Autism spectrum disorder (ASD) is a composite heterogeneous neurodevelopmental condition frequently characterized by a broad spectrum of clinical manifestations, including impairments in social and communicative interactions alongside restricted, repetitive behaviors. A myriad of genetic loci have been implicated in pathomechanisms of ASD; however, they converge to a few molecular pathways that have been engaged in the neurobiology of ASD. Synaptopathies are one of the common neuronal processes associated with ASD as a result of genetic variations during early brain development, involving altered glutamatergic and GABAergic neurotransmission that may disrupt the excitatory and inhibitory equilibrium. One pivotal protein that plays a role within the post-synaptic density of glutamatergic neurons is the SHANK3 gene. It binds the cytoskeleton in neuronal cells to glutamate receptors and thereby plays a crucial role in synaptic transmission and dendritic spines. Several point mutations associated with ASD have been identified in the N-terminal region of SHANK3, which consists of the Shank/ProSAP N-terminus (SPN) and Ankyrin Repeat Region (ARR) domains in close contact with each other. In vitro, studies of numerous of these mutations have identified that abnormal dendritic spine morphology and function is a hallmark feature associated with the expression of these SHANK3 mutations. The SHANK3 N-terminal domain has been shown previously to interact with essential proteins involved in signaling cascades and have direct implications on the dynamics and organization of the actin cytoskeleton, further demonstrating the importance of the N-terminal domain for correct SHANK3 and synaptic functioning. Additionally, most of these mutations affect the stabilization and dynamics of the SHANK3 protein or alter the SHANK3 protein function. However, the structural and functional relevance of these mutations is still unclear because the N-terminal domain is much less studied than the other SHANK3 domains and motifs. In light of this, the computational approach is a sensible strategy for comprehending the structural and functional implications of these mutations and their impacts on the structure

and stability of the SHANK3 protein. In the current study, two designed point mutations in the N-terminal were constructed, the first one at position E71 and the second at position N52. The two SHANK3 mutants were studied to assess their effects on the stability and dynamic of SHANK3, particularly SPN-ARR domains. In addition, two mutations that have been found in ASD and ADHD-like phenotype patients were investigated, namely P141A and L270M mutants, in comparison to SHANK3 WT using molecular dynamics (MD) simulations to examine protein equilibrium, flexibility, compactness, and intramolecular interactions. Consequently, to gain insights into the potential effects of deleterious mutation on the protein binding implicated in the neuronal transaction associated with ASD pathogenesis. MD simulations unveiled that the E71S mutation perturbed the stability and folding of SHANK3, leading to the disturbance of intramolecular contacts between the SPN and ARR domains, thereby opened-up the SPN-ARR tandem; as a result, influencing the binding with αCaMKII and α-Fodrin to their sites on the SHANK3. Similarly, the SHANK3 N52R mutant hindered the stabilization and folding of SHANK3, as well as the intramolecular interactions between SPN and ARR were affected, which impeded the binding of αCaMKII to SHANK3 N52R mutant. Whereas the SHANK3 N52R mutant increased the binding to α-Fodrin. In contrast, the results of two SHANK3 L270M and SHANK3 P141A mutants disclosed elevated values of radius gyration over time of the simulation of 200 ns in comparison to the SHANK3 WT protein. However, the SHANK3 P141A mutant has the highest value; therefore, the SHANK3 P141A mutant refers to a less compact and unfolded state. Noteworthy, the SHANK3 L270M mutation is positioned within the hydrophobic part of the ARR domain without displaying significant characteristics of an unfolding state, as the majority of the interactions within the SHANK3 N-terminal region remain preserved. Thereby, the plausible interpretation from our findings is that the SHANK3 L270M mutation subtly modifies the surface characteristics of the ARR domain in a manner incompatible with δ-catenin binding.

The SHANK3 P141A mutant showed increased distance between SPN and ARR domains, suggesting an impact on domain interactions and the potential open-up of the SPN-ARR fold over time. The previous findings were confirmed by intra-molecular hydrogen bond analysis, which revealed that the SHANK3 P141A mutant had the lowest intra-molecular hydrogen interactions. Meanwhile, SHANK3 WT has heightened intra-molecular hydrogen bonds. SHANK3 WT protein demonstrated a higher affinity for docking with αCaMKII than the interactions observed in the SHANK3 P141A mutant with αCaMKII. Conversely, our findings indicated that intramolecular interaction prevented α-Fodrin from accessing its location on the ARR domain in SHANK3 WT, whereas SHANK3 P141A significantly boosted the connecting of α-Fodrin to SHANK3. A potential scenario might be that the open conformation facilitates α-Fodrin binding.

Consequently, the SHANK3 P141A mutant exhibited a dramatic impact, hindering the stabilization and folding of SHANK3, as well as disrupting intramolecular interactions between SPN and ARR, influencing its binding with αCaMKII and α-Fodrin similarly to the SHANK3 N52R and SHANK3 E71S impacts. In contrast, the SHANK3 L270M mutant resulted in moderate stability and intramolecular hydrogen interaction compared to the SHANK3 WT. These findings emphasize the intricate dynamics of SHANK3 mutations and suggest their potential contributions to neurodevelopmental disorders such as ASD.

Genetic modifications to synaptic proteins can stimulate the immune system in the brain, referred to as "immune-synaptopathy." Malfunctional synapses, maternal immune activation, and other agents can induce immune activation, including microglia and astrocytes, resulting in neuroinflammation. It is a complicated process in which the brain responds to different types of stimuli and acts as a prominent hallmark of many other pathological conditions. Accumulating studies support the involvement of neuroinflammation in the etiology of ASD, with evidence from sustained activation of microglia and astrocytes

in postmortem brains, as well as increased levels of cytokines and chemokines, which leads to robust pathological changes and neurobehavioral complications. In this study, a comprehensive bioinformatic analysis of gene expression profiles was conducted to identify significant differentially expressed genes (DEGs) in two different types of tissues from ASD patients. Moreover, a Venn diagram was utilized to detect 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 DEGs, meeting the criteria of P-value < 0.01 and with cut-off -1> Log2FC >1. Among these, 520 genes exhibited significant upregulation, whereas 61 genes displayed downregulation. The functional enrichment analysis implicated processes such as positive regulation of cytokine production, response to bacterial molecules, and involvement in the TNF signaling pathway.

In contrast, the blood dataset exhibited sixty significant DEGs with 20 genes downregulated and 40 genes upregulated, associated with inflammatory responses, including chemokine response and the interaction of cytokines and cytokine receptors. Remarkably, eight common genes were identified 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.