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STRING-based Network and Structural Analysis of Acetylcholinesterase and Superoxide Dismutase Proteins highlights Neurodegenerative Cascades in the Mouse Brain

Mamta Meena[1], Riddhi Garg[1], Reena Kumari[1], Komal Rathore[1], Renu Bist[2]*, P.C. Mali[3]

[1]Research Scholar, Department of Zoology, University of Rajasthan, Jaipur, India
[2]Associate Professor, Department of Zoology, University of Rajasthan, Jaipur, India, and Chief –Coordinator, Empowering Youth for Value Added Translational Research (EYUVA) Centre, University of Rajasthan, Jaipur, India
[3]Professor and HOD, Department of Zoology, University of Rajasthan, Jaipur, India

***Address for Correspondence:** Dr. Renu Bist, Associate Professor, Department of Zoology, University of Rajasthan, Jaipur-302004, India
E-mail: renu_bisht22@yahoo.co.in

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ABSTRACT

Background: Acetylcholinesterase (AChE) and Superoxide Dismutase 1 (SOD1) are pivotal enzymes in cholinergic neurotransmission and redox homeostasis, respectively. Aberrations in their expression and function are closely associated with neurodegenerative conditions such as Alzheimer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS). The present study aims to perform STRING-based network and structural analysis of AChE and SOD1 proteins to highlight neurodegenerative cascades in the mouse brain.

Methods: This study employed in silico tools to compare the protein–protein interaction (PPI) networks of mouse AChE and SOD1 using the STRING database and evaluated their tertiary structures using AlphaFold-predicted models and PyMOL visualization.

Results: STRING analysis revealed distinct PPI landscapes, with AChE associated with synaptic and neuromuscular proteins and SOD1 strongly linked with redox regulatory proteins. Structural modeling demonstrated unique functional folds: AChE possesses a deep catalytic gorge and β-sheet-rich structure, while SOD1 forms a metal-binding β-barrel core.

Conclusion: The combined network and structural insights reinforce the significance of these proteins in neuromuscular integrity and oxidative defense, providing a foundation for further experimental validation and therapeutic exploration.

Key-words: Acetylcholinesterase, AlphaFold, Neurodegeneration, Oxidative stress, Protein–protein interactions, STRING database, Superoxide Dismutase

INTRODUCTION

Acetylcholinesterase (AChE) and Superoxide Dismutase 1 (SOD1) are two pivotal enzymes involved in neural function, though operating in distinct biological domains. AChE is responsible for the rapid hydrolysis of acetylcholine in the synaptic cleft, thereby ensuring the timely termination of cholinergic neurotransmission [1,2].

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The enzyme is highly expressed in neuromuscular junctions and central synapses, and its dysregulation has been associated with Alzheimer's disease (AD), Parkinson's disease, and various forms of neurocognitive decline [3–5]. Neurocognitive decline, including memory impairments and behavioural changes caused by dementia, severely impairs a person's ability to live independently [6].

Therapeutic modulation of AChE forms the basis of current symptomatic treatments for AD, particularly through the use of AChE inhibitors like donepezil and rivastigmine [7,8]. AChE is also implicated in non-cholinergic functions such as cell adhesion and apoptosis, further extending its neurobiological significance [9,10].

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SOD1, in contrast, catalyzes the dismutation of superoxide anions (O_2^-) into molecular oxygen and hydrogen peroxide, forming the first line of defense against reactive oxygen species (ROS)-mediated toxicity [11,12]. It plays a crucial role in maintaining redox homeostasis in the brain and peripheral tissues. Mutations in the SOD1 gene, such as A4V and G93A, are causative of familial Amyotrophic Lateral Sclerosis (fALS), with toxic gain-of-function effects due to protein misfolding and aggregation [13–15]. Additionally, oxidative stress and mitochondrial dysfunction involving SOD1 have been reported in a range of neurodegenerative diseases, including AD, Huntington's disease, and multiple sclerosis [16,17].

Mouse (*Mus musculus*) models have been extensively used to study the functions of AChE and SOD1 due to their high sequence homology and physiological relevance to humans [18,19]. Deciphering the structural and interaction networks of these enzymes can illuminate therapeutic avenues, especially for oxidative neurodegeneration and cholinergic dysfunction.

In this study, we present a comparative in silico bioinformatics analysis of AChE and SOD1 in the mouse model. The approach involves protein-protein interaction (PPI) network mapping using the STRING database and 3D structure prediction via AlphaFold, followed by visualization and structural annotation with PyMOL. This integrative study seeks to provide a molecular framework for understanding their roles in neurophysiology and disease.

MATERIALS AND METHODS

Place of the study- The present study was conducted in February 2025 by a research team of the Molecular Neuroscience and Drug Designing Lab of the Department of Zoology, University of Rajasthan.

Protein Selection- The UniProt entries for mouse AChE (P21827) and SOD1 (P08228) were used for all analyses. These proteins were selected due to their relevance in neurodegeneration and the availability of validated structural and functional data.

STRING Interaction Analysis- The STRING database (v12.0; https://string-db.org) was employed to analyze PPI networks. Parameters were adjusted to a high confidence interaction score (≥0.700). Evidence channels considered included experimental data, curated databases, co-expression, and text mining. Ten top interactors were selected for visualization.

3D Structure Prediction and Visualization- Predicted 3D models were retrieved from the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/). Structural visualization and annotation were performed using PyMOL (v2.5.2). Active sites and conserved motifs were highlighted. Comparison of structural domains was based on topology and secondary structure elements. Metal ion binding sites in SOD1 were evaluated by aligning with crystallographic data (PDB ID: 2C9V).

Ethical Consideration- All analyses were conducted *in silico* and did not involve live animals or human participants.

RESULTS

The AChE interaction network (Fig. 1) revealed high-confidence links to proteins involved in synaptic anchoring (PRIMA1), neuromuscular junction organization (COLQ), and receptor clustering (RAPSN). These associations reflect its role in neuromuscular cholinergic transmission. Additional interactors included butyrylcholinesterase (BChE) and DOK7. Protein–protein interaction network highlighting high-confidence interactors (confidence ≥ 0.7): PRIMA1, COLQ, RAPSN, ACHE dimerization partners, and neuromuscular junction components. Edge thickness correlates with interaction confidence.

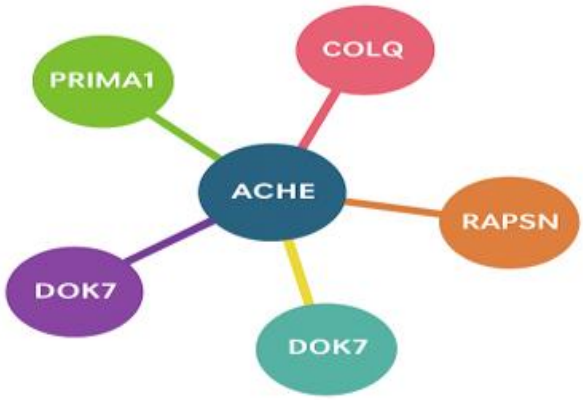

Fig. 1: STRING network of mouse AChE

The SOD1 network included interactions with copper chaperone for SOD (CCS), glutathione peroxidase 1 (GPX1), and catalase (CAT). These proteins are integral to maintaining oxidative balance and scavenging ROS. High

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interaction scores were also noted with peroxiredoxins and thioredoxin (Fig. 2). STRING network illustrating key oxidative defense interactions: CCS, GPX1, CAT, SOD1 dimerization. Strong experimental and database evidence shown by the network edges.

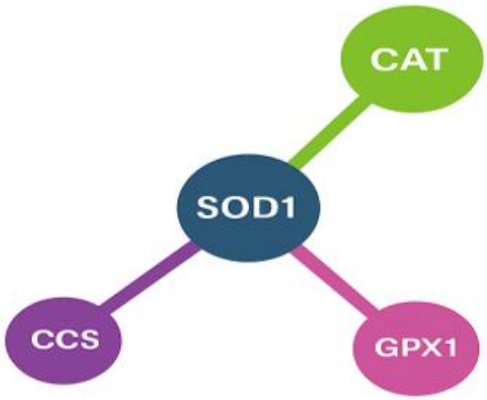

Fig. 2: STRING network of mouse SOD1

The AlphaFold-predicted structure of AChE (Fig. 3) exhibited a globular architecture with a central β-sheet flanked by α-helices. The active site is located within a deep and narrow gorge composed of conserved residues: Ser203, His447, and Glu334. This arrangement is critical for substrate specificity and rapid catalysis. Cartoon representation of the AChE monomer: central β-sheet core and surrounding α-helices. The catalytic triad (Ser203, Glu334, His447) is highlighted in stick form (red).

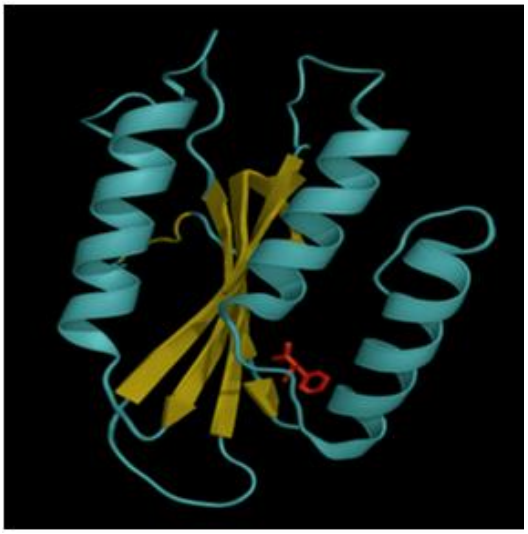

Fig. 3: AlphaFold-predicted 3D structure of mouse AChE

The SOD1 model (Fig. 4) revealed a compact β-barrel core with a Greek-key topology. Metal-binding sites were visible with Cu^{2+} and Zn^{2+} coordinated by conserved histidine residues. These structural features facilitate redox catalysis and oligomerization. Ribbon diagram showing the β-barrel fold of SOD1 with bound Cu and Zn ions at the active site (represented as colored spheres: Cu in orange, Zn in gray). Metal-coordinating residues are shown in sticks (green).

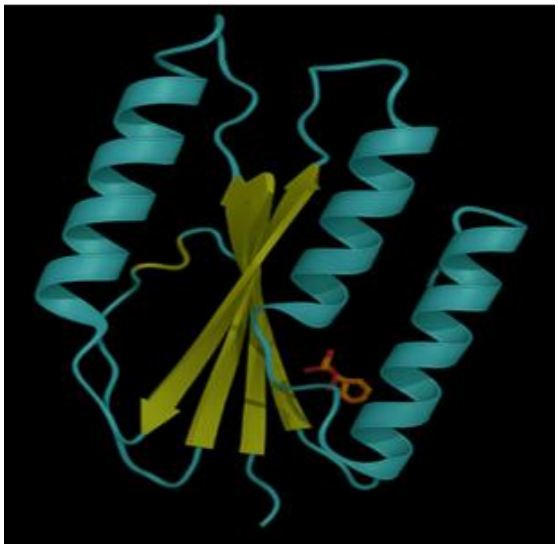

Fig. 4: AlphaFold-predicted 3D structure of mouse SOD1

DISCUSSION

The comparison of AChE and SOD1 in mice highlights distinct yet critical structural and functional characteristics, each tailored to their neurophysiological roles. AChE plays an essential part in synaptic transmission by catalyzing the hydrolysis of acetylcholine at cholinergic synapses, thereby ensuring signal fidelity and preventing overstimulation [1]. The interaction of AChE with synaptic proteins such as PRIMA1, COLQ, and RAPSN underlines its anchoring mechanism at the neuromuscular junction and central synapses [4,20].

Structurally, AChE is defined by a deep, narrow catalytic gorge lined by aromatic residues, facilitating selective substrate guidance to the active site, which comprises the catalytic triad Ser203, Glu334, and His447 [21]. This unique arrangement allows for rapid substrate hydrolysis. The peripheral anionic site (PAS) of AChE also plays a crucial role in modulating enzyme activity and has been implicated in amyloid-β fibrillogenesis in AD, further linking cholinergic dysfunction with neurodegeneration [22,23].

SOD1, on the other hand, is central to cellular antioxidant defense. It catalyzes the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen, thus mitigating oxidative stress—a key

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factor in the progression of neurodegenerative diseases [11]. The β-barrel structure of SOD1 and its Greek-key motif confer high structural stability, crucial for redox reactions [24].

Mutations in the SOD1 gene can compromise metal ion coordination—especially Cu^{2+} and Zn^{2+} binding—and promote protein misfolding, aggregation, and ultimately motor neuron toxicity, as evidenced in fALS [15]. Additionally, misfolded SOD1 has been found to exert toxic effects through mitochondrial dysfunction, glial activation, and disruption of axonal transport in ALS models [25].

Together, these findings support a dual therapeutic strategy: targeting AChE to restore or modulate cholinergic neurotransmission, and enhancing SOD1 stability or mimicking its activity to combat oxidative stress in the central nervous system (CNS). Such integrative approaches may offer disease-modifying potential in AD, ALS, and related neurodegenerative disorders.

CONCLUSIONS

The STRING network bioinformatic analysis of AChE and SOD1 highlights their distinct protein interactions and structural characteristics in the mouse brain, which reveals the pathways that may contribute to neuronal function and vulnerability. AlphaFold-based structural modelling further demonstrated functional conformational differences, providing insights into their roles in oxidative stress and synaptic regulation. These findings enhance our understanding of how AChE and SOD1 contribute to neurodegenerative processes and may inform the design of targeted preclinical interventions. By integrating network and structural analyses, the current study establishes a comprehensive framework for exploring therapeutic strategies aimed at modulating these proteins.

The findings open avenues for experimental validation in murine models to clarify roles in neuronal signalling, oxidative stress, and synaptic maintenance. Integration with multi-omics data (transcriptomics, metabolomics) may uncover novel neurodegenerative pathways and support targeted therapies to mitigate neuronal damage and improve brain health.

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CONTRIBUTION OF AUTHORS

Research concept- Renu Bist
Research design- Renu Bist
Supervision- Renu Bist
Materials- Mamta Meena, Riddhi Garg, Komal Rathore
Data collection- Mamta Meena, Reena Kumari, Komal Rathore
Data analysis and interpretation- Mamta Meena, Renu Bist
Literature search- Mamta Meena
Writing article- Mamta Meena and Reena Kumari
Critical review- Renu Bist
Article editing- Riddhi Garg
Final approval- Renu Bist

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