Computational investigation on the biomarkers and the role of SHANK3 in Autism Spectrum Disorder

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Hiba Almaadani

Registration No: TZ203949 of 2023

Department of Molecular Biology and Biotechnology

School of Sciences, Tezpur University,

Tezpur-784028 Assam, India

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CHAPTER 9

Summary and Future Prospects

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9.1 Overall conclusion

Autism spectrum disorder is a neurodevelopmental condition with a complex genetic background. SHANK3 has been documented as one of the high-risk genes in ASD that directly impacts synaptic activities, especially during early developmental stages. Moreover, protein interactions between SHANK3 and other postsynaptic proteins have made SHANK3 critical in the process of dendrite and spine formation, vesicle release, synaptic transmission, and synaptic plasticity. Numerous SHANK3 variants have been identified in ASD. However, The N-terminus of SHANK3, including the SPN and ARR domains, has emerged as a hotspot of missense mutations and provided insights into the potential role of these mutations in ASD pathophysiology.

We designed two point mutations to study their effects on the stability and dynamic conformation and how they impact the interaction between SHANK3 and other post synaptic proteins, such as αCaMKII and α-Fodrin. We tried to investigate the impact of E71S mutation situated in the SPN domain. The study revealed crucial consequences, notably impeding the stabilization and folding processes of the SHANK3 protein. The SHANK3 E71S mutant disrupted the intramolecular interactions between the SPN and ARR domains, thereby increased the distance between these domains and led to the open construction of the SPN-ARR tandem. Consequently, negatively influences the binding of SHANK3 E71S mutant with αCaMKII, whereas increased binding α-Fodrin to its sites on the SHANK3.

Similarly, we tried to study the influence of N52R point mutation. The findings shed light on the structural consequences of the N52R mutation in SHANK3, emphasizing its role in influencing intramolecular interactions with stabilization and led to open conformation.

On the other hand, we tried to detect the effect of two point mutations that have been found in ASD patients L270M and P141A. The P141A mutation significantly disrupted SHANK3 stabilization and caused a disturbance in intramolecular connections between SPN and ARR domains. The alteration affected the αCaMKII binding as one of the pivotal protein partners. On the contrary, the SHANK3 L270M mutation resulted in moderate stability conformation.

These findings emphasize the intricate dynamics of SHANK3 mutations and suggest

their potential relevance to ASD.

The second aim of this thesis focused on identifying the significant DEGs in ASD and detecting potential biomarkers. However, the quest for potent and precise biomarkers for the detection and diagnosis of ASD remains an unmet challenge. In this study, a comprehensive bioinformatic analysis of gene expression profiles from ASD patients was conducted to identify common genes that serve as potential and reliable biomarkers for early diagnosis of ASD, particularly from accessible tissues. The analysis of brain datasets revealed 581 differentially expressed genes, with functional enrichment implicating processes such as positive regulation of cytokine production, response to bacterial molecules, and involvement in the TNF signaling pathway.

In contrast, the blood dataset revealed sixty significant DEGs linked to the inflammatory responses, including chemokine response and the interaction of cytokines and cytokine receptors. Remarkably, eight common genes were detected between the two datasets. Among these common DEGs, UPB1, CXCL1, CXCL10, CSF1, CCL2, and IL1B were identified as novel genes associated with ASD, while FFAR2 and WWC2-AS2 were novel genes not previously linked to ASD. The common genes were enriched in the TNF signaling pathway. The findings revealed the relevance of immunity dysregulation in the neurodevelopmental of ASD. Additionally, these common genes hold promise as prospective biomarkers for early detection from accessible tissues and represent potential targets for future pharmacological interventions in ASD.

9.2. Future Prospects:

- Investigate the post-synaptic proteins that play a role in controlling the closed and open conformation of SHANK3, which can be important in future pharmaceutical intervention.
- Study the impact of interactions between SHANK3 and other post synaptic proteins, such as sharpin.
- Investigate experimentally the level of the previous biomarkers in larger sample sizes to validate these findings and enable accurate early diagnosis of ASD.