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Next Genration Sequencing Data Analysis of A25T Transthyretin Structure Associated with Parkinson's Disease

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Abstract: Parkinson's disease (PD) is a complex neurodegenerative disorder, with Leucine-Rich Repeat Kinase 2 (LRRK2) playing a pivotal role in its pathology. This study integrates Next-Generation Sequencing (NGS) and structural bioinformatics to investigate the A25T Transthyretin crystal structure of LRRK2, aiming to elucidate its functional and evolutionary significance. A comprehensive computational approach incorporating RasWin, PyMOL (B-factor analysis), InterProScan, Ramachandran plot assessment, STRING, Prosite, BLAST, and ERRAT was employed to assess protein stability, structural integrity, conserved motifs, gene ontology, and functional interactions. BLAST analysis identified conserved regions, while InterProScan and Prosite provided insights into domain architecture, homologous superfamilies, and functionally relevant binding sites. B-factor analysis highlighted structurally dynamic regions, whereas Ramachandran plot validation confirmed stereochemical accuracy. STRING analysis, including the A25T transthyretin variant, identified key protein-protein interactions and gene ontology associations, suggesting a potential link between LRRK2 and neurodegenerative pathways. ERRAT quality assessment further reinforced the structural reliability of 6TXV. Furthermore, Multiple Sequence Alignment (MSA) revealed conserved regions across homologous sequences, shedding light on evolutionary relationships and functionally critical domains relevant to LRRK2's role in PD. These findings enhance our understanding of LRRK2's molecular function in PD pathogenesis and provide a foundation for targeted drug discovery and therapeutic interventions.

Keywords: Parkinson's Disease, LRRK2, NGS, Structural Analysis, A25T Transthyretin, Sequence analysis, Prosite, MSA

Introduction:

Neurodegenerative diseases represent a diverse array of conditions characterized by the progressive degeneration of neuronal structures. These disorders pose significant challenges to healthcare systems globally, mainly due to their increasing prevalence with aging populations. Among these, Parkinson's disease (PD) is one of the most common, motivating extensive research into its molecular mechanisms and potential therapeutic targets [1, 2]. This disorder is primarily defined by the degeneration of dopaminergic neurons in the substantia nigra, leading to classic motor symptoms such as bradykinesia, rigidity, and tremors [3, 4]. The multifactorial etiology of Parkinson's disease encompasses both genetic and environmental influences, with numerous risk factors identified ranging from exposure to pesticides to genetic predisposition [5, 6].

Recent advances in genomic research, particularly next-generation sequencing (NGS), have greatly enhanced our understanding of the molecular underpinnings of neurodegenerative diseases, including PD. By enabling detailed characterization of the genetic variants associated with these conditions, NGS has facilitated the discovery of several genes implicated in PD, such as SNCA, PARK7, and PINK1 [7, 8]. This high-throughput sequencing technology not only aids in identifying rare variants but also allows for a comprehensive exploration of the non-coding regions of the genome, which may harbor significant regulatory elements [9, 10]. Moreover, the integration of NGS data with bioinformatics tools can elucidate complex genetic interactions and pathways that contribute to PD pathogenesis, ultimately guiding the development of novel therapeutic strategies [11, 12].

In parallel with genetic approaches, understanding protein structure and function also plays a critical role in elucidating the mechanisms of Parkinson's disease. One particularly notable protein linked to PD is the 6TXV protein. This protein has been recognized as integral to the pathophysiology of PD, yet its structural characteristics remain inadequately explored [13]. Structural biology techniques, including X-ray crystallography and cryo-electron microscopy, can provide crucial insights into the three-dimensional architecture of the 6TXV protein, unveiling potential sites of interaction with other cellular components and identifying regions that may serve as targets for drug development [14, 15]. The elucidation of the 6TXV protein's structure could yield important information about its functional repertoire in dopaminergic signaling and its role in the aggregation processes that contribute to neurodegeneration [16, 17].

Deficiencies in autophagic processes and proteasomal degradation are hallmark features of neurodegenerative diseases, including PD, leading to the accumulation and aggregation of misfolded proteins [18, 19]. The aggregation of α-synuclein, a protein closely associated with PD, is particularly significant, as its misfolding can initiate toxic cascades resulting in neuronal death [20, 21]. Structural analysis of α-synuclein and related proteins is essential in developing strategies to inhibit their aggregation and enhance degradation pathways. Furthermore, targeting the molecular chaperones involved in the protein folding process may offer promising therapeutic avenues [22, 23].

Research into neurodegenerative diseases is seeing a push toward combinatorial approaches that incorporate both genetic insights from NGS and structural data. The interplay between genetic predispositions and protein misfolding mechanisms suggests a need for comprehensive models that address these interactions collectively [24]. Investigating the implications of genetic variations on protein structure-function relationships could provide vital connections between observed genetic alterations and their phenotypic expressions in Parkinson's disease [25, 26]. Such models can contribute to the development of targeted therapies that not only address symptomatic relief but also slow the underlying disease progress.

The integration of NGS technologies with cutting-edge structural biology principles forms a vital frontier in the quest to unravel the complexities of PD. Future research endeavours must aim to elucidate the interactions between the 6TXV protein and key pathological processes in neurodegeneration. This dual approach could yield valuable insights that pave the way for innovative treatment paradigms, ultimately addressing the profound impact of Parkinson's disease on affected individuals and society alike [27, 28, 29]. As we push forward in this research landscape, a concerted effort must be made to integrate findings across various disciplines, capitalizing on the depth and breadth of modern biotechnologies for a comprehensive understanding of Parkinson's disease at both the genetic and structural levels.

MATERIALS AND METHODOLOGY

The three-dimensional structure of the protein was retrieved from the Protein Data Bank (PDB) by accessing the database and downloading the structure in PDB format, which contains atomic coordinates essential for subsequent analyses. The molecular visualization and analysis were performed using RasMol, where the 6TXV PDB file was loaded into the application to explore its structure through interactive tools that enabled rotation, zooming, and panning. This facilitated a detailed examination of molecular surfaces and secondary structural elements, allowing for an analysis of various types of bonds present in the protein [30].

B-factor analysis was conducted using PyMOL by loading the 6TXV structure file with the fetch command. The visualization of B-factors was achieved by selecting the label tab and opting for B-factor representation. Regions with high B-factors were identified to determine areas exhibiting structural flexibility or disorder, which could be significant in understanding the protein's function. Functional annotation of the protein was carried out using InterProScan, where the amino acid sequence of 6TXV was submitted for analysis. The results were examined to identify functional domains and potential protein families, with significant findings summarized to highlight functional annotations related to the protein's biological role [31].

Ramachandran plot analysis was performed using PROCHECK available on the UCLA SAVES server. The structure of 6TXV was uploaded to assess the distribution of phi (ϕ) and psi (ψ) dihedral angles, which provided insights into the stereochemical quality of the protein. The plot was analyzed to detect outliers and evaluate any unusual conformations. Protein-protein interaction analysis and gene ontology (GO) annotations were conducted using the STRING database. The protein's name was entered into the STRING platform to visualize its interactions with other proteins. The resulting interaction network was examined, emphasizing direct and indirect associations and identifying key proteins within the network that may have biological relevance to 6TXV. The function tab was accessed to retrieve GO analysis results [32, 33].

Domain and motif identification were carried out using Prosite by submitting the amino acid sequence of 6TXV for analysis. The detected patterns and functional domains were examined to infer their significance concerning the protein's function and interaction capabilities. The structural quality of the protein was assessed using ERRAT by uploading the 6TXV PDB file to the UCLA SAVES server. ERRAT evaluated the non-bonded atomic interactions within the protein, providing a quality score and identifying potential structural concerns that needed further investigation [34].

Sequence similarity search was performed using BLAST, where the amino acid sequence of 6TXV was entered into the BLASTp program with appropriate search parameters. The results were analyzed to identify sequence similarities with other proteins, focusing on high-scoring alignments that could provide functional insights based on known homologs. Root Mean Square Deviation (RMSD) calculations were conducted in PyMOL by loading the original and target structures using the fetch command. The structures were superimposed using the align command, aligning them based on atomic positions. RMSD values were analyzed, with lower values indicating structural similarity and higher values suggesting conformational variations [31, 35,39].

Multiple Sequence Alignment (MSA) was performed by retrieving the amino acid sequence of 6TXV from the PDB or UniProt in FASTA format. Homologous sequences with high similarity to 6TXV were identified through a BLAST search on the NCBI website and downloaded in FASTA format. These sequences were submitted to Clustal Omega for alignment using default parameters, ensuring accurate handling of gaps and scoring. The alignment results were examined to identify conserved motifs and functional significance based on conserved regions and gaps [36,37,38,39]

Table 1: The summary of software and tools used in this study

Analysis Type	Software/Tool Used
Molecular Visualization	RasMol, PyMOL
B-factor Analysis	PyMOL
Functional Annotation	InterProScan
Ramachandran Plot Analysis	PROCHECK
Protein-Protein Interaction Analysis	STRING
Domain and Motif Identification	Prosite
Structural Quality Assessment	ERRAT
Sequence Similarity Search	BLAST
RMSD Calculation	PyMOL
Multiple Sequence Alignment	Clustal Omega

This methodological framework ensured a comprehensive analysis of the 6TXV protein, facilitating structural, functional, and interaction-based insights crucial for understanding its biological significance.

RESULT AND DISCUSSION

1.Structural Analysis of A25T Transthyretin structure in complex with Tolcalpone

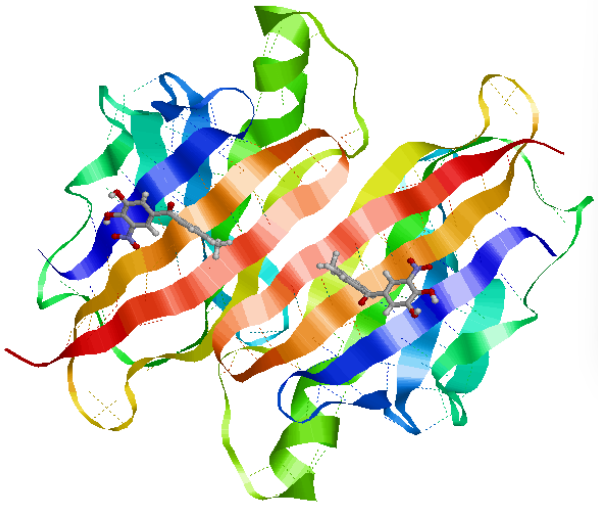

Figure 1: Structural analysis of 6TXV protein visualizing H-bonds

The structure of this protein was resolved using X-ray crystallography, a powerful technique that provides atomic-level detail, allowing for a precise understanding of its molecular architecture. This particular protein consists of six chains, suggesting an oligomeric assembly, likely a tetrameric transthyretin (TTR) structure, with possible additional fragments present. In terms of composition, the structure contains 232 groups, with 187 being unique, representing distinct amino acids and other structural components. The total number of atoms is 1,845, including both backbone and side-chain atoms, while 1,976 covalent bonds hold the entire framework together. Structurally, the protein exhibits a β-sheet-dominant architecture, characterized by 24 β-strands, while α-helices are minimal, with only two detected, which aligns with known TTR folding patterns. Interestingly, there are no significant loop regions, as reflected in the absence of detected turns in the RasMol analysis. The structural stability of this protein is largely maintained by hydrogen bonds within the β-sheets, which are essential for its integrity. However, mutations, such as the substitution of Alanine to Threonine at position 25 (A25T), can interfere with these stabilizing interactions. Such disruptions have been linked to amyloid formation, a characteristic feature observed in conditions like familial amyloid polyneuropathy (FAP) or systemic amyloidosis, making this structural analysis particularly significant for understanding disease mechanisms.

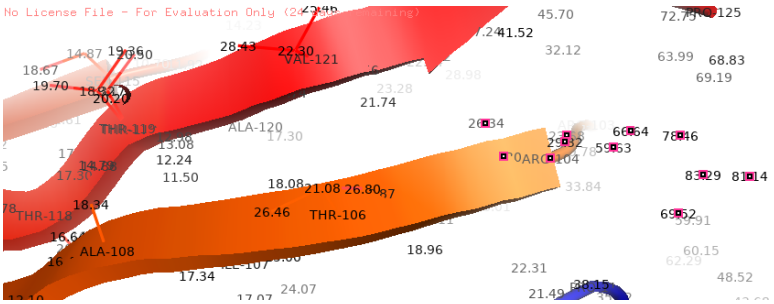

Figure 2: B-Factor analysis of Proteomic sample visualizing areas with high deformity/mutations

The B-factor analysis provides valuable insight into the flexibility and stability of different regions within the protein structure. Areas highlighted in red and orange correspond to regions with higher B-factors, indicating that these segments are more dynamic or disordered. In contrast, regions shown in blue and white have lower B-factors, signifying greater stability and rigidity. As expected, the β-strands forming the core of the protein exhibit low B-factor values, reinforcing their role in maintaining the structural integrity of the protein. On the other hand, loop regions and residues exposed on the surface tend to have higher B-factors, suggesting that these parts of the molecule are more flexible and possibly involved in protein interactions. Certain residues, such as THR-106, ALA-108, THR-119, and VAL-121, show moderate-to-high B-factors, hinting at a potential role in ligand binding or structural movement. Additionally, regions with elevated B-factors may indicate possible binding sites or areas susceptible to mutation-induced conformational changes. Notably, increased flexibility in loop regions has been linked to protein misfolding, which could contribute to the development of neurodegenerative disorders such as Parkinson's and Alzheimer's disease.

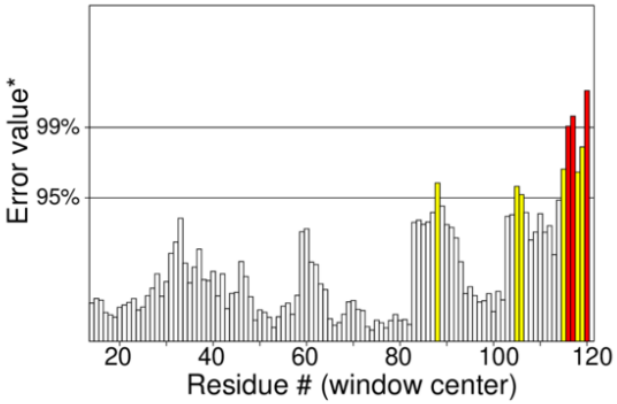

Figure 3: Structure Validation analysis for 6TXV Protein

The Structure validation of the parkinson proteomic structure reveals a quality factor of 92.093, indicating a highly reliable crystallographic model. Typically, structures with an ERRAT score above 90% are considered structurally sound, aligning well with high-resolution protein models. In the analysis, the x-axis represents the residue positions, while the y-axis quantifies error values, helping to pinpoint potential structural deviations. The majority of residues fall below the 95% error threshold, confirming the overall accuracy of the model. However, specific regions display elevated error values, particularly between residues 80-100, where yellow bars indicate moderate structural deviations—possibly due to flexibility or localized disorder. More significantly, residues beyond position 120 exhibit red bars, surpassing the 95% rejection limit, which could indicate loop disorder, misalignment, or crystallization artifacts. These areas may correspond to surface-exposed loops or binding regions, which naturally display higher flexibility. The stable core of the protein, characteristic of beta-sheet-rich structures like transthyretin, is well-resolved with minimal errors. Notably, the regions with higher errors may hold functional significance, potentially marking binding sites or mutation-prone areas, such as A25T. If linked to transthyretin amyloidosis, these structural variations could play a role in misfolding and aggregation, contributing to neurodegenerative disorders like Parkinson's disease.

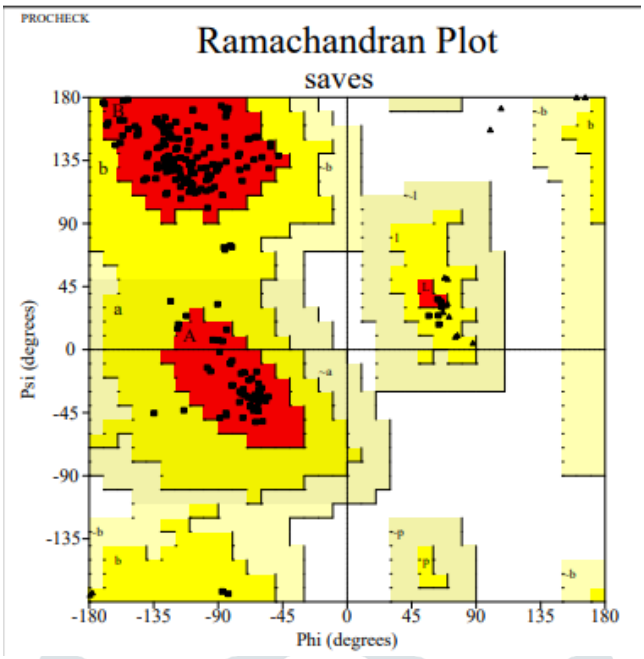

Figure 4: Ramachandran Plot Analysis for 6TXV

The Ramachandran plot for 6TXV indicates that the protein structure is of high quality, with 92.1% of its residues positioned in the most favoured regions. This suggests that the backbone conformations are well within acceptable limits, which is a strong indicator of structural reliability. Additionally, 7.9% of residues fall into the additional allowed regions, which, while slightly deviating from the ideal phi (ϕ) and psi (ψ) angles, are still considered acceptable. Notably, no residues are found in the generously allowed or disallowed regions, meaning there are no sterically strained conformations that could compromise the model's integrity. The presence of 14 glycine residues, known for their conformational flexibility, and 14 proline residues, which contribute to structural rigidity, further supports the protein's stable and well-folded nature. Overall, these results confirm that the 6TXV protein structure is well-refined, meeting high-resolution structural standards and ensuring its reliability for further analysis.

1. **Comparative Analysis with Homologous Proteins and Functional Insights**

Blast analysis has been performed for inferring functional and evolutionary relationships between sequences. The BLAST analysis of the 6TXV protein shows a strong resemblance to known human transthyretin (TTR) structures. The top five closest matches are 3I9I_A (100%), 6R6I_A (100%), 3I9A_A (100%), 7Q9L_A (99.14%), and 5E4O_A (99.14%). These structures correspond to different forms of transthyretin, some representing its natural state, while others are associated with disease-related variations or ligand interactions. The exceptionally high similarity confirms that 6TXV is nearly identical to human transthyretin, supporting its role in transporting thyroid hormones and its potential link to amyloid disorders.

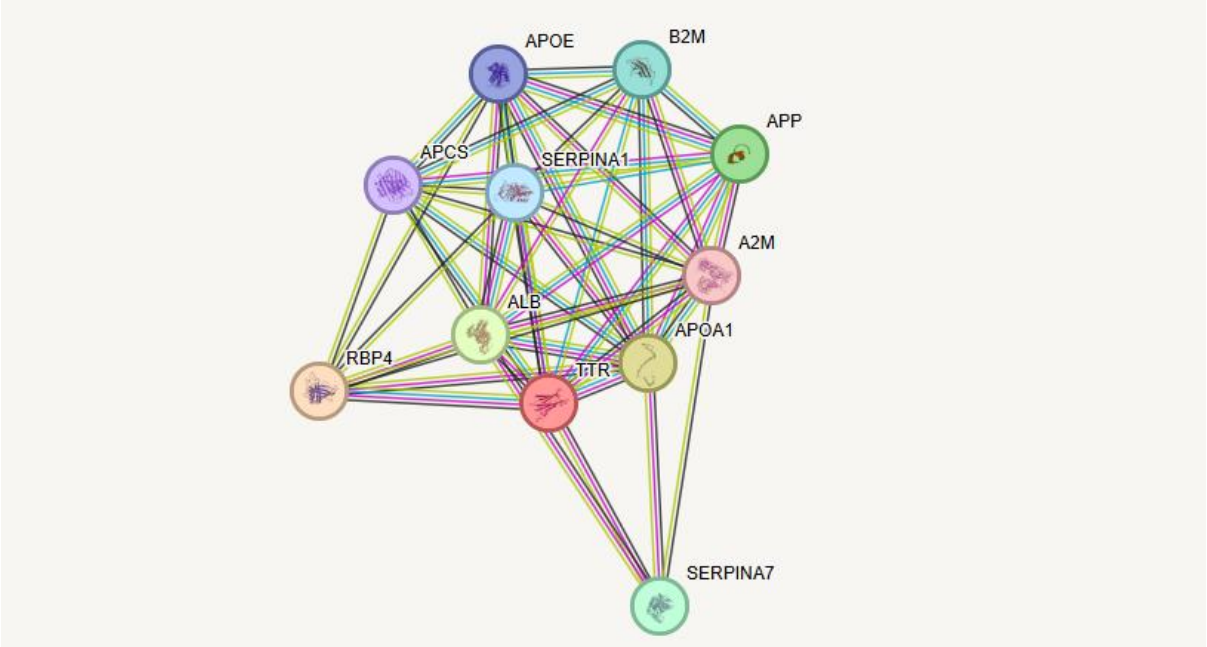

Figure 5: Protein enrichment Analysis for 6TXV

The analysis of the protein interaction network highlights key connections between transthyretin (TTR) and several proteins implicated in Parkinson's disease, including albumin (ALB), apolipoproteins (APOA1 and APOE), amyloid precursor protein (APP), and alpha-2-macroglobulin (A2M). These interactions suggest that TTR may play a crucial role in neuroprotection, amyloid regulation, and metabolic processes that impact Parkinson's pathology. Its connection with APP is particularly significant, as TTR has been suggested to bind amyloidogenic proteins, potentially preventing the aggregation of toxic species that contribute to neurodegeneration. Additionally, its interaction with apolipoproteins hints at a link between lipid metabolism and neuronal health, processes that are often disrupted in Parkinson's disease. The association with protease inhibitors such as A2M and SERPINA1 suggests a role in modulating inflammation and proteolytic activity, which are key contributors to neuronal damage in Parkinson's. With multiple layers of functional interactions, this network supports the idea that TTR could influence neurodegenerative processes by impacting protein homeostasis, lipid transport, and inflammatory control, all of which are relevant to the progression of Parkinson's disease.

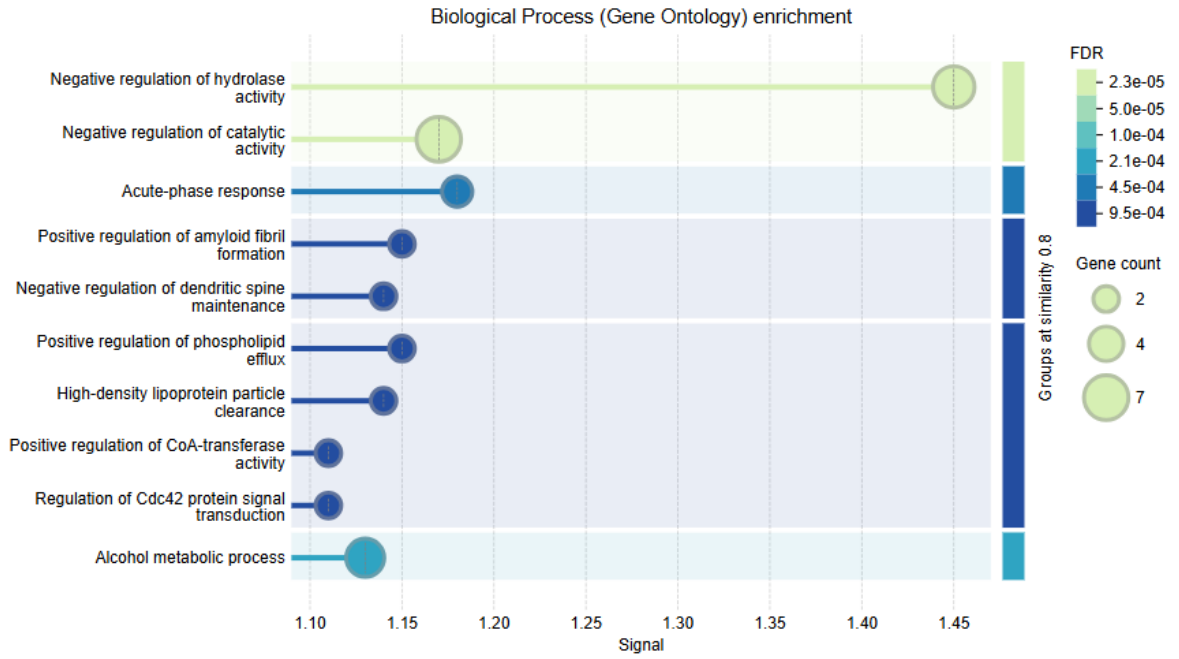

Figure 6: Gene Ontology analysis of A25T Transthyretin structure

The Gene Ontology (GO) enrichment analysis for proteomic sample, performed using STRING, highlights its involvement in several key biological processes. Notably, the protein appears to play a role in acute-phase responses, amyloid fibril formation, and lipid metabolism, all of which are relevant to neurodegenerative diseases. Its connection to "positive regulation of amyloid fibril formation" suggests a potential influence on protein aggregation, a hallmark of conditions like Parkinson's and Alzheimer's. Additionally, the enrichment of lipid metabolism-related processes, such as "high-density lipoprotein particle clearance" and "positive regulation of phospholipid efflux," points to a link with cholesterol transport, which has been associated with neurodegeneration. The regulation of hydrolase and catalytic activity indicates that this protein may also be involved in enzymatic processes related to protein breakdown and inflammation. Interestingly, the mention of "negative regulation of dendritic spine maintenance" suggests a potential impact on synaptic plasticity, which is essential for proper neuronal communication and survival. Taken together, these findings suggest that 6TXV is involved in multiple physiological pathways, particularly those related to inflammation, lipid metabolism, and protein aggregation, all of which play a role in neurodegenerative diseases like Parkinson's.

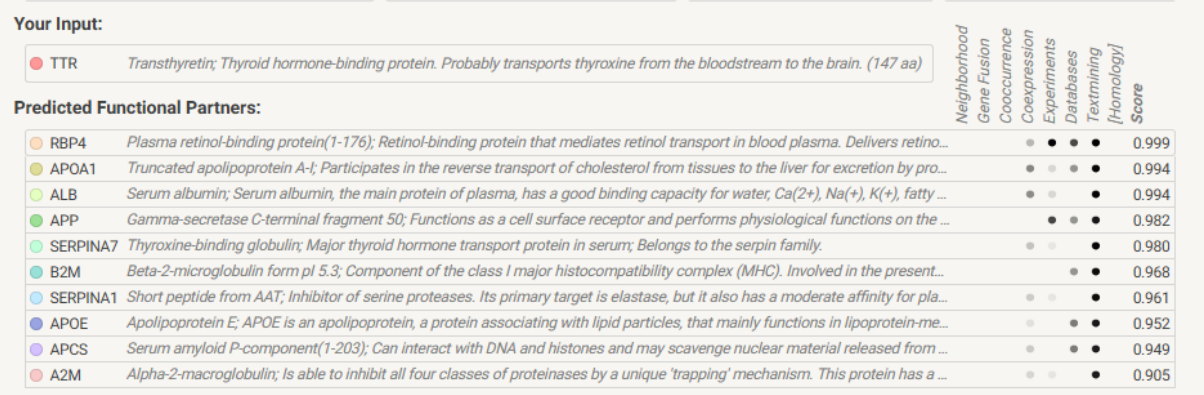

Figure 7: Predicted Functional Partners showing protein enrichment analysis

The predicted functional partners of transthyretin (TTR) highlight its diverse roles in transport, lipid metabolism, and disease-related processes. Its strongest association is with RBP4, reinforcing its function in retinol transport, as TTR carries the RBP4-retinol complex in the bloodstream. Connections with APOA1 and APOE suggest a link to lipid transport and cholesterol metabolism, which are crucial for maintaining cellular health and have implications for neurodegenerative diseases. The interaction with ALB (albumin) supports TTR's role in transporting small molecules and hormones, while the association with APP (amyloid precursor protein) is particularly significant due to its connection with amyloid fibril formation, reinforcing TTR's potential role in neuroprotection against conditions like Alzheimer's and Parkinson's. The presence of protease inhibitors such as SERPINA1 and A2M indicates TTR may also help regulate inflammation and immune responses. Additionally, the interaction with B2M, a component of the immune system, suggests a broader role in immune defense. The connection with SERPINA7, a thyroid hormone-binding globulin, aligns with TTR's primary function of transporting thyroxine. Lastly, APCS, which is involved in amyloid formation, further ties TTR to amyloidosis-related pathways. Together, these interactions underscore TTR's significance in physiological functions, from hormone and lipid transport to potential protective roles in neurodegeneration and inflammation.

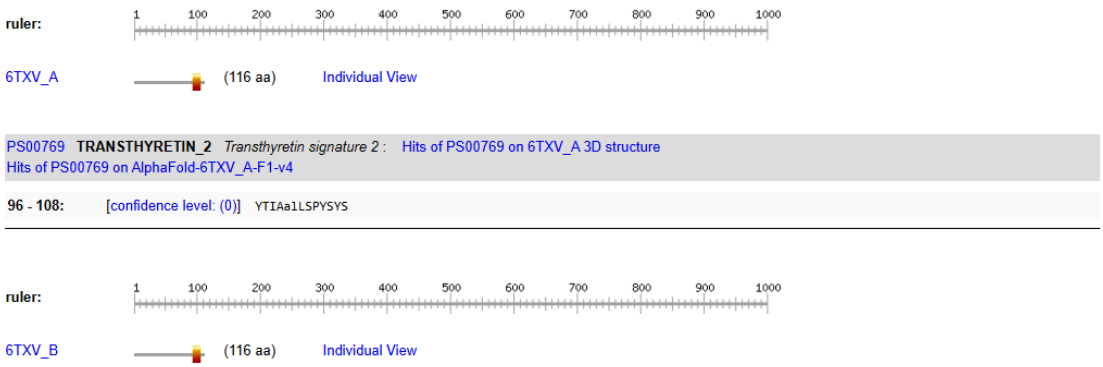

Figure 8: Prosite analysis of 6TXV protein

The Prosite analysis of the 6TXV protein structure detected the **Transthyretin signature 2 (PS00769)**, a conserved motif typically found in transthyretin proteins. This particular sequence was identified between **residues 96-108**, but with a **confidence level of 0**, meaning the match is weak and lacks strong statistical support. While this suggests the sequence aligns with the transthyretin signature, it does not provide conclusive evidence of functional significance. However, the presence of the **YTIAALLSPYSYS** sequence in this region may still play a role in the structural stability or interactions of the protein. To better understand its relevance, further analysis using other bioinformatics tools or experimental validation would be needed.

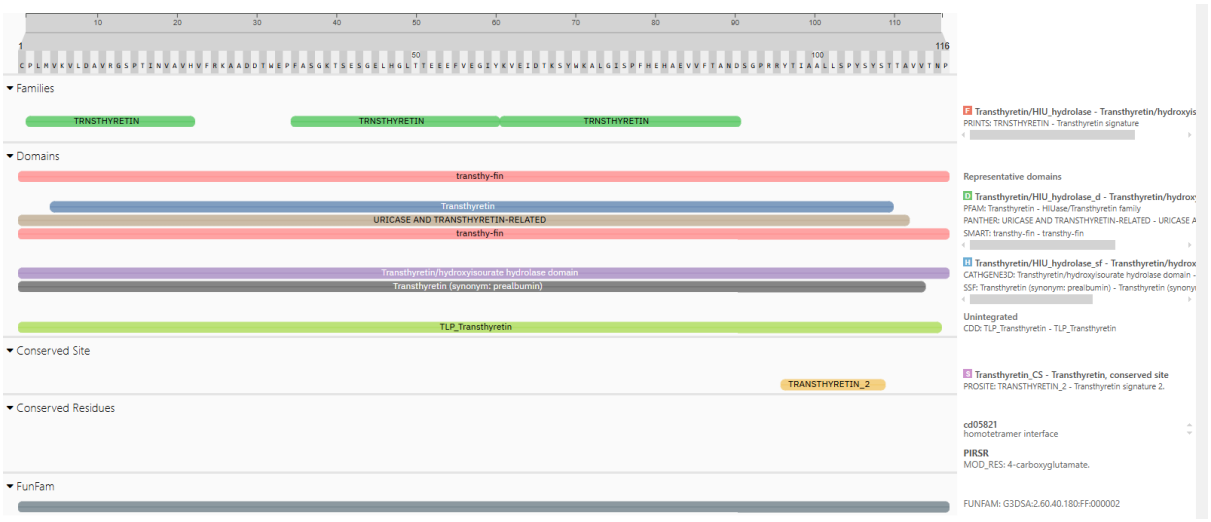

Figure 9: Sequence scanning analysis of 6TXV protein

The 6TXV protein, identified as transthyretin, exhibits a well-defined structural and functional organization. Several key domains span the protein sequence, including the transthyretin/hydroxyisourate hydrolase domain (1-116), the TLP_Transthyretin region (1-115), and the uricase-associated transthyretin-related domain (1-111). Additionally, the protein contains the transthyretin (prealbumin) domain (1-113) and the transthy-fin domain (1-116), which further support its functional role in transport and enzymatic activity. The HIUase/Transthyretin family domain (5-109) also reinforces its connection to related protein families. Furthermore, transthyretin features three signature regions at positions 2-22, 35-60, and 61-90, highlighting conserved functional motifs that define its family characteristics. A particularly significant conserved site, spanning positions 96-108, has been identified as TRANSTHYRETIN 2, suggesting a role in maintaining the protein's structural stability and biological function. Together, these features underscore transthyretin's critical role in hormone transport and protein interactions, contributing to its broader physiological significance.

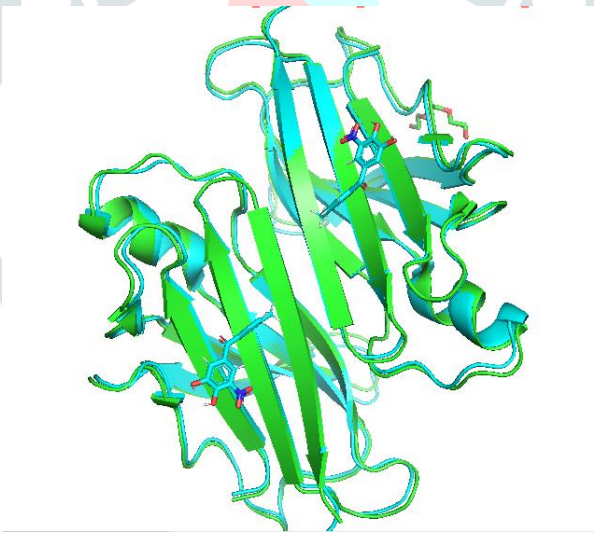

Figure 10: RMSD Score analysis of 6TXV and 3I9I protein

The structural comparison between 6TXV and 3I9I in PyMOL reveals a strong similarity, with a Root Mean Square Deviation (RMSD) of 0.578 Å. This low RMSD value suggests that the two proteins share an almost identical overall fold, as structural deviations below 2 Å typically indicate a high degree of conservation. During the alignment process, 1793 atoms were matched. The slight differences observed could be due to minor conformational changes, mutations (such as A25T in 3I9I), or ligand interactions. Overall, this analysis confirms that 6TXV retains the characteristic structure of transthyretin, making it a reliable model for further studies.

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CLUSTAL O(1.2.4) multiple sequence alignment


6TXV_1|Chains      ----------------------------CPLMVKVLDAVRGSPTINVAVHVFRKAADDT 31
3I9I_1|Chains      ----------------------------CPLMVKVLDAVRGSPTINVAVHVFRKAADDT 31
6R6I_1|Chains      MASHRLLLLCLAGLVFVSEAGPTGTGESKCPLMVKVLDAVRGSPTINVAVHVFRKAADDT 60
3I9A_1|Chains      --------------------GPTGTGESKCPLMVKVLDAVRGSPTINVAVHVFRKAADDT 40
                                       ******************************

6TXV_1|Chains      WEPFASGKTSESGELHGLTTEEEFVEGIYKVEIDTKSYWKALGISPFHEHAEVVFTANDS 91
3I9I_1|Chains      WEPFASGKTSESGELHGLTTEEEFVEGIYKVEIDTKSYWKALGISPFHEHAEVVFTANDS 91
6R6I_1|Chains      WEPFASGKTSESGELHGLTTEEEFVEGIYKVEIDTKSYWKALGISPFHEHAEVVFTANDS 120
3I9A_1|Chains      WEPFASGKTSESGELHGLTTEEEFVEGIYKVEIDTKSYWKALGISPFHEHAEVVFTANDS 100
                   ************************************************************

6TXV_1|Chains      GPRRYTIAALLSPYSYSTTAVVTNP--  116
3I9I_1|Chains      GPRRYTIAALLSPYSYSTTAVVTNP--  116
6R6I_1|Chains      GPRRYTIAALLSPYSYSTTAVVTNPKE  147
3I9A_1|Chains      GPRRYTIAALLSPYSYSTTAVVTNPKE  127
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Figure 11: MSA of 6TXV and its homologues using Clustal Omega

The multiple sequence alignment of 6TXV with its homologs 3I9I, 6R6I, and 3I9A, performed using Clustal Omega, reveals a high degree of sequence similarity, suggesting strong structural and functional conservation. The presence of asterisks throughout the alignment highlights regions where residues are fully conserved, emphasizing their critical role in maintaining protein stability and function. Notably, the N-terminal region of 6R6I contains additional residues, which could be due to differences in the experimental construct or an extended isoform. Meanwhile, the C-terminal region exhibits slight variations, which may influence ligand interactions or overall protein stability. The alignment also reveals a near-perfect match between 3I9I and 3I9A, indicating their close structural relationship with 6TXV. The color coding provides further insight into the biochemical nature of the residues—red marks hydrophobic residues important for protein folding, blue represents acidic residues involved in charge interactions, pink (magenta) highlights basic residues crucial for binding, and green denotes residues with hydroxyl, sulfhydryl, or amine groups, often participating in hydrogen bonding or enzymatic function. Overall, this high sequence similarity suggests that 6TXV and its homologs share a conserved fold and function, characteristic of transthyretin-related proteins, with only minor variations at the terminal regions potentially affecting specific interactions.

Conclusion

Our findings suggest that transthyretin and its variants may play a crucial role in PD pathogenesis by modulating amyloid aggregation and protein homeostasis. The presence of conserved domains and functional motifs, identified through multiple sequence alignment (MSA) and Prosite analysis, further reinforces its significance in neurodegenerative pathways. Moreover, the comparison of 6TXV with homologous structures provides insights into potential mutation-induced structural deviations, which could impact its function in disease progression. This study contributes to the growing body of knowledge on the molecular mechanisms underlying PD and underscores the importance of structural bioinformatics in drug discovery. Future research should focus on validating these findings through experimental approaches, including molecular docking and in vitro studies, to explore transthyretin's potential as a therapeutic target in neurodegenerative diseases.

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