid cascade hypothesis," which was based on the identification of genetic abnormalities that cause autosomal familial AD. Additionally, the biochemical analyses of presenilin and amyloid precursor protein (APP) have significantly improved our knowledge of the molecular mechanisms underlying the production of Aß [33]. This research supported the methodical creation of Aß pathway-based disease-modifying treatments. The effectiveness of focussing on a particular facet of Aß biology (genesis, aggregation, clearance, etc.) in AD patients is presently being assessed in several studies. The notion of Alzheimer's is guided by three main hypotheses-(a)Tau Hypothesis,(b) Amyloid beta hypothesis, and (c) Neuroinflammation.[29]

Gaps In Research- The mechanism of aggregation of Phosphorylation of Tau is not fully understood. The reason for this resilience of tau is not understood. Since tau is unexpectedly diverse and comparable, it is challenging to pinpoint certain alterations as AD biomarkers and treatment targets. Summingly, When tau is aberrantly altered in Alzheimer's disease, it aggregates to form neurofibrillary tangles [25]inside neurons, upsetting the transport system and seriously harming brain cells, which adds to the cognitive loss linked to the illness. A protein known as amyloid beta also forms plaques in the brain, and tau protein is overphosphorylated in Alzheimer's disease. Tau proteins break away from their axons due to injury. Exosomes can also accelerate these diseases.[12]

1.1 Rationale of the Study

The study aims to analyze computational data to explore the mechanisms behind the phosphorylation of tau protein in AD, as well as the evolution in tau protein. Additionally, the study seeks to investigate the potential for utilizing more biomarkers to improve the differential diagnosis of Alzheimer's by mapping relevant genes knowing their regulation and pathways.

This involves comparing genetic study datasets to better understand risk factors and other key aspects.

1.2 Objectives

The study involves examining the sequence data of the MAPT for the human gene interaction involved processes involved comparing its variations across different species, and analyzing the similarities between them. Understanding the tau protein's evolution as well as the protein interactions linked to Alzheimer's disease are among its goals. Additionally, the study focuses on identifying biomarkers that could help in the varied detection of Alzheimer's.

Chapter 2

Neuroinflammation and Alzheimer's Disease

Neuroinflammation is characterized as an inflammatory response in the brain or spinal cord that is mediated by the production of cytokines, chemokines, reactive oxygen species, and secondary messengers.

These mediators are produced by endothelial cells, peripherally derived immune cells, and resident CNS glia (including microglia and astrocytes).

Causes: autoimmune diseases, stress, viral infections, and industrial chemicals (DCE). Pathogens, tissue damage, aberrant stimulation, neurotoxins, infection, or injury. [8] Microglia can even physically attack healthy neurons through phagocytosis or by secreted apoptosis factors.[34]

Following activation, microglia gather, multiply, migrate, phagocytes, present antigens to T-cells, release a range of oxidants, and activate different genes and proteins, including reactive oxygen species (ROS), pro-inflammatory cytokines like Interleukin 1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), COX-1, COX-2, and potentially neurotoxic substances that result in cell death and neuronal dysfunction.[37] When it comes to chronic neuroinflammation, these cells can stay active for prolonged periods, generating neurotoxic chemicals and cytokines that lead to long-term neurodegeneration.

The release of adhesion molecules and pro-inflammatory cytokines like TNF-α and IL-1β determines the cytokine pathway. TNF-α and IL-1β are essential for pathological inflammation and the progression of illness. [8]

They have the potential to disrupt the blood-brain barrier (BBB), increase the expression of adhesion molecules, and promote the flow of harmful chemicals like nitric oxide (NO). The development of chronic neurodegenerative disorders is significantly influenced by IL-1β.

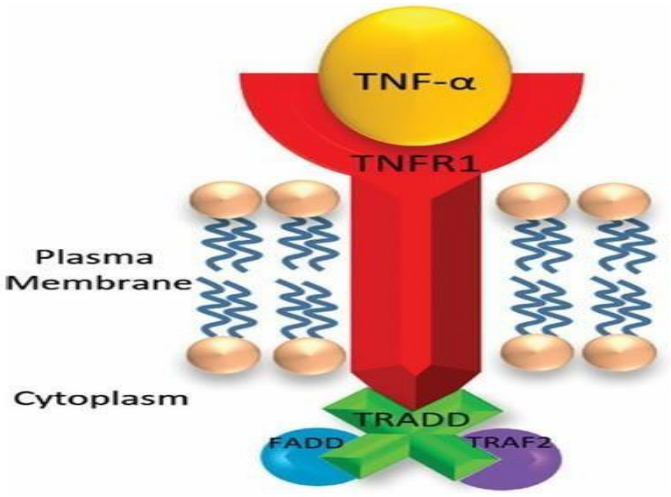

Figure2-Figure Showing Cytokine Pathway of Neuroinflammation[37]

2.1(a) Mechanism of Neuroinflammation

1. Microglia Activation: The immune cells in the central nervous system, or microglia, are responsible for triggering neuroinflammation.

2. Release of Inflammatory Mediators: Inflammatory mediators, including histamines, cytokines, chemokines, and reactive oxygen species (ROS), are released by the brain and peripheral blood cells

3. Injury To The Blood-Brain Barrier: The blood-brain barrier may become compromised due to the production of inflammatory mediators, which may further exacerbate inflammation. [17]

4. Immune cell migration: Neutrophils, monocytes, and lymphocytes are examples of peripheral immune cells that can enter the central nervous system.

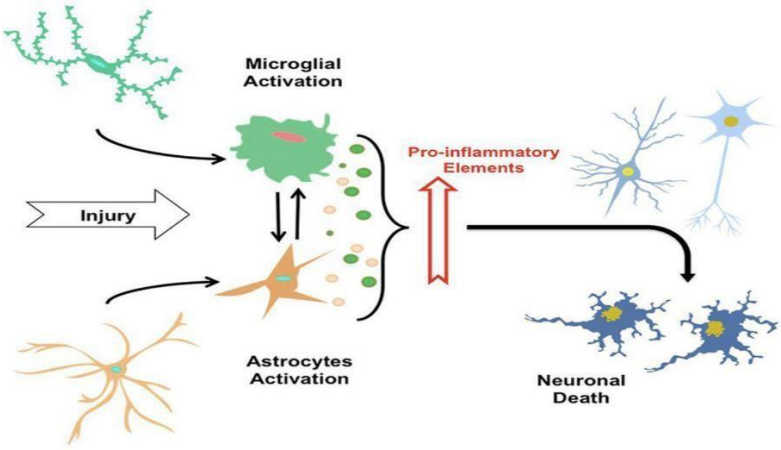

Figure3- Figure Showing Mechanism Of Neuroinflammation[49]

2.1(b)More Pathways Of Neuroinflammation- A variety of TLRs expressed by microglia activate these cells and start a neuroinflammatory response. TLRs have a Toll/IL-1 receptor (TIR) domain in the cytosolic area that is involved in the signaling cascade, as well as an extracellular leucine-rich repeat domain that is important in particular pathogen detection. An adapter protein called myeloid differentiation factor 88 (MyD88) attaches itself to TLRs through their TIR domains, triggering several signal transduction pathways that ultimately result in inflammation and NF-κB activation. MyD88 activates all TLRs except TLR3, albeit it may be limiting TLR3 signaling. The MyD88 pathway contributes to CNS infection and the activation of astrocytes that follow.

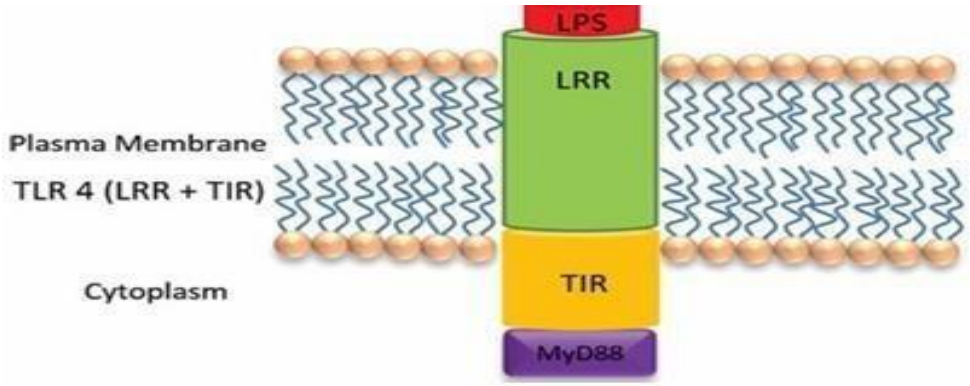

Figure 4-Figure Showing NF κB Activation And Neuroinflammation[37]

2.2 Neuroinflammation And Alzheimer's Disease

An increasing loss of cognitive and functional abilities is a hallmark of Alzheimer's disease, a neurodegenerative illness that is one of the main causes of impairment in the elderly. Degeneration of cholinergic neurons and accumulation of amyloid b (Ab) plaques are linked to the course of the disease. In AD, mitochondrial dysfunction is thought to be one of the most noticeable characteristics seen in the brains of susceptible neurons. Neuroinflammation is caused by mitochondrial malfunction in microglia, which results in an excess of ROS generation [38]. This leads to a redox imbalance and triggers the transcription of pro-inflammatory genes and the release of cytokines including IL-1β, IL-6, and TNF-α. In AD patients, oxidative protein modification, oxidative DNA/RNA damage, and large amounts of DNA breaks are seen. A strong correlation exists between activated microglia-mediated neuroinflammation and the pathogenesis of AD.

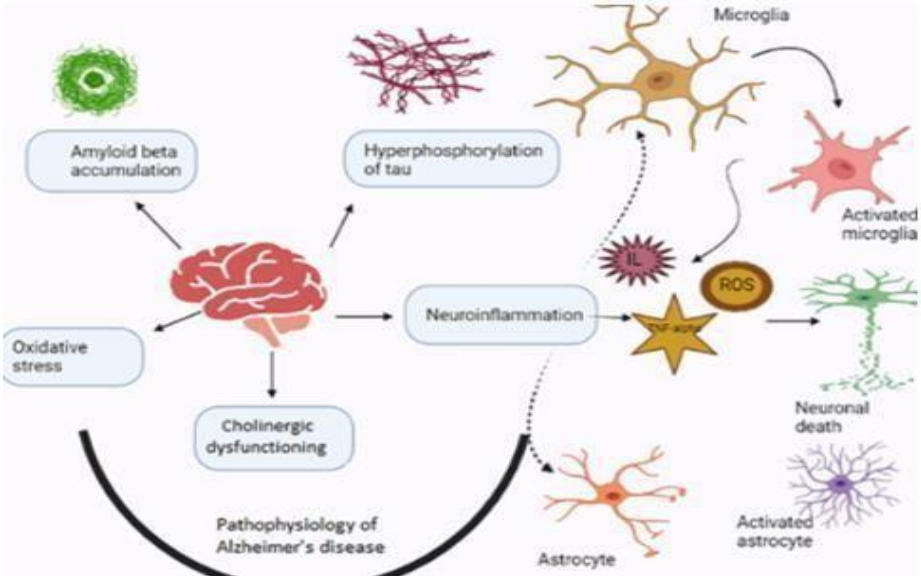

Figure 5- Figure Showing Neuroinflammation in Alzheimer's Disease[50]

Chapter 3

Review Of Literature

Tau is an inherently disordered protein with six major isoforms [18] that are extremely soluble. The tau in Alzheimer's disease (AD) is approximately three to four times more hyperphosphorylated than tau in the normal adult brain. Tau polymerizes into paired helical filaments (PHF) along with straight filaments (SF) in this hyperphosphorylated state, resulting in neurofibrillary tangles.

3.1 Hypothesis Regarding Alzheimer's Disease

According to the most widely accepted explanation, the main cause of AD is the buildup of Aβ that results in amyloid plaque. Aggregation of glial cells in the central nervous system causes neuroinflammation, an inflammatory reaction. According to the tau hypothesis, tau is a protein that may depolymerize microtubules, form neurofibrillary, and aggregate and damage axons. Pathogenic characteristic of AD, detergent-insoluble Tau aggregated in NFTs, is the subject of studies examining the PTMs of AD Tau. Tau from brains having disease had changes in 43e55 unique phosphorylation, 19 acetylations, 14e17 ubiquitination, and 4 methylation sites, [2] according to Steen and colleagues. Tau aggregation growth is associated with the decline in coordinate function and, in turn, in AD patients. In the tau sequence, other PTMs like methylation and glyconacetylation are not very common. Glycosylation that is O-linked starts can compete with phosphorylation on Ser or Thr. [4] Low but comparable levels of methylation on Lys residues are found in AD and control brains; It was proposed that Tau aggregation is enhanced by methylation. The very prevalent type of dementia[13], AD, frequently affects adults over 65. In AD, internal and extracellular protein aggregates build up. Amyloid beta is a peptide with 39 to 42 amino acids and is a constituent of amyloid plaque. Synaptic dysfunction causes PHF and NFT to accumulate, which eventually results in neuronal death.[10]The nervous system is protected via neuroinflammation, which is the mind's method of triggering the innate immune system. against damaging taunts, pain, or disease. The disease is directly connected to neuroinflammation.[38]

3.2 Tau And CSF: A Disease State Biomarker

Both AD and healthy individuals have soluble tau in their CSF, and the amount of tau in the CSF is associated with the state of the condition. As indicators for AD stages and progression

of the illness prediction, CSF concentrations of total and phosphorylation Tau, together with A-beta, can differentiate AD from other neurodegenerative illnesses. It has recently been demonstrated that tau in specimens of blood may be a biomarker.[3] Clinical studies employ the amount and phosphorylation in CSF tau as biomarkers to identify disease stages and monitor the effectiveness of AD medication because of its association with brain amyloid burden and AD condition in AD patients. [6]

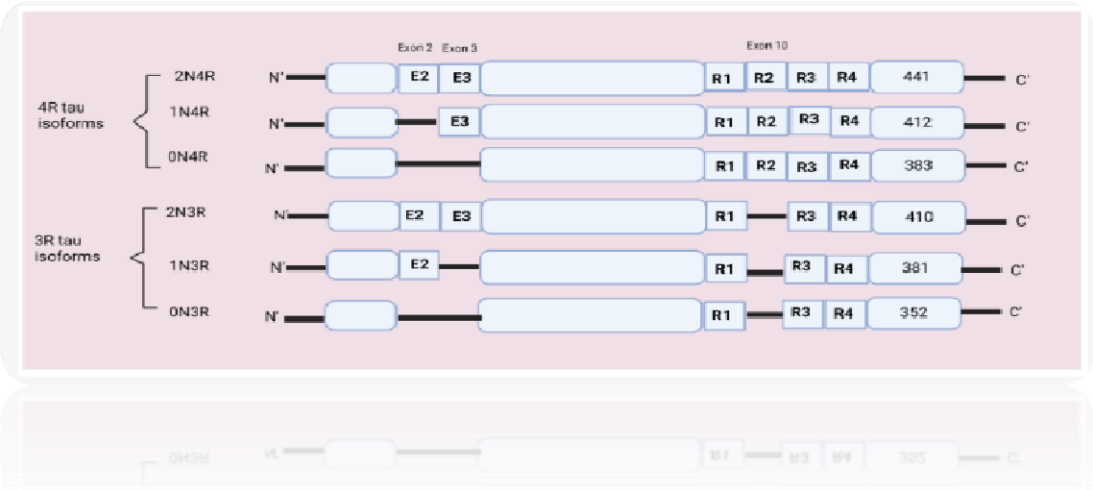

Figure 6- Figure Showing Some Isoforms of Tau[2]

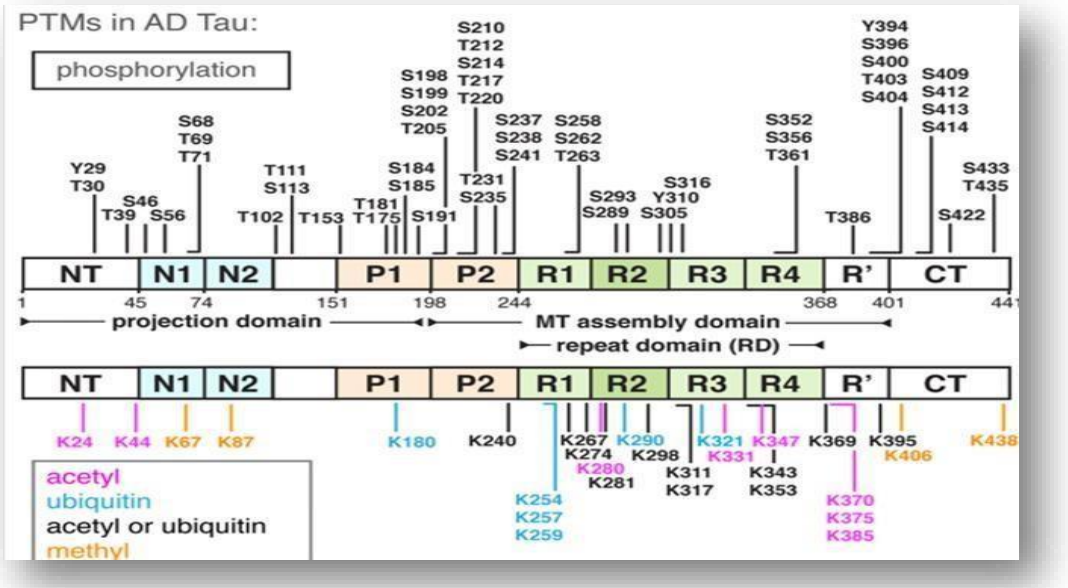

Fig 7- Figure Showing Posttranslational Modifications In Tau[2]

The aberrantly high tau in brains with AD can: (1) sequester normal tau, MAP1 and MAP2, and microtubules; (2) assemble themselves into PHF/SF Due to oligomerization, the cytosolically hyperphosphorylated tau loses its capacity to sequester normal MAPs when it self-assembles into PHF/SF, making it sedimentable. In AD, the tau is encouraged to self-assemble because a piece of it is truncated. PTMs exhibits alterations in 14–17 ubiquitination, 19 acetylation, 4 methylation, and 43–55 [19] unique phosphorylation. Together via A-beta, CSF levels of both phosphorylated and the protein act as markers for AD stages and the forecasting of development of the disease. However, there have been conflicting findings from experimental studies on the amount of APP expression in CSF of AD patients. Because of the conflicting results from different research, the possibility that CSF-APP could be a useful biomarker for AD was ruled out. PBT imaging suggests that the peptide's deposition in plaques (sometimes referred to as "amyloid sinks") is the primary reason behind the drop in CSF-Aβ42 levels seen in AD. [13]The reduced Aβ42/Aβ40 ratio is much more obvious in the diagnosis of AD. Other techniques include plasma biomarkers, urine biomarkers, and imaging techniques. This also applies to other MAPs in the MAPT/MAP2/MAP4 family area. It has numerous repetitions of a very similar motif that is 18 amino acids long, as well as the main microtubule-interacting sites. Tau is encoded by a single gene located on chromosome 17q21 of the human genome. Six different CNS isoforms are created by alternatively splicing three exons. The nervous system expresses the protein in a variety of different conformations, the longest of which is 441 amino acids long and encoded by 11 exons. On top of the numerous splice variants, tau is also impacted by phosphorylation, O-glycosylation[39], ubiquitination, methylation, acetylation, sumoylation, proteolytic cleavage, and other PTMs.[11] ,[16]These PTMs produce a wide variety of proteoforms that may have distinct localization and functional specialization.

3.3 How Bioinformatics Affects Alzheimer's Disease Investigations-

Bioinformatics plays a major role in breaking down the complex molecular and cellular mechanisms of Alzheimer's illness (AD).

a) Genetic Variables- For late-onset AD, the primary genetic risk factor is the APOE ε4 allele. Other genes: APP, PSEN1, and PSEN2 mutations result in early-onset AD that runs in families.

b) Molecular Mechanisms And Synaptic Dysfunction: A loss of synaptic connections is one of the main causes of cognitive decline.[9]

c) Mitochondrial Dysfunction and Oxidative Stress: AD is frequently linked to increased oxidative damage and impaired energy generation.

d) Protein Misfolding and Clearance Deficits: The etiology of illness is enhanced by Aβ misfolding and reduced tau clearance.[29]

Fluid Biomarkers- Fluid in the brain (CSF) and blood tests for neurofilament light chain (NfL), tau, and Aβ are being developed for early diagnosis.

Imaging Biomarkers like Aβ accumulation, tau pathological conditions, and brain atrophy are detected by PET and MRI studies. The migration of hyperphosphorylated tau and amyloid-β in the brain as the illness progressed was traced by **Braak and Braak [21]**in 1991, illustrating the division of tau migration into six stages (I–VI) and amyloid movement into three stages (A–C).

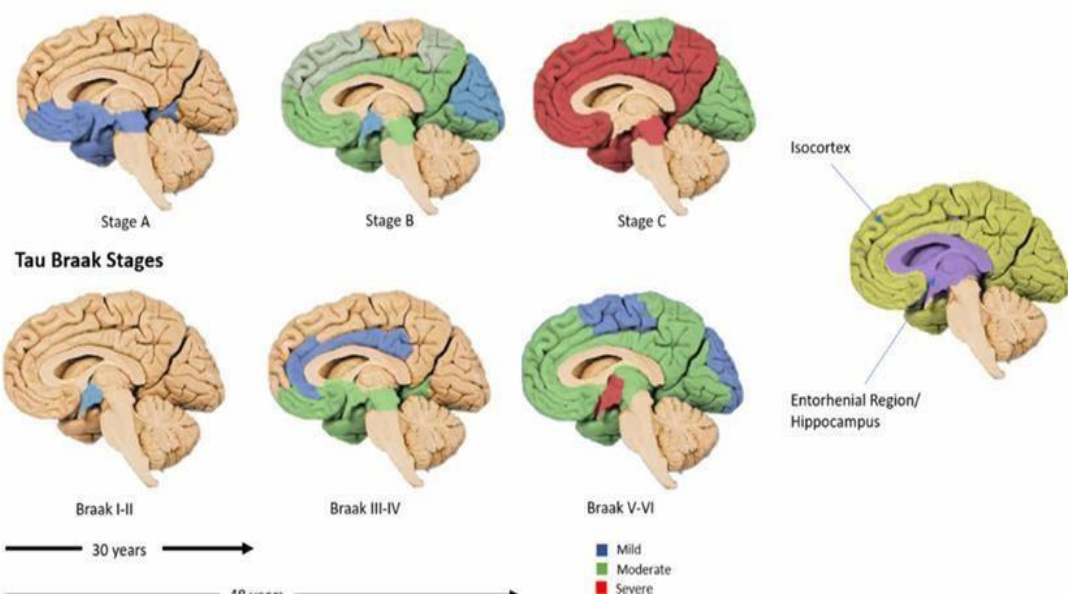

Figure 8-Figure Showing Different Stages of Alzheimer's

3.4 Therapeutic Approaches-

3.4.1 Amyloid-targeting Therapies: Drugs like aducanumab aim to reduce Aβ plaques.

3.4.2 Tau-targeting Therapies: Efforts focus on preventing tau aggregation.

3.4.3 Anti-inflammatory Approaches: Modulating microglial activity to reduce neuroinflammation.[32]

Lifestyle Interventions: Diet, exercise, and cognitive training may help delay onset or slow progression.[31]

3.5 Emerging Areas of Research

3.5.1 Role of Non-Coding RNAs-MicroRNAs and other regulatory RNAs influence gene expression in AD.[26]

3.5.2 Gut-Brain Axis- The gut microbiome may play a role in modulating neuroinflammation.[36]

3.5.3 Dysfunction-Blood-brain barrier breakdown contributes to disease pathology. Studies also emphasize how genetic mutations, post-translational modifications, and interactions with other proteins influence tau's pathological evolution, making it a key target for potential therapeutic interventions. Research on the tau protein across different species reveals variations in its sequence that may influence susceptibility to Alzheimer's disease. While the basic structure and function of tau are conserved, differences in specific amino acid sequences can affect how the protein undergoes phosphorylation, aggregation, and the formation of neurofibrillary tangles. Species less prone to Alzheimer 's-like pathology, such as rodents, often exhibit tau sequences that resist abnormal modifications. In contrast, human tau is more prone to hyperphosphorylation and aggregation, contributing to disease progression. Understanding these interspecies differences helps in studying disease mechanisms and developing better models for Alzheimer's research. The MAPT gene contains four types of repetitive sequences: transposable elements. Alzheimer's condition (AD) and other tauopathies are caused by atypical phosphorylation of tau, which results in the loss of neurons and synapses. pticAD patients have tau phosphorylation[7] that is at least three times greater than usual. Packed helical filaments (PHFs)-tau is insoluble and hyperphosphorylated, phosphorylated tau (P-tau) is readily soluble and hyperphosphorylated, and AD tau is not hyperphosphorylated and resembles normal tau. These three types of tau are discovered in diseased people. Damage to brain cells results from tau buildup, however, the specific cause of tau toxicity is yet unknown.[34] ,[15]In AD, tau declines and its capacity to attach to microtubules, most likely as a result of many sites of hyperphosphorylation, which causes tau to separate from microtubules, interfering with intracellular transport and causing neuronal degeneration. Tau levels in cerebral fluid were discovered to have increased by Vigo Pelfrey and colleagues, most likely as a result of dying neurons.[37] Tau missorting from axonal to somatodendritic areas in

AD also suggests a malfunction in tau's appropriate compartmentalization, which might result in synaptic degeneration. Proteasome and autophagy, or two protein breakdown processes that are essential in avoiding aberrant protein buildup, are both compromised in AD.[28]

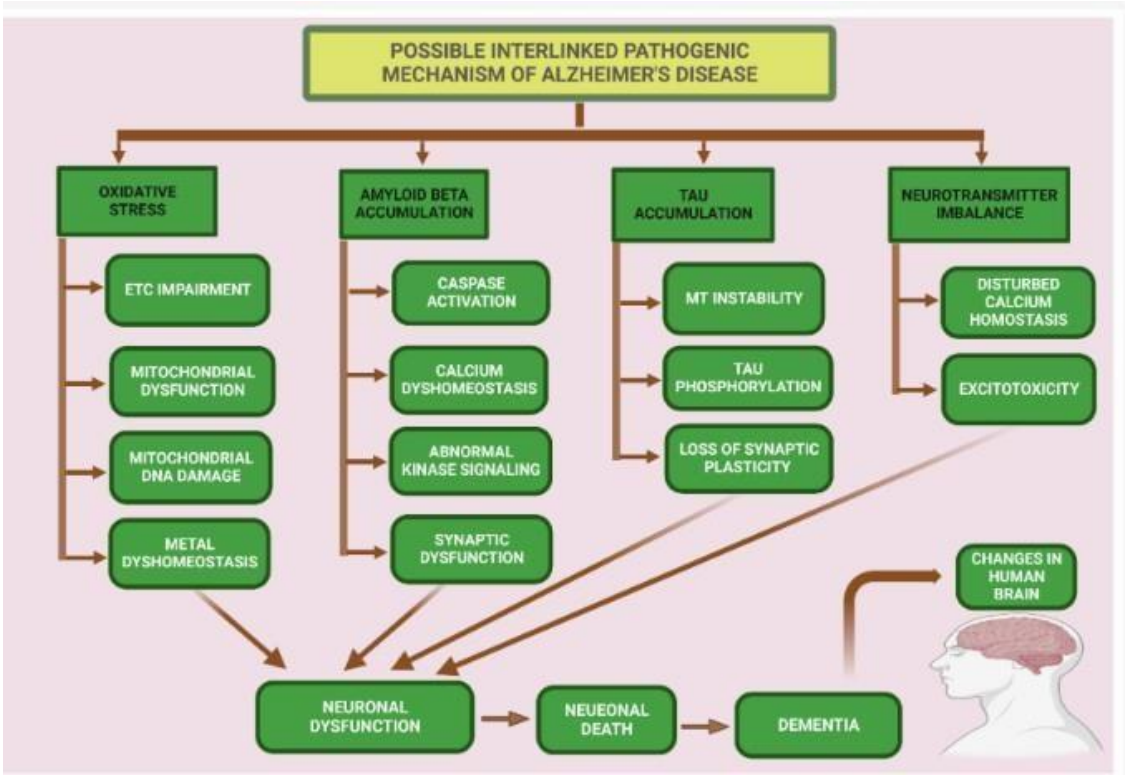

Figure 9-Figure Showing Possible Mechanisms of Alzheimer's Disease[5]

The increased interaction between phosphorylated tau (P-tau) and amyloid-beta (Aβ) may cause neuronal damage in AD patients, which can lead to cognitive impairment. Tau hyperphosphorylation is promoted by Aβ aggregates through CDK-5 activation. Amyloid beta oligomers were obtained from the LOAD of the patient and found that these oligomers are enough for increasing tau hyperphosphorylation at disease sites , destabilizing the microtubule cytoskeleton, and interfering with neuronal processes.[5] Beta-amyloid peptides rise as a result of caspases, sometimes referred to as cysteine aspartate proteases, cleaving amyloid precursor protein (APP) during death. Tarnished proteins speed up tau aggregation, whereas Aβ speeds up tau cleavage and subsequent aggregation, according to studies done in vitro by Chris. .Aβ-induced neurotoxicity requires tau, as demonstrated by Amadoro and colleagues using cellular and transgenic animal models.Though they produce toxicity through different routes, studies have clearly linked tau and Aβ to AD toxicity. [23]Lars and colleagues hypothesized three potential relationships between tau and Aβ.Aβ is the catalyst for tau disease, and both tau and Aβ are detrimental. Aβ and tau both have detrimental effects (b), tau may mediate (a), and tau

induces tau pathology . This idea suggests that neurons are more susceptible to amyloid beta damage when tau levels in dendrites are greater. The SILK technique(stable isotope labeling kinetics) [35]allows us to quantify the formation and removal of Aβ from the central nervous system. In this labeled protein aids in analyzing the effects, both positive and negative, of various medications that block the synthesis of Aβ in order to show any reduction in amyloid production.[35] The exact process underlying the elimination of amyloid is yet unknown, although it may be connected to several elements, such as APOE or improper macrophage or microglial cell activity for the depletion of amyloid blockages.

Chapter -4

Methodology

4.1 Softwares Utilized in the Study –

- Clustal W-Widely For Multiple Sequence Alignment Generation.
- Uniprot-Used to retrieve and analyze different proteins
- String Database-Used To analyze protein interactions.
- Consurf Database-Used to study Evolutionary Relationship between proteins
- Scan Prosite - Used to check for different motifs in a protein.
- QuickGo-Check for different important gene ontology terms.
- NetPhos-Used to predict phosphorylation regions within proteins
- PONDR-Helps to predict disordered regions.
- Iupred 2a-Predicts long and short region disorder
- DEPP-Predicts the phosphorylation sites within disordered regions.
- gProfiller-Used for GO annotation.
- KEGG and Reactome-Databases used for functional and pathway analysis.

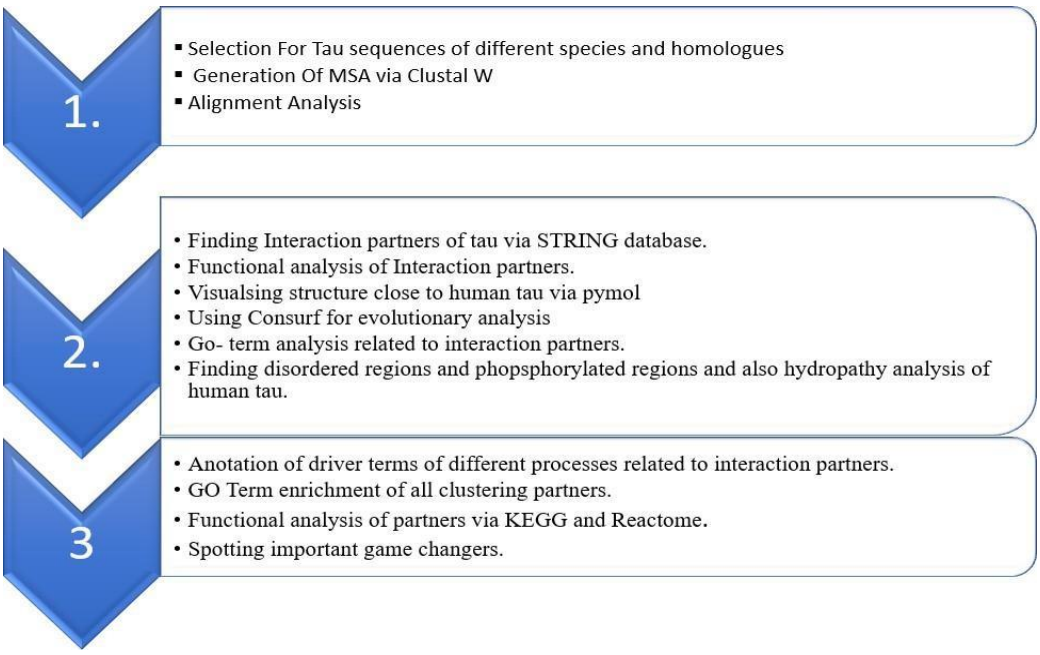

Figure 10-Figure Showing Methodology of The Research

3.2 Steps A) Through GO-term analysis, the associated human proteins' IDs of tau's interaction partners were recovered using UniProt. Following that, their QuickGO annotations were searched for Gene Ontology (GO) keywords associated with the biological process, molecular function, and cellular components (independent of area). The STRING database was used for data analysis and visualization.

B) With tau regions, the structure may be seen in the discovery studio mapped with human Tau.

C) Prediction Of Phosphorylation sites using Net PHOS and Pondr and Depp

D) Tau Sequence Selection Across Different Species also tau homologs After retrieving MAPT sequences from UniProt, Clustal W was used to perform MSA and was used to create alignment.

E) Disorder prediction is to be performed using several algorithms: IUPred2A (for long and short disordered regions), PONDR (neural networks predicting disorder), SLIDER (predicts long disordered segments), and Phosphorylation prediction software used and NetPhos 3a(for predicting phosphorylation sites). Sequence alignment isdone with Clustal W followed by manual editing, and sequence gaps are found. Hydropathy analysis is also done via Pondr

F) Go term Enrichment Via G Profiler of some driver terms and KEGG pathways and Reactome tracing via G profiler.

Chapter 5

Results And Discussion

5.1 Results

5.1(a)Results For Previously Different Species

Alignment Results

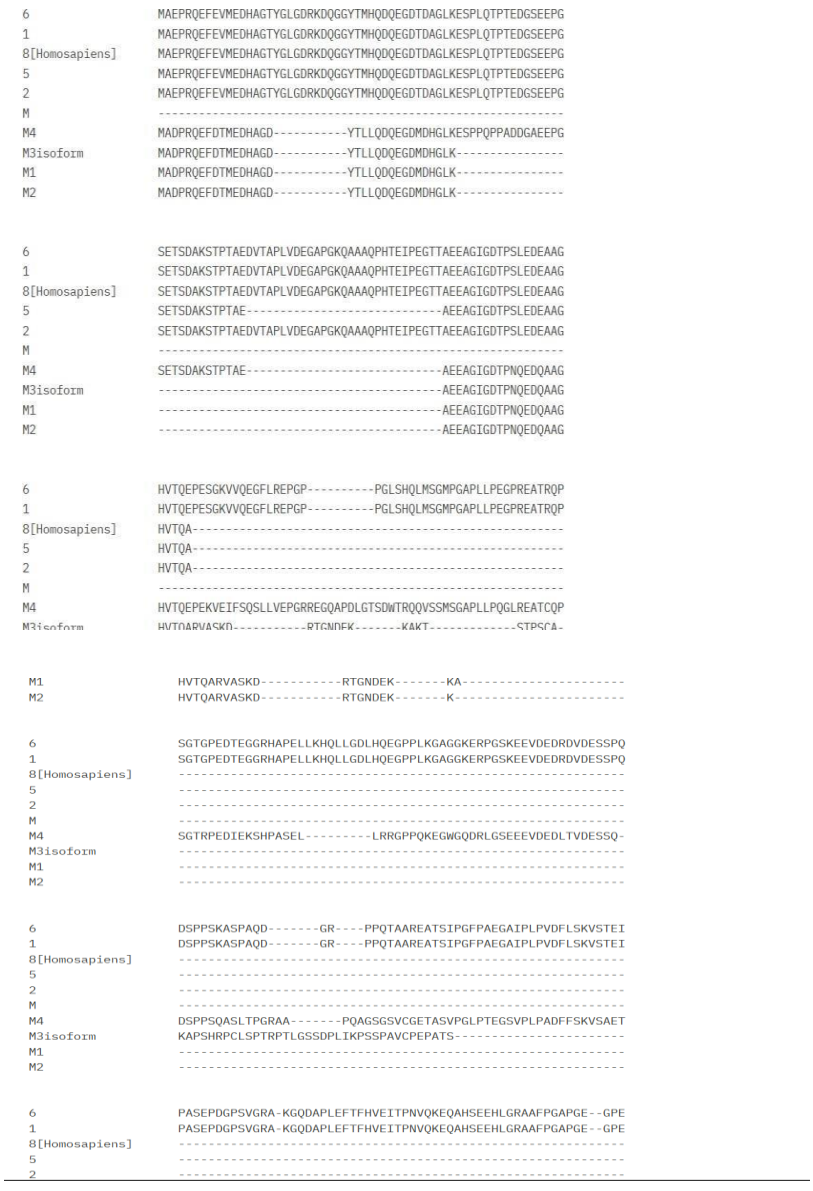

Figure11-Figure Showing Alignment Of 10 Species

Observation

Hydrophilic residues like Q are abundant which is why so much ambiguity and gap variability. Positions 668 to 8oo are nearly conserved and they likely underpinning functional ones. Symbols above the aligned sequences indicate the level of conservation at that position. Such symbols include an " " (asterisk), ":" (colon), and "." (period). "denotes residues completely conserved across all sequences, ":" denotes strong residue similarity where the residues share similar properties, and "." denotes weaker similarity where the residues are somewhat similar. This alignment shows that even the human isoforms are quite evolutionarily different. The last few residues are conserved.

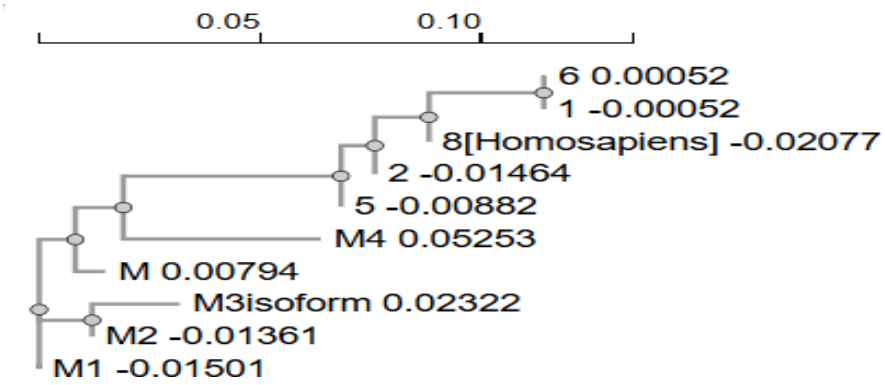

Figure12-Figure Showing Phylogenetic Tree of Alignment

Observations

The tau isoform8 (Homosapiens 8)is closely related to 1(homo-sapiens isoform1) and 6(Homosapeins isoform 8) and out of the mouse family mouse isoform 10(M4) is the most distant whereas M(mouse isoform4) M1(mouse isoform 5), M2(mouse isoform 26), M3(mouse isoform 15) are progressively less distant.

5.1(b)Further Results of Homologous Alignment

```
Gorilla(WesternLong)            MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMLQDQEGDTDAGLKESPLQTPTEDGSEEPG
Chimpanzee                      MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPG
Orangutan(Sumatran)             MAEPHQEFDVTEDHAGTYGLGDRKDQGGYTMLQDQEGDTDAGLKESPLQTPAEDGSEEPG
Orangutan(Bornean)              MAEPHQEFDVTEDHAGTYGLGDRKDQGGYTMLQDQEGDTDAGLKESPLQTPAEDGSEEPG
Bonobo                          MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPG
Human                           MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPG
X3(Siamang)                     MAEPRQEFDVMEDHAGTYGLGDRKDQGGYTMLQDQEGDTDAGLKESPLQTPAEDGSEEPG
Gibbon(WhiteCheeked)            MAEPRQEYDVMEDHAGTYGLGDRKDQGGYTMLQDQEGDTDAGLKESPLQTPAEDGSEEPG
Gibbon(Javan)                   MAEPRQEFDVMEDHAGTYGLGDRKDQGGYTMLQDQEGDTDAGLKESPLQTPAEDGSEEPG
X4(Siamang)                     MAEPRQEFDVMEDHAGTYGLGDRKDQGGYTMLQDQEGDTDAGLKESPLQTPAEDGSEEPG
Gelada                          MAEPRQEFDVMEDHAGTYGLGDRKDQEGYTMLQDQEGDTDAGLKESPLQTPAEDGSEELG
Baboon(Olive)                   MAEPRQEFDVMEDHAGTYGLGDRKDQEGYTMLQDQEGDTDAGLKESPLQTPAEDGSEELG
Macaque(SouthernPigTailed       MAEPRQEFDVMEDHAGTYGLGDRKDQEGYTMLQDQEGDTDAGLKESPLQTPAEDGSEELG
Colobus(UgandanRedColobus)      MAEPRQEFDVMEDHAGTYGLGDRKDQEGYTMLQDQEGDTDAGLKESPLQTPAEDGSEELG
FranÃ§oisLangur(LeafMonkeys)    MAEPRQEFDVMEDHAGTYGLGDRKDQEGYTMLQDQEGDTDAGLKESPLQTPAEDGSEELG
                                ****:**::* *************** **** ********************:****** *

Gorilla(WesternLong)            SETSDAKSTPTAEDVTAPLVDEGAPGEQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG
Chimpanzee                      SETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG
Orangutan(Sumatran)             SETSDAKSTPTAEDVTAPLVDERAPGEQAAAQPHMEIPEGTTAEEAGIGDTPSLEDEAAG
Orangutan(Bornean)              SETSDAKSTPTAEDVTAPLVDERAPGEQAAAQPHMEIPEGTTAEEAGIGDTPSLEDEAAG
Bonobo                          SETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG
Human                           SETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG
X3(Siamang)                     SETSDAKSTPTAEDVTAPLVDERAPGEQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG
Gibbon(WhiteCheeked)            SETSDAKSTPTAEDVTAPLVDERAPGEQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG
Gibbon(Javan)                   SETSDAKSTPTAEDVTAPLVDERAPGEQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG
X4(Siamang)                     SETSDAKSTPTAEDVTAPLVDERAPGEQAAAQPHTEIPEGTTAEEAGIGDTPSLEDEAAG
Gelada                          SETSDAKSTPTAEDVRAPLVDERAPGEQAAAQPHMEIPEGTTAEEAGIGDTPSLEDEAAG
Baboon(Olive)                   SETSDAKSTPTAEDVTAPLVDERAPGEQAAAQPHMEIPEGTTAEEAGIGDTPSLEDEAAG
Macaque(SouthernPigTailed       SETSDAKSTPTAEDVTAPLVDERAPGEQAAAQPHMEIPEGTTAEEAGIGDTPSLEDEAAG
Colobus(UgandanRedColobus)      SETSDAKSTPTAEDVTAPLVDERAPGKQDAAQPHMEIPEGTTAEEAGIGDTPSLEDEAAG
FranÃ§oisLangur(LeafMonkeys)    SETSDAKSTPTAEDVTAPLVDERAPGEQDAAQLHMEIPEGTTAEEAGIGDTPSLEDEAAG
                                *************** ****** ***:* *** * ************************

Gorilla(WesternLong)            HVTQEPESGKVVQEGFLREPGPPGLSHQLMSGMPGAPLLPEGPREATRQPSGTGPEDTEG
Chimpanzee                      HVTQEPESGKVVQEGFLREPGPPGLSHQLMSGMPGAPLLPEGPREATRQPSGTGPEDTEG
Orangutan(Sumatran)             HVTQEPESGKVVREGFLGEPGPPGLSHQLVSGMPGAPLLPEGPREATRQPSGIGPEDTEG
Orangutan(Bornean)              HVTQEPESGKVVREGFLGEPGPPGLSHQLVSGMPGAPLLPEGPREATRQPSGIGPEDTEG
Bonobo                          HVTQEPESGKVVQEGFLREPGPPGLSHQLMSGMPGAPLLPEGPREATRQPSGTGPEDTEG
Human                           HVTQEPESGKVVQEGFLREPGPPGLSHQLMSGMPGAPLLPEGPREATRQPSGTGPEDTEG
X3(Siamang)                     HVTQEPESGKVVWEGFLGEPGPPSLSHQLVSGMPGAPLLPEGPREATRQPSGTGPEDTEG
Gibbon(WhiteCheeked)            HVTQEPESGKVVREGFLGEPGPPGLSHQLVSGMPGAPLLPEGPREATRQPSGTGPEDTEG
Gibbon(Javan)                   HVTQEPESGKVVREGFLGEPGPPSLSHQLVSGMPGAPLLPEGPREATRQPSGTGPEDTEG
X4(Siamang)                     HVTQEPESGKVVWEGFLGEPGPPSLSHQLVSGMPGAPLLPEGPREATRQPSGTGPEDTEG
Gelada                          HVTQEPESGKVVQEVFLGEPGPPGLSHQLVSSMPGAPLLPEGPREATRQPSGTGPEDTEG
Baboon(Olive)                   HVTQEPESGKVVQEVFLGEPGPPGLSHQLVSSMPGAPLLPEGPREATRQPSGTGPEDTES
Macaque(SouthernPigTailed       HVTQEPESGKVVQEVFLGEPGPPGLSHQLVSSMPGAPLLPEGPREATRQPSGTGPEDTEG
Colobus(UgandanRedColobus)      HVTQEPESGKVVQEVFLGEPGPPGLSHQLVSGMPGAPLLPEGPREATRQSSGTGPEDTEG
FranÃ§oisLangur(LeafMonkeys)    HVTQEPESGKVVQEVFLGEPGPPGLSHQLVSGMPGAPLLPEGPREATRQPSGTGPEDTEG
                                *********** * ** *****.*****:*.***************** ** ******.

Gorilla(WesternLong)            GRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQDSPPSKASPA
Chimpanzee                      GRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSLQDSPPSKASPA
Orangutan(Sumatran)             GRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQDSPPSKVSPA
Orangutan(Bornean)              GRHAPELLKHQLLGDLHQEGPPLKGAGDKERPGSKEEVDEDRDVDESSPQDSPPSKVSPA
Bonobo                          GRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSLQDSPPSKASPA
Human                           GRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQDSPPSKASPA
X3(Siamang)                     GRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQDSPPSKVPPA
Gibbon(WhiteCheeked)            RRHAPELLKHQLLGDLHQEGPPLKGAGGKERPRSKGEVDEDRDVDESSPQDSPPSKFSPA
Gibbon(Javan)                   GRHAPELLKHQLLGDLHQEGPPLKRAGSKERPGSKEEVDEDRDVDESSPQDSPPSKVSPA
X4(Siamang)                     GRHAPELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQDSPPSKVPPA
Gelada                          GHHAPELLKHQLLGDLHQEGPPLKGAGGKERLGSKEEVDEDRDVDESSPQDSPPSRVSPV
Baboon(Olive)                   GHHTPELLKHQLLGDLHQEGPPLKGAGGKERLGSKEEVDEDRDVDESSPQDSPPSRVSPV
Macaque(SouthernPigTailed       GQHAPELLKHQLLGDLHQEGPPLKGAGGKERLGRKEEVDEDRDVDESSPQDSPPSRVSPV
Colobus(UgandanRedColobus)      GQHAPELLKHQLLGDLHQEGPPLKGAGSKETLGSKEEVDEDRDVDESSPQDSPPSGVSPV
FranÃ§oisLangur(LeafMonkeys)    GHHAPELLKHPLLGDPHQEGLPLKGAGSKERLGSKEEVDEDRDVDESSPQDSAPSGVSPV
                                 :*:****** **** **** *** **.**    * ************ *** **   *.
```

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Gorilla(WesternLong)            QDGRPPQTAAREATSIPGFPAKGAIHLPVDFLSKVSTEIPASEPDGPSAGRAKGQDAPLE
Chimpanzee                      QDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDGPSAGRAKGQDAHLE
Orangutan(Sumatran)             QDGWPPQAAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDRPSAGGAEGQDAPPE
Orangutan(Bornean)              QDGWPPQAAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDRPSAGRAEGQDAPPE
Bonobo                          QDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDGPSAGRAKGQDAHLE
Human                           QDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDGPSVGRAKGQDAPLE
X3(Siamang)                     HDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDGPSAGRAEGQDAPPE
Gibbon(WhiteCheeked)            HDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDGPSAGRAEGQDAPPE
Gibbon(Javan)                   HDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDGPSAGRAEGQDAPPE
X4(Siamang)                     HDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIPASEPDGPSAGRAEGQDAPPE
Gelada                          QDGQPPQTAAREATSVPGFPAEGAIALPVDFLSKVSTEIPASEPEGPSAGWAEGQDVPPE
Baboon(Olive)                   QDGQPPQTAAREATSVPGFPAEGAIALPVDFLSKVSTEIPASEPEGPSAGWAEGQDVPPE
Macaque(SouthernPigTailed       QDGQPPQTAAREATSVPGFPAEGAIALPVDFLSRVSTEIPASEPEGPSAGWAEGQDVPPE
Colobus(UgandanRedColobus)      QDGRPPQTTAREATSVPGFPAEGAIPLPVDFLSKVSTEIPASEPQGPGAGQAEGQDVPPG
FranÃ§oisLangur(LeafMonkeys)    QDGRPPQTTAREATSVPGFPAEGAIPLPVDFLSKVSTEIPASEPEGPGAGRAEGQDVPPE
                                :** ***::******:*****:*** *******:**********: *..* *:***.

Gorilla(WesternLong)            FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPESSEKQPA
Chimpanzee                      FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
Orangutan(Sumatran)             FTFHVEITPNVQKEQAHSEEHLRRAAFPGAPGEGPEAQGPSLGEDAKEADLPEPSEKQPA
Orangutan(Bornean)              FTFHVEITPNVQKEQAHSEEHLRRAAFPGAPGEGPEAQGPSLGEDTKEADLPEPSEKQPA
Bonobo                          FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
Human                           FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
X3(Siamang)                     FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
Gibbon(WhiteCheeked)            FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
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Baboon(Olive)                   FTFHVEITPNVQKEQAHPEEDSGRAAFPGAPGEEPEARGPSLGEDTKEADLPEPTEKQPA
Macaque(SouthernPigTailed       FTFHVEITPNVQKEQAHPEEDSGRAAFPGAPGEEPEARGPSLGEDTKEAELPEPTEKQPA
Colobus(UgandanRedColobus)      FTFHVEITPNVQKEQAHSE-DSGRAAFPGAPGEEPEARGPSLGEDTKEADLPEPSEKQPA
FranÃ§oisLangur(LeafMonkeys)    FTFHVEITPNVQKEQAHSEEDSGRAAFPGAPGEEPEARGPSLGEDTKEADLAEPSEKQPA
                                ***************.* * .  ********** ***:*******:***:*   :*****

Gorilla(WesternLong)            FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPESSEKQPA
Chimpanzee                      FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
Orangutan(Sumatran)             FTFHVEITPNVQKEQAHSEEHLRRAAFPGAPGEGPEAQGPSLGEDAKEADLPEPSEKQPA
Orangutan(Bornean)              FTFHVEITPNVQKEQAHSEEHLRRAAFPGAPGEGPEAQGPSLGEDTKEADLPEPSEKQPA
Bonobo                          FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
Human                           FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
X3(Siamang)                     FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
Gibbon(WhiteCheeked)            FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
Gibbon(Javan)                   FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEAQGPSLGEDTKEADLPEPSEKQPA
X4(Siamang)                     FTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPA
Gelada                          FTFHVEITPNVQKEQVHPEEDSGRAAFPGAPGEEPEARGPSLGEDTKEADLPAPTEKQPA
Baboon(Olive)                   FTFHVEITPNVQKEQAHPEEDSGRAAFPGAPGEEPEARGPSLGEDTKEADLPEPTEKQPA
Macaque(SouthernPigTailed       FTFHVEITPNVQKEQAHPEEDSGRAAFPGAPGEEPEARGPSLGEDTKEAELPEPTEKQPA
Colobus(UgandanRedColobus)      FTFHVEITPNVQKEQAHSE-DSGRAAFPGAPGEEPEARGPSLGEDTKEADLPEPSEKQPA
FranÃ§oisLangur(LeafMonkeys)    FTFHVEITPNVQKEQAHSEEDSGRAAFPGAPGEEPEARGPSLGEDTKEADLAEPSEKQPA
                                ***************.* * .  ********** ***:*******:***:*   :*****

Gorilla(WesternLong)            TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Chimpanzee                      TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Orangutan(Sumatran)             TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Orangutan(Bornean)              TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Bonobo                          TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Human                           TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
X3(Siamang)                     TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Gibbon(WhiteCheeked)            TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Gibbon(Javan)                   TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
X4(Siamang)                     TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Gelada                          TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Baboon(Olive)                   TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Macaque(SouthernPigTailed       TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
Colobus(UgandanRedColobus)      TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
FranÃ§oisLangur(LeafMonkeys)    TDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL
                                ************************************************************
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Figure13-Figure Showing Alignment Results For 15 Different Homologues

Observations-

1. At certain positions different amino acids are present for example, at the 8th position only white-cheeked gibbons have tyrosine in place of tyrosine.

2. At the 503rd to 521st position on chimpanzee, gorilla, and siamang, isoform 3 has residues; the rest of them have gaps. This can be due to evolutionary divergence 3. Most ambiguity in the alignment is due to polar residues; a possible explanation for this is as follows-

1. Similarities in Physicochemistry-Typically, hydrophobic residues are similar in size, structure, and non-polarity. As a result, they are structurally "exchangeable" with little impact on the stability and functionality of proteins.

2. Conservation of Evolution at a Functional Level-Sequence is less important to evolution than function. As long as it's oily and water-resistant, it doesn't matter if a hydrophobic environment is required or if the leucine is an isoleucine.

3. The Tolerance of Structure- Such exchanges can occur more readily in protein cores, where hydrophobic residues will assemble. Even if the particular residue were different, the fold would remain stable. Therefore, precise identification becomes hazy in MSA.

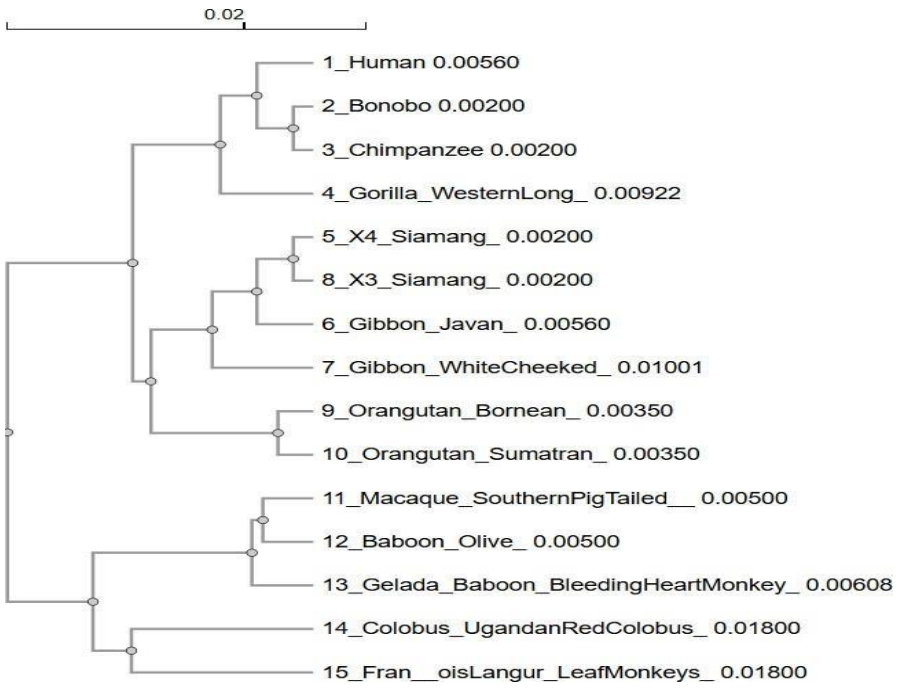


Figure14- Figure Showing Phylogenetic Tree Of Alignment

Discussion

The phylogenetic tree affirms our closest living relatives by placing Homo sapiens in the middle of a tightly connected clade with Pan troglodytes (chimpanzees) and Pan paniscus (bonobos), showing low genetic difference (~0.002). After that, the Gorilla shows signs of an

early split from the human lineage with a fairly divergent sequence (~0.009) of its own. Pongo species, sometimes known as orangutans, represent a more distant branch of the old.

Asian giant apes.Siamangs and gibbons, which are smaller apes, separated before great apes, forming a separate family (Hylobatidae) and having a higher genetic distance. The species with the highest divergence (to 0.018) from the human lineage include macaques, baboons, colobus, and langurs. Overall, the tree provides compelling evidence for the evolutionary theory that, with increasing distance from our ancestry, humans are most closely linked to African big apes.

5.2 STRING Results-

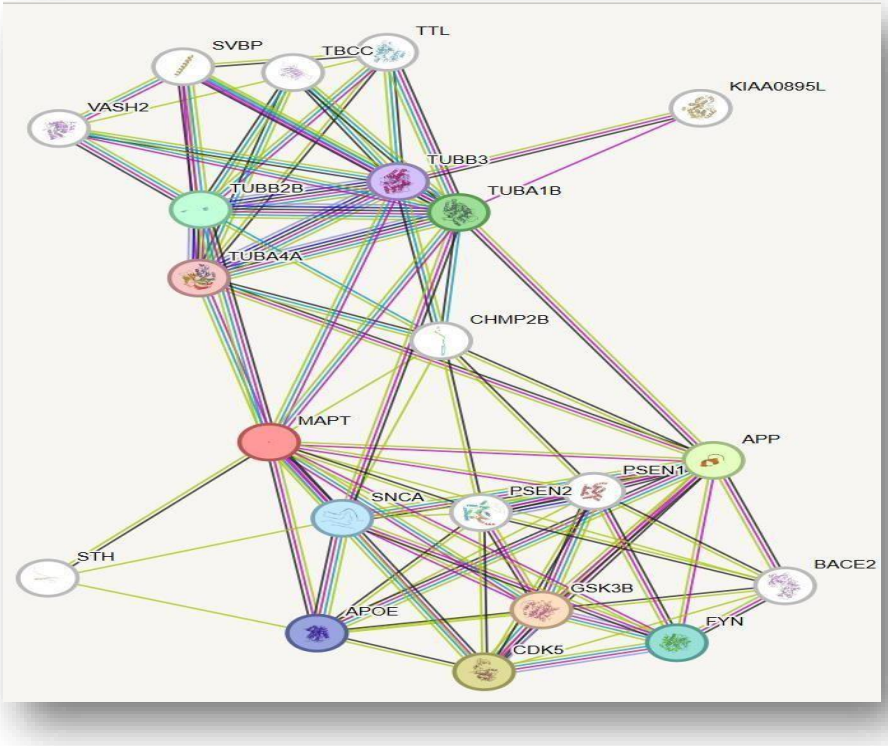


Figure15-Figure Showing Tau Interaction Partners Via String Database

5.2.1 Table 1- Tau Interaction Partners and Their Functions

S.No	Interaction Partner	Function
1	FYN	Tyrosine-protein kinase Fyn; Non-receptor tyrosine-protein kinase that plays a role in many biological processes including regulation of cell growth and survival, cell adhesion, integrin-mediated signaling, cytoskeletal remodeling, cell motility, immune response and axon guidance.
2	Cdk5	Cyclin-dependent-like kinase 5; Proline-directed serine/threonine-protein kinase essential for neuronal cell cycle arrest and

		differentiation and may be involved in apoptotic cell death in neuronal diseases by triggering abortive cell cycle re-entry. Interacts with D1 and D3-type G1 cyclins.
3	APP	On the surface of neurons, gamma-secretase C-terminal fragment 50 serves as a cell surface receptor and carries out physiological processes related to neurite development, neuronal adhesion, and axonogenesis. Synaptogenesis is facilitated by interactions between APP molecules on nearby cells. Via protein-protein interactions, involved in transcription control and cell motility
4	GSK3B	Glycogen synthase kinase-3 beta is a constitutively active protein kinase that phosphorylates and inactivates glycogen synthase, working as a negative regulator of the hormonal regulation of glucose homeostasis[45], [46]Wnt signaling, transcription factor and microtubule regulation.
5	APOE	Apolipoprotein E, often known as APOE, is a protein linked to lipid particles that primarily participates in lipoprotein-mediated lipid transfer between organs through interstitial fluids and plasma. A key element of plasma lipoproteins, APOE plays a role in their synthesis, conversion, and removal.
6	SNCA	Alpha-synuclein is a neuronal protein that regulates synaptic vesicle trafficking and the consequent release of neurotransmitters, among other functions in synaptic activity. Enhances vesicle priming, fusion, and exocytotic fusion pore dilatation to participate as a monomer in synaptic vesicle exocytosis.
7	MAPT	Tau is a microtubule-associated protein that facilitates microtubule stability and assembly and may have a role in establishing and maintaining neuronal polarity.[14],[20]
8	TUBA1B	The main component of microtubules is tubulin, which is a detyrosinated tubulin alpha-1B chain. It binds two moles of GTP, one at the alpha chain's non-exchangeable site and one at the beta chain's exchangeable site.
9	TUB2B	The main component of microtubules is tubulin beta-2B chain. is essential for appropriate axon guidance in the peripheral and central axon tracts. a member of the tubulin family and implicated in neuronal migration.
10	TUBB3	The main component of microtubules is tubulin beta-3 chain. For appropriate axon guidance and maintenance, TUBB3 is essential.
11	TUBA-4A	The main component of microtubules is the tubulin alpha-4A chain. It is necessary for microtubule polymerization because it binds GTP at certain locations.

12	PSEN1	A catalytic component of the gamma-secretase complex, the presenilin-1 CTF subunit is in charge of intramembrane cleavage of proteins including APP and Notch receptors.
13	PSEN2	Presenilin-2 is a likely catalytic member of the gamma-secretase complex that cleaves integral membrane proteins like APP intramembrane.
14	BACE-2	Beta-secretase 2 is in charge of APP's proteolytic processing. Releases beta-cleaved soluble APP after cleaving APP.
15	CHMP-2B	. The core of the ESCRT-III complex, charged multivesicular body protein 2b, is involved in the creation of multivesicular bodies and the sorting of endosomal cargo.
16	STH	Saitohin is a tiny, human-specific gene that is found in the tau gene (MAPT) region. Its biological function is still being studied.
17	VASH-2	By removing the C-terminal tyrosine from alpha-tubulin, tubulin-Tyr carboxypeptidase 2 controls spindle activity, chromosomal segregation, and microtubule dynamics.
18	SBVP	Little vasohibin-binding protein increases the activity of VASH1 and VASH2, which are essential for mitotic spindle control and microtubule detyrosination.
19	TBCC	The tubulin-specific chaperone C ensures appropriate tubulin heterodimer construction by participating in the last stage of the tubulin folding process. .
20	TTL	Tubulin–tyrosine ligase: Enables detyrosinated alpha-tubulin to have a tyrosine added to its C-terminal.
21	KIAA0895 like	KIAA0895-like protein; Encoded by the KIAA0895L gene. Part of an initially uncharacterized group of genes from large-scale cDNA projects.

5.3(a)Structural Analysis Of Tau

Figure16-Analysis Of Tau Sequence Of Human Via Scan PROSITEWith Discovery Studio

Observations-Comparison of structural results of the tau protein via PDB and Scan PROSITEThe PDB motif is found in the ScanPROSITE sequence from 584 to 629 position

5.3(b)Consurf Results[30]

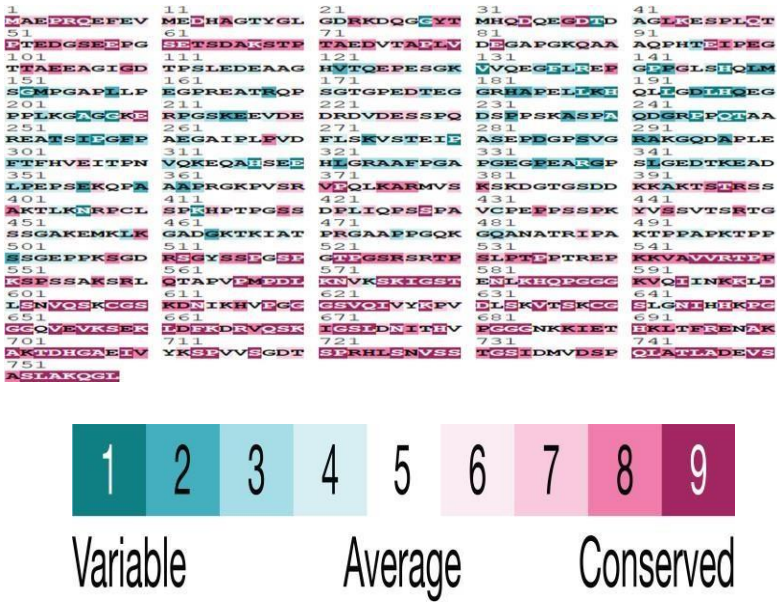


Figure17-Figure Showing Sequence Results Via Consurf of Olive Baboon and Human Respectively

Observation

Olive Baboon and Human: Thirteen sequence variations and a conservation rate of over 70% in functionally relevant locations were revealed by this analysis. Even though the majority of the protein is conserved, the variations found need to be experimentally verified because they may be responsible for minute functional or regulatory differences.

5.3` GO Term Analysis Via STRING

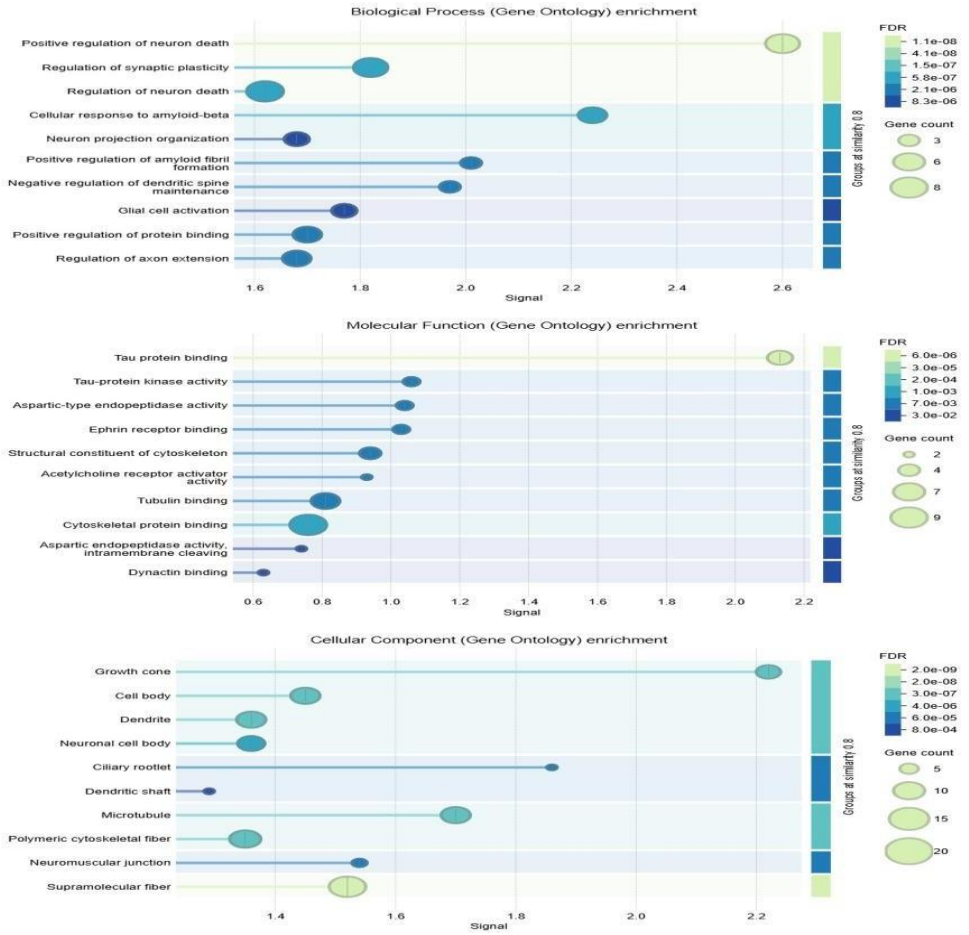


Figure18-GO Term Analysis Using String Graph

Observation**: The higher the FDR less significant the results.**

Discussion-Positive control of neuron death, modulation of synaptic plasticity, and cellular response to amyloid-beta all showed significant enrichment signals (e.g., FDR = 1.1e-08) in the Biological Process category, and up to eight genes were involved. Gene numbers 2 through 9 were shown to be enriched in tau-related activities, including tau protein binding and tau-protein kinase activity (FDR = 6.0e-06), according to molecular function. In the Cellular Component category, up to 20 genes were found to be strongly associated with structural neuronal components, including growth cone, polymeric cytoskeletal fiber, and supramolecular fiber (FDR = 2.0e-09). All of these findings highlight how tau affects synaptic structure, cytoskeletal dynamics, and neuronal death—three key aspects of Alzheimer's disease pathophysiology

5.4 Disorder Regions

5.4.1 Disorder Analysis Using PONDR[43]

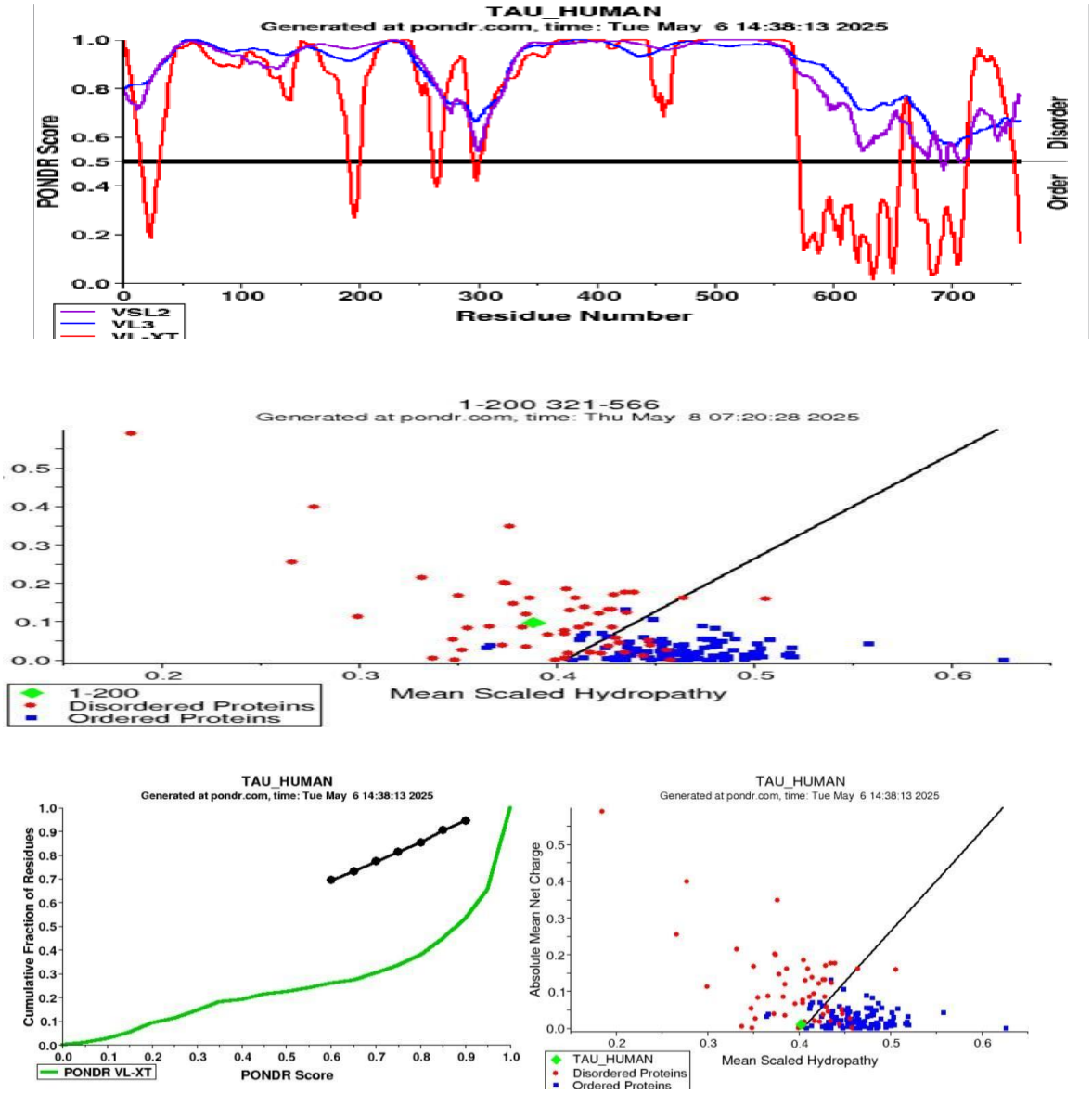


Figure 19-Figure Showing Disorder and Hydropathy Plot at Different Positions

a) Left plot-PONDR VL-XT

Interpretation: Because so many of the Tau protein's residues are over 0.5, there are chaotic areas in the protein. In physiological situations, these regions usually don't have a consistent three-dimensional fold and attach to signal proteins or scaffolding.

b) Right Plot-Charge-Hydropathy Plot Interpretation the disorder side, although not far from the line, the tau protein is located at the border. According to this, Tau is an inherently

disordered protein (IDP), although it may also have partial order, or regions that fold when bound or in specific circumstances.

Observation

Analysis of Disorder Prediction for Human Tau -

All predictors show that most of the Tau protein sequence is larger than 0.5. In particular, all lines stay substantially above the 0.5 threshold between residues ~50–450, confirming lengthy intrinsically disordered regions (IDRs).

Regions of Short Order- A few dips below 0.5, especially in the VL-XT line (e.g., at residues ~250, ~350, ~600–700), indicate brief segments that fold upon binding or are momentarily ordered. The VSL2 and VL3 predictors indicate large areas of disorder across the protein, particularly in the middle and N-terminal regions. Although transiently ordered segments are reflected by localized minima smaller than 0.5 in the VL-XT predictor,[33] these regions may be molecular recognition features (MoRFs) that acquire structure upon interaction. Tau's remarkable structural flexibility, which is necessary for its multifunctionality—including microtubule association and involvement in neurodegenerative disease pathologies—is shown by the overall disorder profile. Comparing the hydropathy Plots at different positions. Now the threshold of hydropathy decreased for human tau as the range of hydropathy analysis have changed which are strongly disordered region.[22]

5.4.2 IUpred 2a Results

Table- Table Showing Disordered Regions in 15 Homologs Of Human Tau[42]

Protein	Start Position	End Position
Human	1	196
Human	198	266
Human	270	321
Human	323	566
Human	571	742
Bonobo	1	196
Bonobo	198	266
Bonobo	270	321
Bonobo	323	566

Bonobo	571	742
Chimpanzee	1	196
Chimpanzee	198	266
Chimpanzee	270	321
Chimpanzee	323	566
Chimpanzee	571	736
Gorilla	1	196
Gorilla	198	266
Gorilla	270	321
Gorilla	323	566
Gorilla	571	760
Borenean Orangotan	1	196
Borenean Orangotan	198	266
Borenean Orangotan	270	321
Borenean Orangotan	323	566
Borenean Orangotan	571	747
Sumatran Orangotan	1	196
Sumatran Orangotan	198	266
Sumatran Orangotan	270	321
Sumatran Orangotan	323	566
Sumatran Orangotan	571	741
Siamang Isoform3	1	196
Siamang Isoform3	198	266
Siamang Isoform3	270	321
Siamang Isoform3	323	566
Siamang Isoform3	571	743
Siamang Isoform4	1	196
Siamang Isoform4	198	266
Siamang Isoform4	270	321
Siamang Isoform4	323	566
Siamang Isoform4	571	743

Macque	1	196
Macque	198	266
Macque	270	321
Macque	323	566
Macque	571	743
Olive Baboon	1	730
Bleeding Heart Monkey	1	744
Colobus	1	740
Leaf Monkey	1	700

Observation

The table shows that most primates (Humans, Bonobo, chimpanzees, gorillas, Orangutans, and Siamang) have the same disordered region pattern, divided into five segments. However, Olive Baboon, Bleeding Heart Monkey, Colobus, and Leaf Monkey have a single continuous disordered region from 1-730 meaning they exhibit greater overall disorder because the variation is less. The red IUPred2 curve stays above 0.5 for most of the sequence, indicating disorderThe strongly disordered regions match well with the table's predictions (especially between 1-196 and 571-745). The regions between 617 and 638, 661 and 675, 701 and 720, and 700 to 758 are lower than the threshold.

5.4.3 Disorder Analysis Via SLIDER

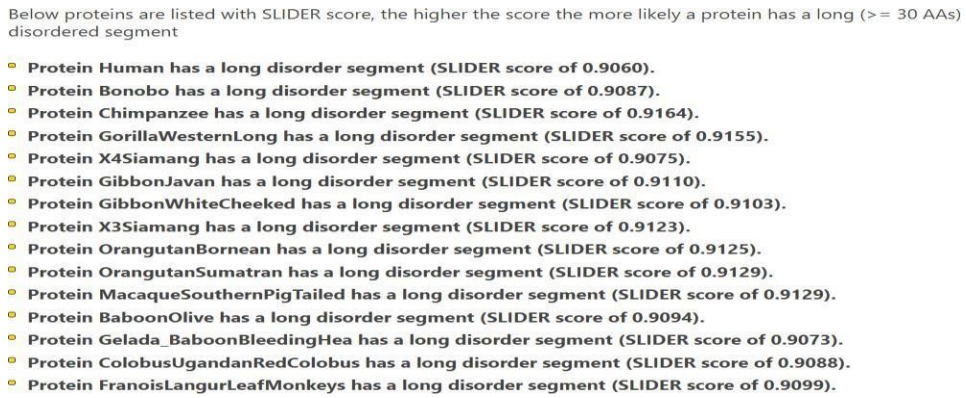


Figure20- Figure Showing SLIDER Prediction

Observation-Comparing human protein with a SLIDER score of 0.9060, the human protein has the lowest score among the other species. The chimpanzee protein, on the other hand, has the highest SLIDER score (0.9164), followed closely by GorillaWesternLong (0.9155) and several other great apes and monkeys, such as Orangutan Sumatran (0.9129) and X3Siamang (0.9123). It's interesting to note that longer disordered protein segments are more likely to be carried by Bonobo (0.9087) and Colobus Uganda Red Colobus (0.9088), two of the most human-like animals with scores.

75.5(a) Phosphorylation Analysis

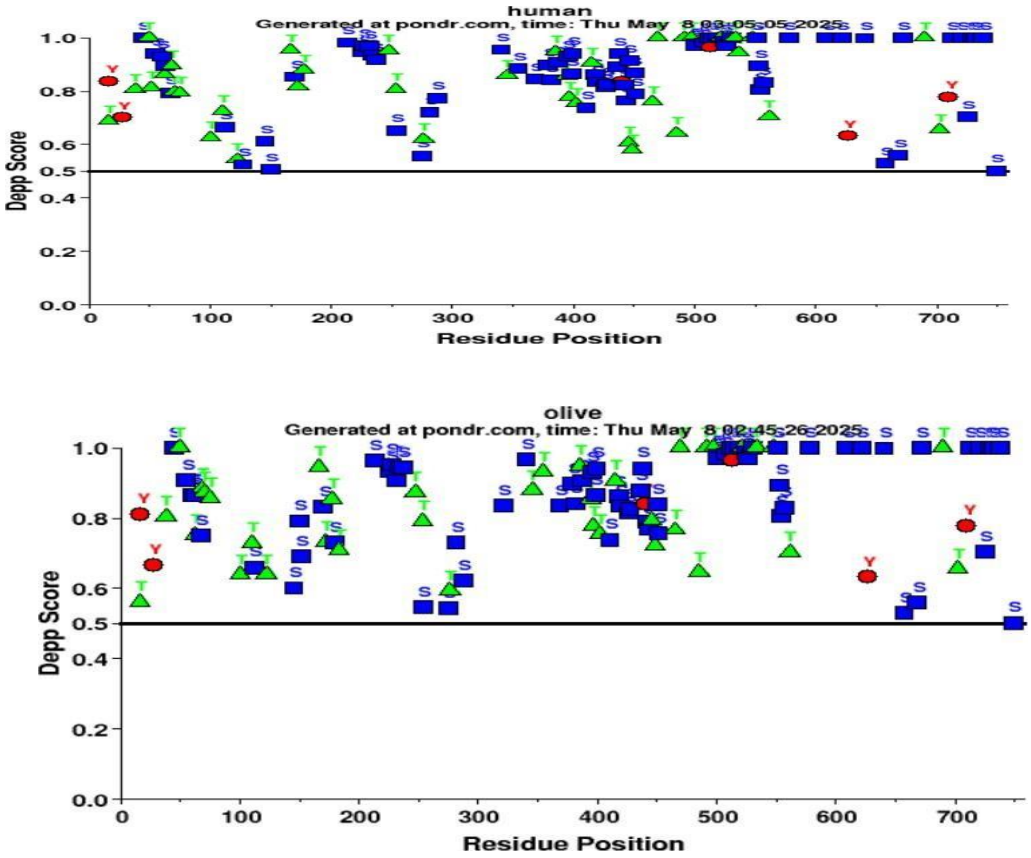


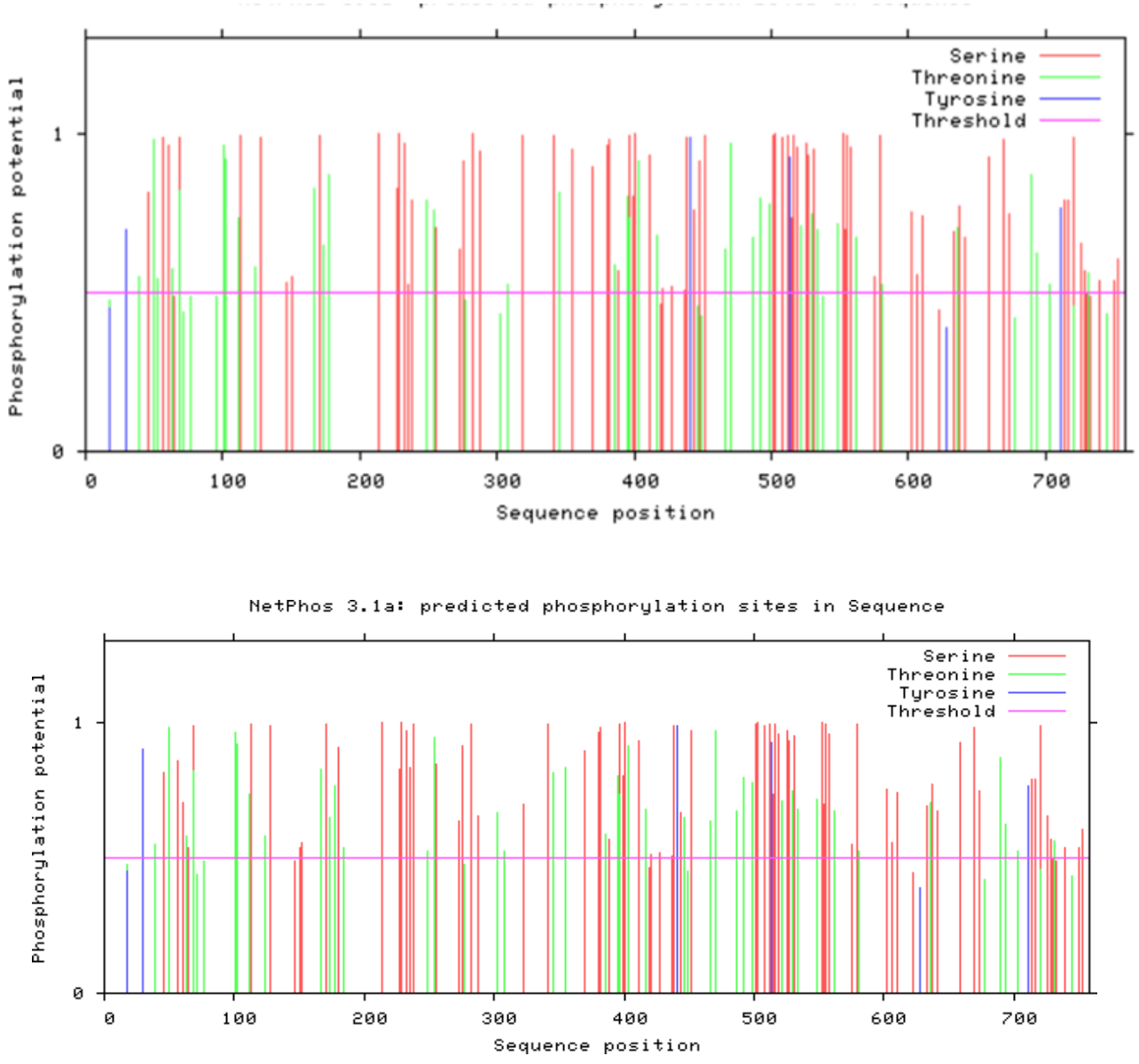
Figure 21- Figure Showing DEPP Disorder In Human And Olive Baboon Respectively

Observation And Discussion

Most density is 100-200, 350-500,600-700 in humans whereas in olive baboons less density is 300-600. More tyrosine sites in humans as compared to baboon and olive baboons show high DEPP scores.

Discussion- Olive baboon has longer disordered segments and a larger number of phosphorylation residues, might resemble some similar amino acid similarity and might have local residues related to phosphorylation, and may have similarity to validated phosphorylation regions explaining high DEPP score in key regions.[30]

5.5(b)Phosphorylation Analysis Via Net Phos



[41] Figure 22- Figure Showing Phosphorylation Via Net Phosphorylation Analysis

Although the two NetPhos 3.1a graphs show similar overall patterns of predicted phosphorylation, there are some notable differences in the number of sites that cross the threshold: the first graph(human) shows a slightly higher number of phosphorylation sites,

particularly for serine residues between positions 200 and 350 and threonine residues in the 100–200 and 400–600 ranges, while the second graph(olive baboon) shows fewer sites crossing the phosphorylation threshold, probably as a result of slight shifts in prediction scores. These differences imply that the first sequence may have a slightly higher phosphorylation potential overall. Serine and Theorinine sites are more than tyrosine as it is more into enzymatic functions.

Table3- Most Common Kinases

Kinase	Function in Alzheimer's Disease
GSK3 (Glycogen Synthase Kinase 3)	Promotes tau hyperphosphorylation, leading to neurofibrillary tangles and neuronal death.
Cdk5 (Cyclin-Dependent Kinase 5)	Involved in tau phosphorylation, neuronal stress, and synaptic dysfunction.
PKC (Protein Kinase C)	Regulates synaptic plasticity and neurotransmitter release; its dysfunction leads to cognitive decline.
CKI (Casein Kinase I)	Modulates circadian rhythms and contributes to tau phosphorylation.
CKII (Casein Kinase II)	Involved in DNA repair and cellular signaling, which are disrupted in Alzheimer's.
p38MAPK (p38 Mitogen-Activated Protein Kinase)	Activated by oxidative stress and inflammation, leading to neuronal damage.
PKA (Protein Kinase A)	Regulates synaptic transmission and memory; its dysregulation contributes to cognitive impairment.
ATM (Ataxia Telangiectasia Mutated Kinase)	Plays a role in DNA damage response; its dysfunction accelerates neurodegeneration.

*Humans have the most unspecified kinases which might be due to structural, biological, and cellular complexity

Most Presented GO terms In Tau Interaction Partners-

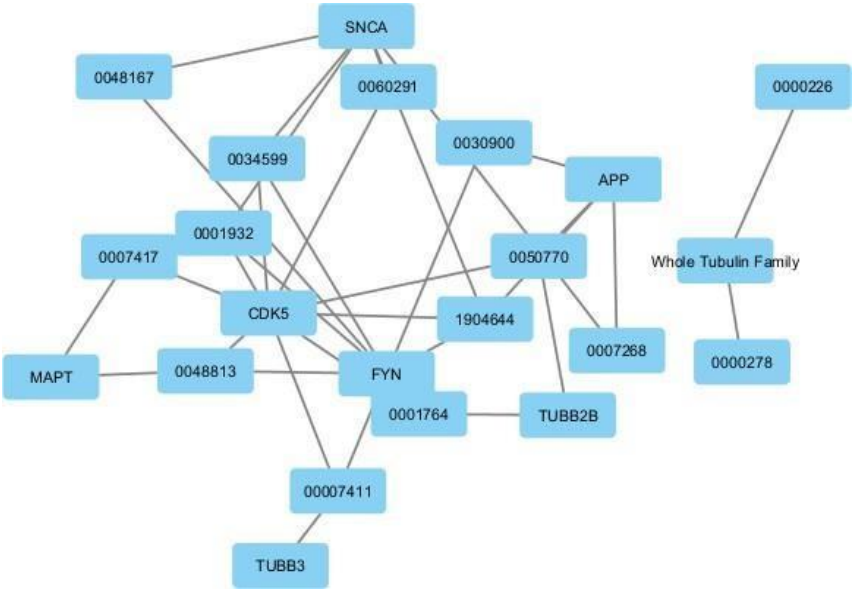
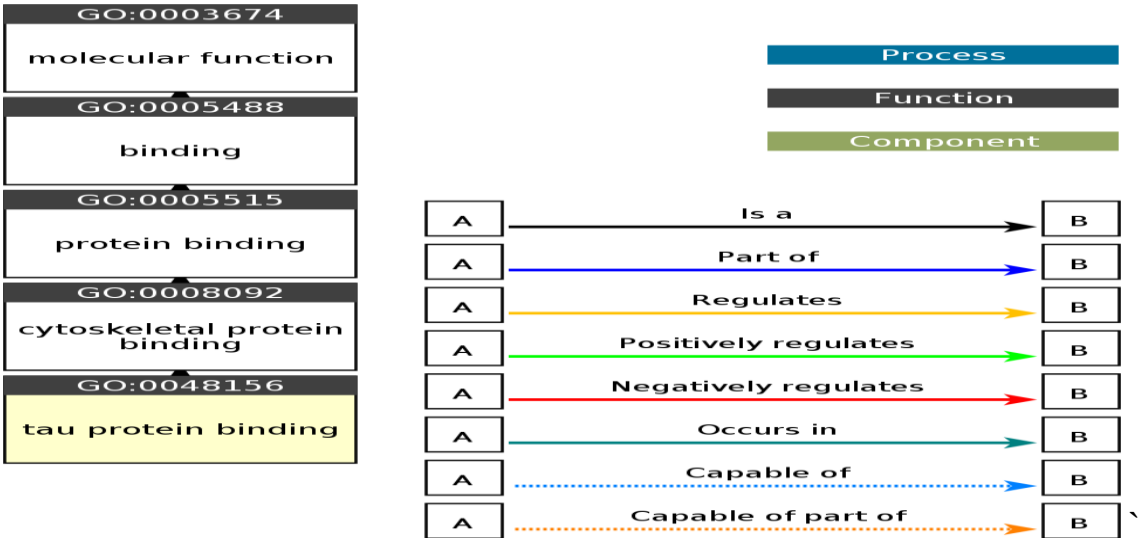


Figure23-Figure Showing Cytoscape Network Showing for Most Presented Term With Maximum Interaction Partners.

Observation

GO:0001764 namely **neuron migration** is present sufficiently but not highly Enriched. This proves that an overrepresented term might not be one of the driver terms.

5.6 GO Annotation Analysis



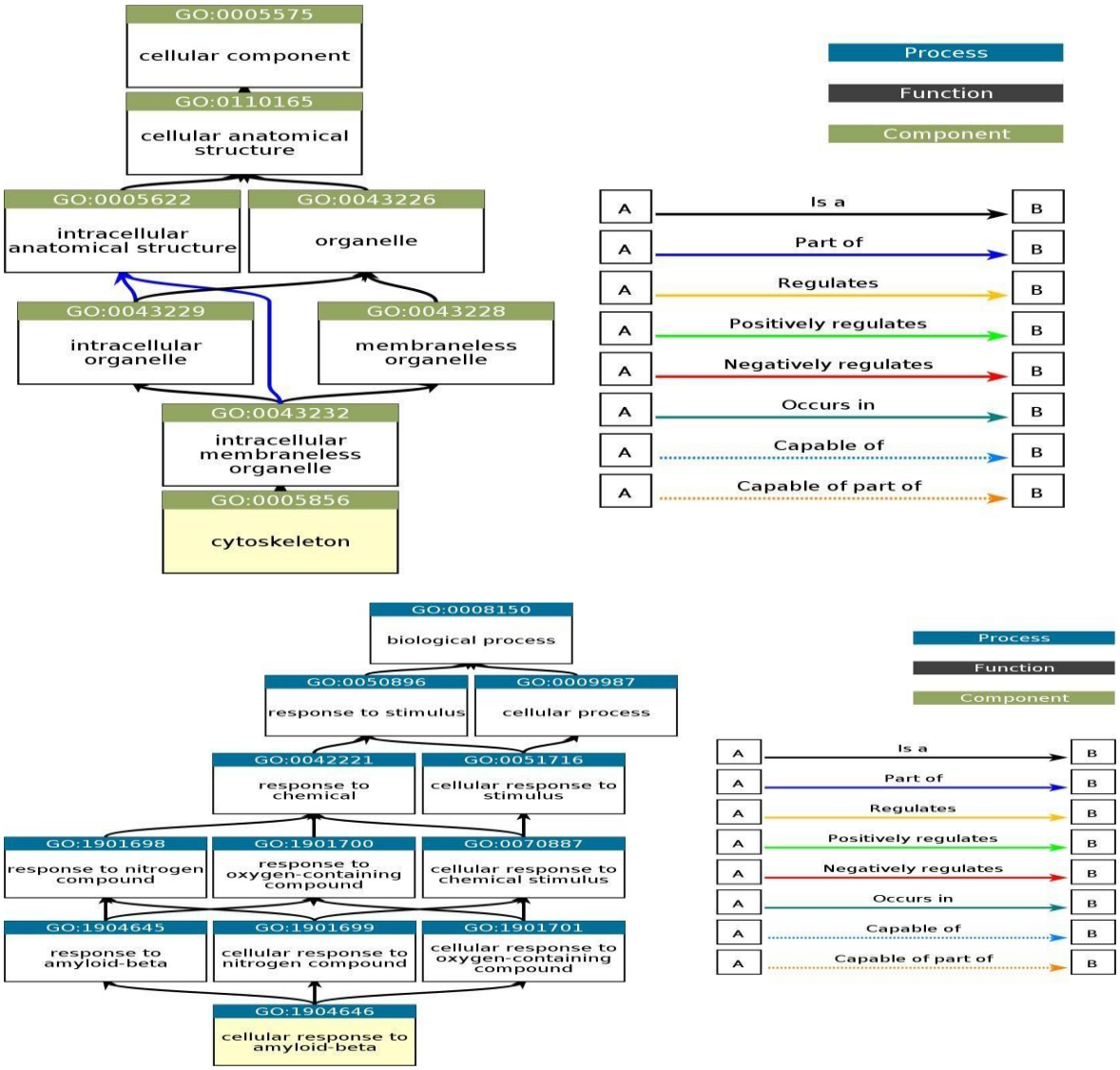


Figure24-Figure Showing Ancestor Charts For Most Significant GO Terms.

*B is a term that is in a certain relationship with A which is a GO term.

Discussion

The images illustrate the hierarchical connection between the two main GO keywords, "cytoskeleton" (GO:0005856) and "cellular response to amyloid-beta" (GO:1904646). A subclass of more encompassing categories such as reaction to stimuli, chemical response, and cellular process, GO:1904646 is a part of the ontology of biological processes. It explains the distinct way in which a cell reacts to the amyloid-beta peptide, a substance linked to neurodegenerative illnesses. GO:1904646 is a kind of reaction to molecules that includes all diseases. The cytoskeleton, GO:0005856, is included in the ontology of cellular components. Its structural role within the cell is indicated by its designation as an intracellular membrane-less organelle, which denotes that it is not membrane-bound.

5.7.1 g Profiler GO Term Enrichment

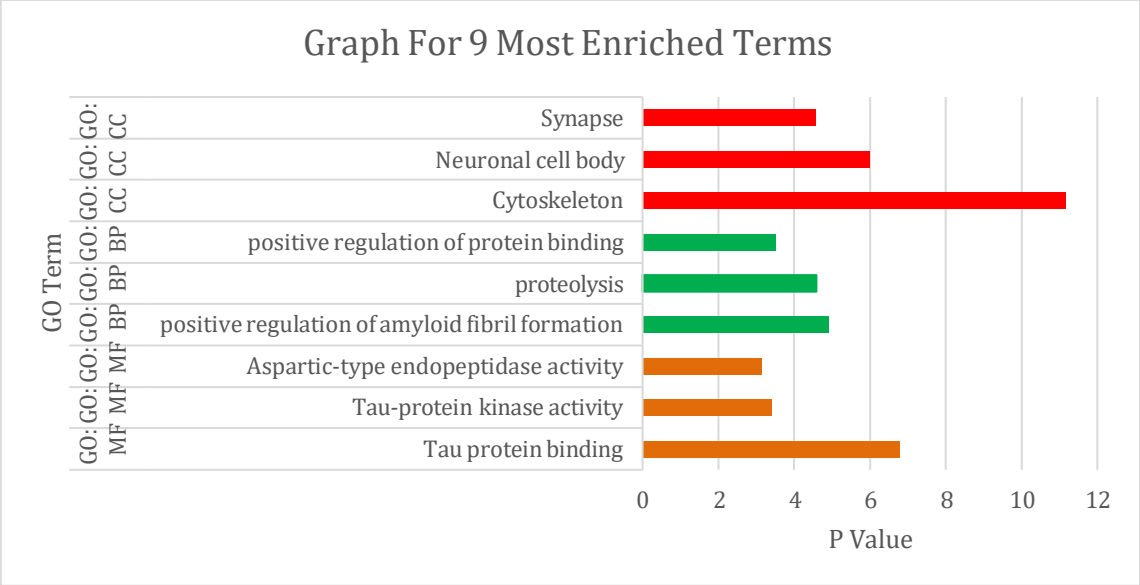


Figure25: Figure Showing Graph Representing Top 9 terms via G Profiler

Discussion

Using g: Profiler, a functional enrichment analysis was conducted on a list of twenty human genes. The research showed a notable enrichment in molecular activities associated with protein binding and microtubule dynamics. Notable enriched phrases were aspartic-type endopeptidase activity, tau-protein kinase activity, and tau protein binding (adjusted $p = 1.6 \times 10^{-7}$). Additional noteworthy roles included proteins serine/threonine kinase activity, heat shock protein binding, and cytoskeletal structure, all of which suggested participation in stress response or neurodegenerative pathways. These findings point to the importance of protein folding and cytoskeletal systems in the biological processes controlled by the input gene set.Using g: profiler for functional enrichment analysis: Several strongly enriched GO: BP phrases were found by the profiler. The processes that were ranked highest were the organization of microtubule cytoskeletons, the organization of neurofilaments, and the control of amyloid fibril production. These processes suggest involvement in both neurodegenerative processes and the preservation of neuronal structure. Roles in tau-protein kinase activity positive regulation and axon growth are also emphasized. The most enriched terms for the Gene Ontology Cellular Component (GO: CC) category were tau-protein kinase complex, neurofibrillary tangle, axon, and microtubule cytoskeleton; these terms were strongly localized to cytoskeletal and neuronal compartments, confirming the biological significance of the gene,

list to neurodegenerative diseases such as Alzheimer's disease. The association with intracellular aggregation and protein misfolding is further supported by the prevalence of terminology like inclusion body and perinuclear area of cytoplasm. These enrichment results often show a strong structural and functional relationship to tau pathology, cytoskeletal dynamics, and neuronal processes—all of which are important indicators of neurodegeneration.

GO:MF

Term name	Term ID	p_{adj}
tau protein binding	GO:0048156	1.588×10^{-7}
tau-protein kinase activity	GO:0050321	3.853×10^{-4}
aspartic-type endopeptidase activity	GO:0004190	7.294×10^{-4}
tubulin-tyrosine carboxypeptidase	GO:0106423	8.517×10^{-4}
structural constituent of cytoskeleton	GO:0005200	1.281×10^{-3}
carbohydrate derivative binding	GO:0097367	6.512×10^{-3}
catalytic activity	GO:0003824	6.538×10^{-3}
tubulin binding	GO:0015631	9.621×10^{-3}

1 to 8 of 8 Page 1 of 1

GO:BP

Term name	Term ID	p_{adj}
cellular response to amyloid-beta	GO:1904646	3.603×10^{-7}
modulation of chemical synaptic transmission	GO:0050804	4.080×10^{-7}
regulation of synapse organization	GO:0050807	2.708×10^{-6}
positive regulation of amyloid fibril formation	GO:1905908	1.187×10^{-5}
proteolysis	GO:0006508	2.588×10^{-5}
positive regulation of protein binding	GO:0032092	2.950×10^{-4}
cognition	GO:0050890	6.678×10^{-4}
receptor metabolic process	GO:0043112	1.003×10^{-3}
smooth endoplasmic reticulum calcium ion homeostasis	GO:0051563	1.388×10^{-3}
regulation of catalytic activity	GO:0050790	3.478×10^{-3}
astrocyte activation involved in immune response	GO:0002265	8.316×10^{-3}
neuron apoptotic process	GO:0051402	8.747×10^{-3}
cerebral cortex development	GO:0021987	1.011×10^{-2}
regulation of generation of precursor metabolites and energy	GO:0043467	1.245×10^{-2}
positive regulation of small molecule metabolic process	GO:0062013	1.282×10^{-2}
regulation of postsynaptic membrane potential	GO:0060078	1.690×10^{-2}
mitotic cell cycle	GO:0000278	1.933×10^{-2}
glial cell activation	GO:0061900	3.021×10^{-2}
regulation of protein catabolic process	GO:0042176	3.397×10^{-2}
regulation of carbohydrate metabolic process	GO:0006109	3.574×10^{-2}
neuron cellular homeostasis	GO:0070050	4.551×10^{-2}
response to oxidative stress	GO:0006979	4.697×10^{-2}

1 to 22 of 22 Page 1 of 1

GO:CC

Term name	Term ID	p_{adj}
cytoskeleton	GO:0005856	6.685×10^{-12}
neuronal cell body	GO:0043025	1.030×10^{-6}
synapse	GO:0045202	2.705×10^{-5}
gamma-secretase complex	GO:0070765	2.616×10^{-3}
endosome	GO:0005768	4.352×10^{-3}
perinuclear region of cytoplasm	GO:0048471	5.605×10^{-3}
membrane raft	GO:0045121	1.937×10^{-2}

1 to 7 of 7 Page 1 of 1

Figure 26- Figure Showing g Profiler Graph

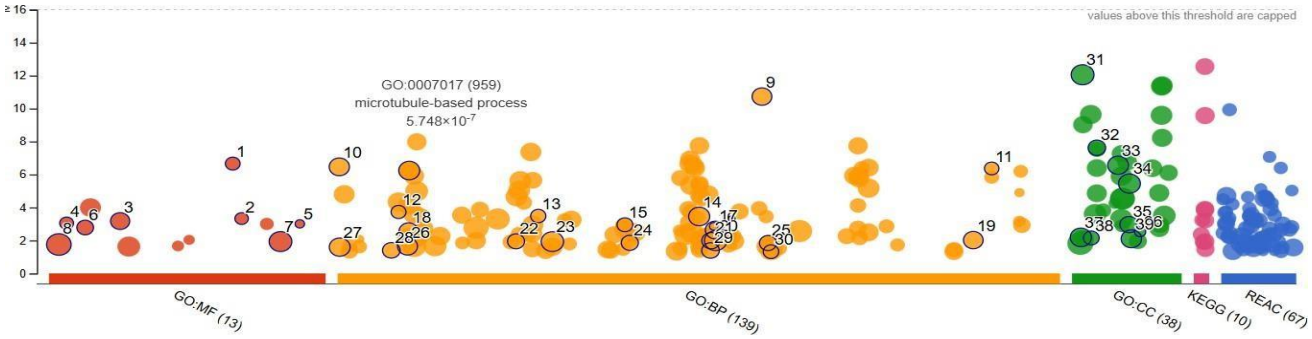


Figure27- Figure Showing g Profiler Graph for KEGG and Reactome Analysis with Annotated Terms[44]

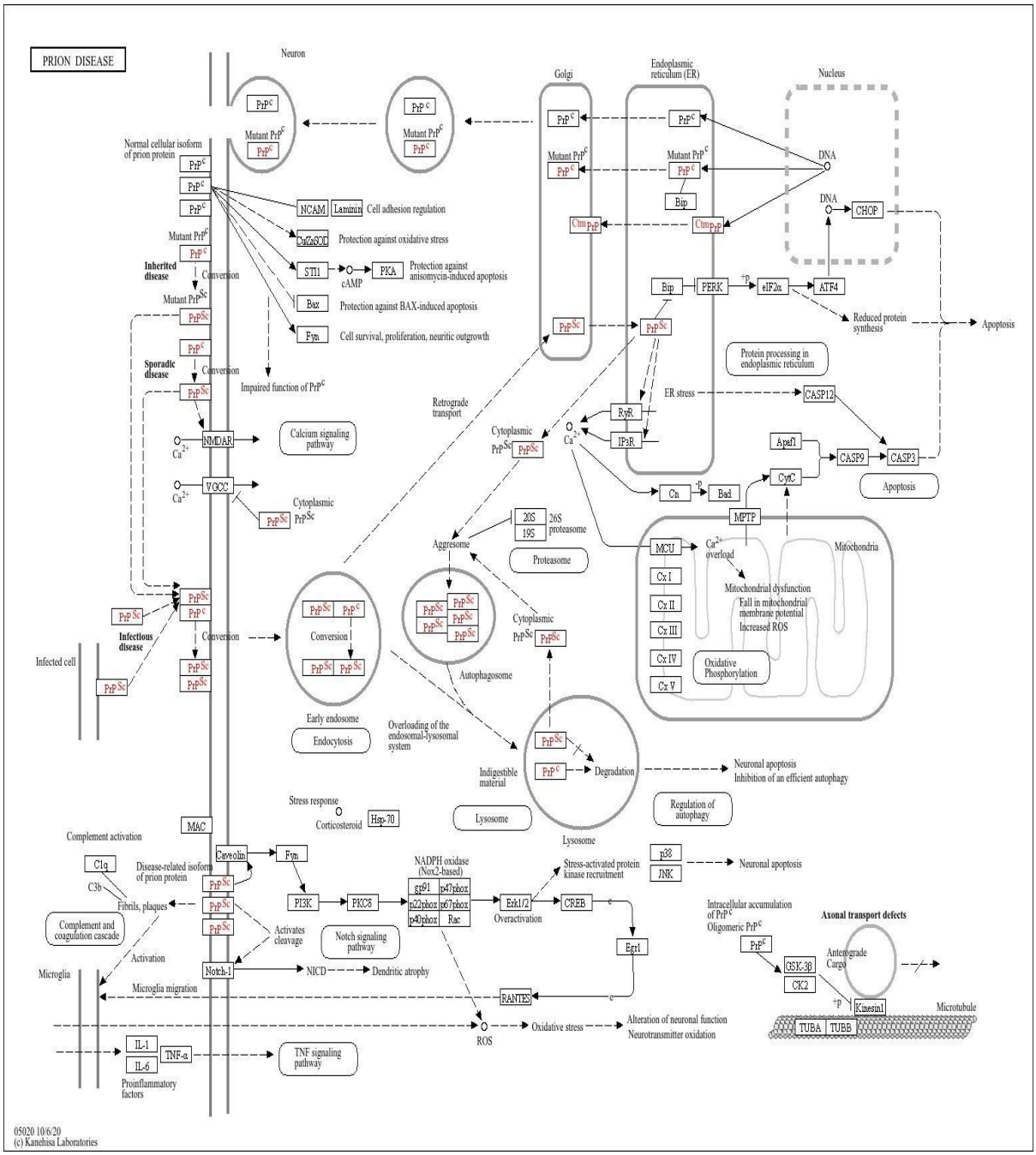
Discussion-Biological Interpretation: According to the enrichment profile, our gene sets are implicated in important pathways linked to Alzheimer's. A characteristic of AD neurofibrillary tangles, dysregulation of tau–microtubule interactions is shown by the strong enrichment of tau protein binding and microtubule/tubulin binding (MF terminology). Aβ-driven disease is suggested by enriched blood words such as cellular response to amyloid-beta, positive control of amyloid fibril production, and synapse-related processes: It is well recognized that oligomeric Aβ species cause synaptic disruption and are very toxic to neurons. Finally, these genes are implicated in neurodegenerative cell death processes based on enrichment of neuron apoptotic process and related keywords. All things considered, the results show that the genes being investigated form a common functional framework for AD and converge on processes that are important to the illness, including tau-mediated cytoskeletal degradation, amyloid beta response, synapse disintegration, and programmed neuronal death. The top 10 most significant terms were determined by the GO enrichment analysis under the headings of Molecular Function (MF), Biological Process (BP), and Cellular Component (CC). These biological topics are important to the dataset. The highest enriched element in the CC category was the cytoskeleton (~11 -log10(Padj)), underscoring the significance of cytoskeletal components for signaling and neural stability. Genes involved in brain architecture may have been enriched, as evidenced by the high features of synapses and the cell body of neurons. The words "cellular response to amyloid-beta," "regulation of synapse architecture," and "modulation of chemical synaptic transmission" all allude to close ties between amyloid-related processes and synaptic function. These pathways are well-established in neurodegenerative illnesses, including Alzheimer's disease. All things considered, the results emphasize mechanisms central to neurodegeneration, including amyloid/tau dynamics, synapse regulation, and cytoskeletal homeostasis. To learn more about their molecular activities, these findings merit further

validation. KEGG correlates to 5 disease-related histories namely Huntington's disease and Gap junction etc whereas Reactome has 35-36 related genes.

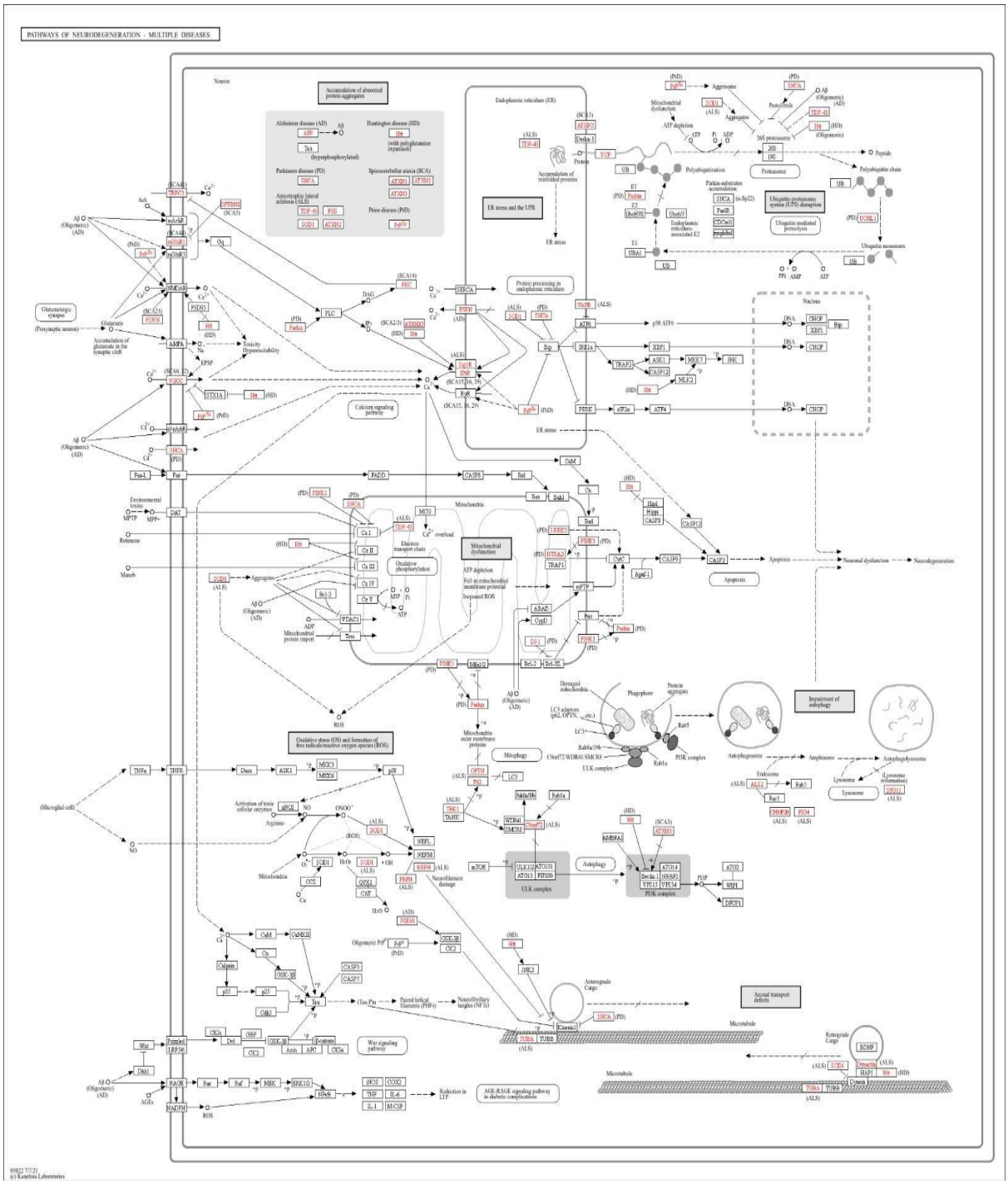
5.7.2 KEGG Analysis

a) Prion Disease-A set of deadly neurodegenerative illnesses that impact humans and several other animal species are known as prism diseases, or transmissible spongiform encephalopathies (TSEs). It is believed that the transformation of the normal protein PrPC into the infectious, pathogenic form PrPSc is linked to the genesis of various disorders. It is believed that the conversion happens after PrPC has reached the plasma membrane or is re-internalized for degradation. It can be caused by prion infections (e.g., variant Creutzfeldt-Jakob disease (vCJD), iatrogenic CJD, Kuru), mutations (familial CJD, Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia (FFI), or unknown factors (sporadic CJD, sCJD). Compared to PrPC, the PrPSc form has higher protease resistance and builds up in afflicted people, frequently manifesting as extracellular plaques.

b) Multiple Nuerogenerative Disorder-Progressive, irreversible neuronal loss is commonly referred to as neurodegeneration, and it can impact the central nervous system (CNS) or the peripheral nervous system. Neurodegenerative diseases (NDs) comprise very incapacitating conditions such as prion diseases (PrD), amyotrophic lateral sclerosis, Huntington's disease, Alzheimer's disease (AD)[47], Parkinson's disease (PD), and spinocerebellar ataxias. The steady buildup of misfolded protein aggregates is the signature event that is believed to be the cause of many disorders. Abnormal protein dynamics resulting from ubiquitin-proteosome-autophagy system deficiencies, oxidative stress and the production of free radicals, endoplasmic reticulum stress, mitochondrial dysfunction, and (secondary) abnormalities in axonal transport are some of the major fundamental processes. Axonal transport is one of the major fundamental processes.



KEGG PATHWAY: Prion disease - Reference pathway



KEGG PATHWAY: Pathways of neurodegeneration - multiple diseases - Reference pathway

Figure 28- Figure Showing Disease Pathways For Prion And Multiple Neurodegenerative Disorders Via KEGG Respectively

Table4-Table Showing Game Changers For GO Term Analysis

GO Category	**Term Name**	**GO ID**
GO: MF	Tau protein binding	GO:0048156
GO: MF	Tau-protein kinase activity	GO:0050321
GO: MF	Aspartic-type endopeptidase activity	GO:0004190
GO: BP	Positive regulation of amyloid fibril formation	GO:1904646
GO: BP	Proteolysis	GO:0050804
GO: BP	Positive regulation of protein binding	GO:0050808
GO: CC	Cytoskeleton	GO:0005856
GO: CC	Neuronal cell body	GO:0043025
GO: CC	Synapse	GO:0045202

*For Disorder regions residues 1-200 are very disordered in nearly all species. For phosphorylation analysis, Serine and threonine are more present than tyrosine, as it is more involved in enzymatic reactions.

Chapter 6

Conclusion

Although phosphorylating alone may not distinguish healthy from diseased tau. Post-translational modifications (PTMs) like acetylation or ubiquitination, and their regulation, likely influence Tau's interactions, function, and aggregation in neurons. To better understand toxic Tau species, combining advanced sample preparation with high-sensitivity mass spectrometry can reveal the composition and stoichiometry of soluble Tau oligomers formed before fibrillary entanglements. Identifying the PTM signatures of these oligomers could help pinpoint early therapeutic targets. Clinical criteria for Alzheimer's diagnosis require dementia, which delays preclinical detection. Sensitive assays to identify amyloid oligomers in the brain and cerebrospinal fluid might facilitate early detection. However, tau phosphorylation levels alone are not enough to distinguish between healthy and disease-associated tau. This protein keeps changing and mutating continuously and has a wide number of hydrophilic residues and the ambiguity is not the same at every level.

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