CHAPTER 2

Introduction

2. Introduction

2.1. Autism spectrum disorder

Autism spectrum disorder (ASD) is a group of neurodevelopmental conditions that affect the development of neurological pathways. According to a recent report, the prevalence rate for ASD is 1 in 100 children [1]. Prior systematic analyses have indicated that the documented increase in ASD prevalence estimates over time may be associated with enhancements in diagnostic criteria, improved research quality, methodological classifications, heightened availability of diagnostic and support services, and elevated awareness of ASD [2]. Furthermore, ASD demonstrates an uneven gender distribution, with males being four times more susceptible to developing the disorder compared to females [3]. Numerous hypotheses have been released to understand this differential prevalence, including that females require a higher genetic dose "defect" than males, which is compatible with the assumption that protective genetic factors have a crucial role in disease progenesis. Moreover, a relationship between the risk of ASD and testosterone levels in males is likely attributed to dramatically elevated inflammatory responses [4], resulting in alterations in neuronal development and influencing the connectivity among various brain regions [5, 6].

2.2. The manifestations of ASD

ASD is characterized by a broad spectrum of manifestations rooted in two fundamental domains of behavior, including:

1. Social deficits encompass a deficiency in social reciprocity and an impaired ability to improve loving relationships through interpersonal communication. In addition, impairments in language and interactions manifested by echolalia, pronominal reversal, and atypical language usage [7].

2. Stereotypical repetitive types of behavior, including obsessed routines habits, abnormal preoccupations, limited interest patterns, abnormal attachments to items, unique motor stereotypies, and idiosyncratic reactions to sensory stimuli [8].

Moreover, seizure disorders often coexist in individuals diagnosed with ASD and cognitive delays. Additionally, ASD is frequently accompanied by hyperactivity, impulsivity, attention impairment, as well as symptoms of anxiety. The diverse array of manifestations observed in ASD underscores its multifaceted etiology, which involves

complex interactions between genetic and environmental factors [9], as shown in **Figure 2.1**.

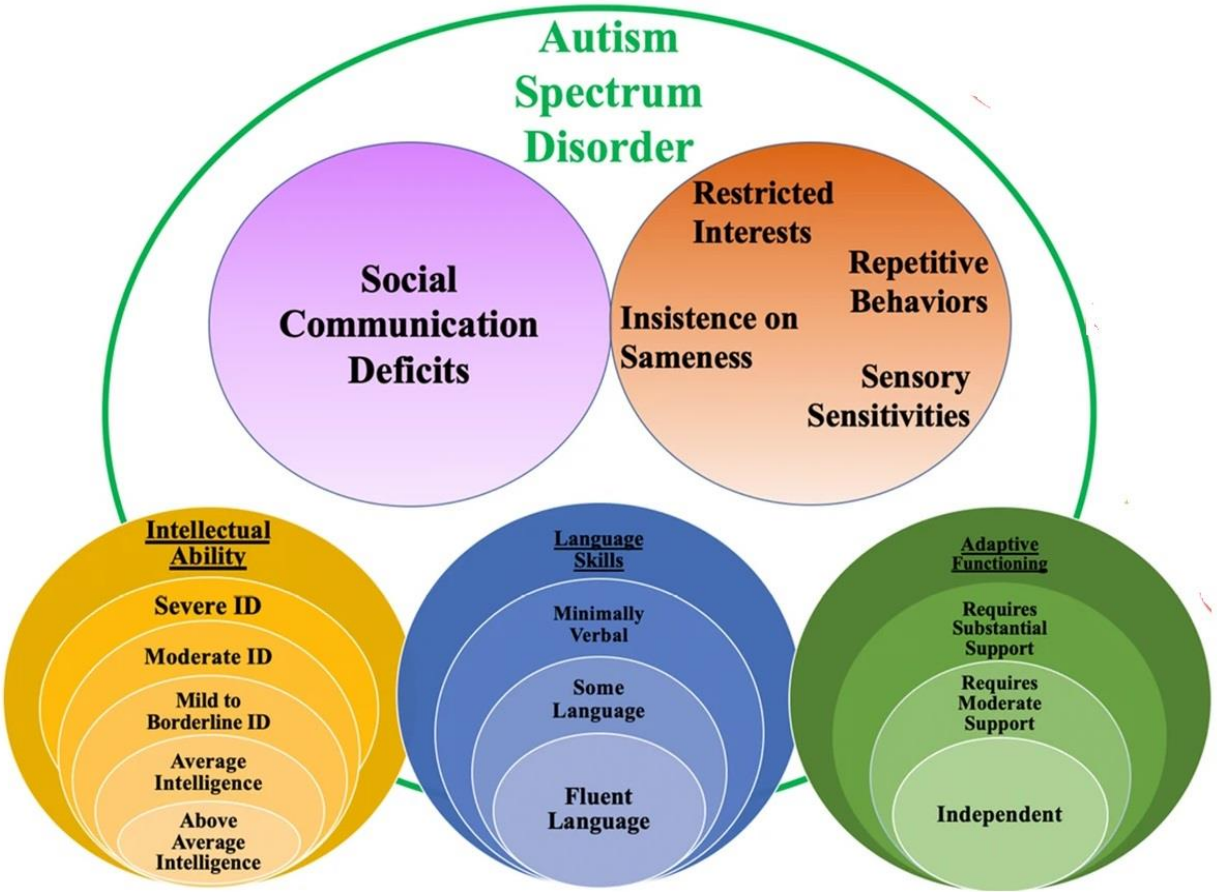

Figure 2.1. *The manifestations categories of autism spectrum disorder (modified from [10]).*

2.3. The etiology of ASD

2.3.1. Non-genetic factors associated with ASD

Epidemiological research continue to pinpoint numerous environmental agents, encompassing biological, chemical, and infectious factors, which independently or synergistically elevate the risk of ASD in affected individuals in conjunction with genetic susceptibility [11, 12]. Numerous studies have revealed various exogenous factors, including the rubella virus and medications such as valproate, which have been revealed to heighten the risk of ASD hundreds of times [13, 14]. Moreover, it has been indicated that maternal infection throughout pregnancy caused by influenza, stress, obesity, and

maternal old age may elevate the incidence of ASD [15-17]. Furthermore, exposure of the mother to chemical or biological substances, like environmental pollutants and pesticides used in agriculture, during the initial phase of fetal neurological development has been associated with a notably

increased likelihood of ASD [18, 19]. However, several clinical epidemiological reports are hampered by methodological constraints and a lack of understanding regarding the cellular and molecular pathways affected by environmental agents implicated in ASD onset. As a consequence, environmental investigations emphasize the crucial need to comprehend the neurobiological basis of ASD, particularly in identifying susceptibility gene transcription through early neurodevelopmental stages.

2.3.2. Genetic factors of ASD

The precise etiology and ASD pathogenesis remain mainly unknown; however, a substantial proportion of ASD cases are attributed to genetic factors. Molecular genetic investigations have revealed a significant genetic predisposition, as evidenced by higher concordance rates among monozygotic twins compared to dizygotic twins (0.98 and 0.53, respectively) [20, 21]. Investigations involving diverse families and various twinning types underscore the significant role of genetics in ASD etiology. A comprehensive review encompassing over two million individuals from six hundred and eighty thousand families across multiple nations estimated heritability at 80%. This finding revealed the potential of genomics as a valuable medical indicator for ASD [22]. The heterogeneity observed in ASD, likely driven by various genetic factors, encompasses monogenetic syndromes such as Fragile X syndrome (FXS). FXS stands out as a notable monogenic contributor to ASD, often exhibiting a significant prevalence of ASD-like manifestations in affected individuals. FXS is primarily linked to a deficiency in the expression of the Fragile X Mental Retardation 1 (FMR1) gene located on the X chromosome [23]. Additionally, Rett syndrome has been linked to many cases of ASD [24]. The considerable genetic heterogeneity observed in ASD underscores the absence of any singular genetic mutation responsible for more than 1–2% of ASD cases. Moreover, the polygenetic type of inheritance, as evidenced by numerous studies, adds a layer of complexity to unraveling the underlying genetic mechanisms associated with the disorder. [25]. Scientists have utilized both traditional and innovative research methods to confront these complex conditions. Chromosomal abnormalities have been identified

in approximately 3–7% of ASD cases, with the most common abnormalities including maternal duplication of 15q11-q13, duplication of 17p11.2, deletion of 2q37, deletion of 22q13.3, and deletion of 22q [26-29]. On the contrary, genome-wide association studies (GWAS) and linkage studies have identified numerous loci or putative regions/genes potentially linked to ASD. Besides this, they underlined the relevance of single nucleotide polymorphisms (SNPs) in the heredity of ASD

[25, 30, 31] (**Figure 2.2**). In accordance with the aforementioned studies, a myriad of genetic loci have been implicated in pathomechanisms of ASD [32]; however, they converge to many molecular pathways that have been engaged in the neurobiology of ASD [33]. Synaptopathies and neuroinflammation are the most common neuronal trajectories associated with ASD [34, 35].

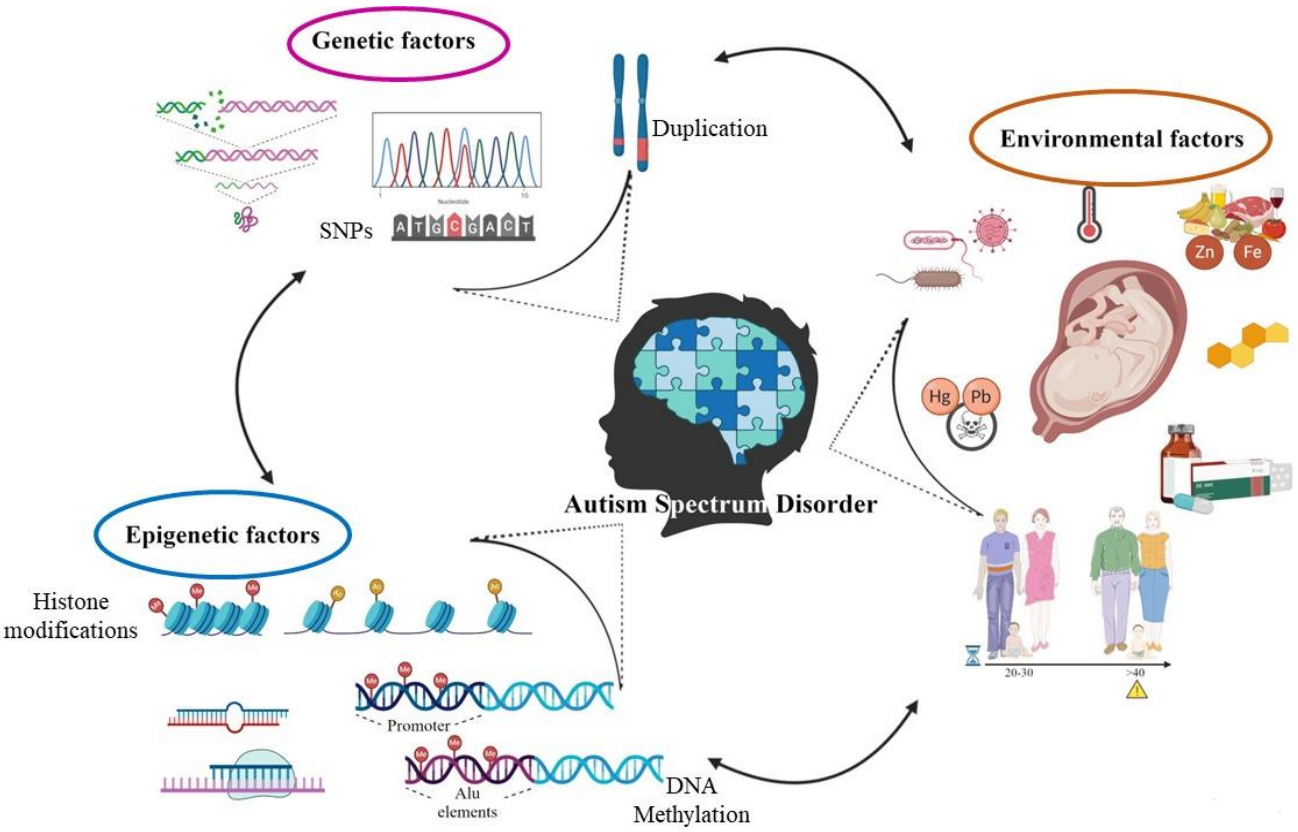

Figure 2.2 *The various genetic, epigenetic, and environmental risk factors involved in ASD (modified from [36]).*

2.4. The pathomechanisms implicated in ASD

2.4.1. Synaptopathy

Synaptogenesis is a complicated and precisely controlled process essential for conserving neuronal homeostasis and ideal brain function [37]. Accurate regulatory systems guide this mechanism to guarantee the proper development and alteration of synapses. Dysfunction, even in a single component as part of this complex architecture, may result in a group of neurological conditions defined by synaptic malfunction, collectively pointed out as synaptopathies [37, 38].

2.4.1.1. Dendritic Spines

Dendritic spines emerge from filopodia extensions originating from dendritic branches of neuronal cells, seeking axonal connection and hosting the required mechanism for post-synaptic functionality [39]. The establishment and maturation of connectivity with the axonal element shape the formation position of the excitatory synapses [39]. This connection serves as a focal point for numerous dynamic processes, especially during the developmental stages of the juvenile brain [40], and decreases during adolescence [41]. The post-synaptic component of the majority of excitatory synapses within the CNS is termed a dendritic spine [42]. The diversity of dendritic spines, which exhibit various morphologies across distinct developmental stages, has been highlighted by early investigations [43], as displayed in **Figures 2.3A** and **2.3B**. These morphologies have been described as maturing mushroom spines with a short neck and distinctly defined spine head or as thin, juvenile spines [39, 44]. The dynamics of the spine play a crucial role in all stages of synapse development, maintenance, and removal [45]. Investigating the regulation of spine plasticity is vital due to its association with several neurodevelopmental, cognitive impairments, and neurological conditions, including ASD [46]. Nevertheless, regulating dendritic spine dynamics is a complicated system associated with actin dynamics and encompasses numerous signaling mechanisms [47].

2.4.1.2. Synaptic plasticity

Neuronal synaptic connections have the ability to endure long-lasting modifications in their strength [48]. Synaptic plasticity refers to the bidirectional capacity of synapses to

enhance or diminish their strength in response to predictable patterns of synaptic activity. This phenomenon is believed to be the fundamental mechanism underlying learning and memory at the cellular level. The alterations that take place precisely at each synapse are able to be observed through experiments as either long-term potentiation (LTP) or long-term depression (LTD) [49, 50]. Long-term potentiation (LTP) is a lasting increase in the strength of synaptic between neurons caused by short, intense stimulation at a high frequency. This process involves an increase in the size of synapses and the addition of Glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. On the other hand, long-term depression (LTD) is a lasting reduction in the strength of synaptic between neurons resulting from longer periods of low-frequency stimulation that leads to the shrinking or elimination of synapses [49, 51-53] (**Figure 2.3C**). Since the dysregulation of excitatory/inhibitory (E/I) neuronal synaptic is implicated in various neurological disorders such as epilepsy and ASD, there is a precise regulation of the size and quantity of excitatory and inhibitory neuronal synaptic. Additionally, long-term plasticity in glutamatergic and GABAergic synaptic transmission takes place coordinately to modulate the harmony of E/I synaptic transmission [54, 55]. Moreover, one of the complicated structures that plays a critical role and serves as a structural scaffold in synaptic plasticity is postsynaptic density [56].

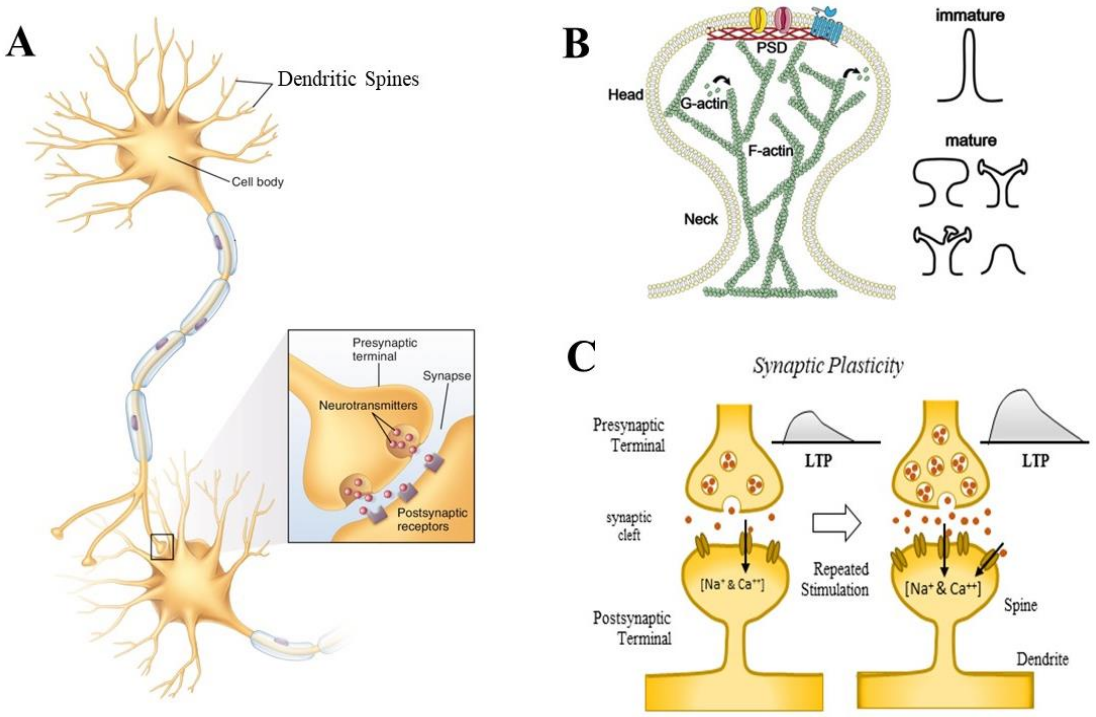

Figure 2.3 (A) *The structure of the synapse,* ***(B)*** *The various morphology types of dendritic spines [57],* ***(C)*** *diagram depicts how repetitive synaptic stimulations LTPs are linked to molecular changes [58].*

2.4.1.3. The post Synaptic density

The postsynaptic density (PSD) represents an intricate protein structure situated within dendritic spines, predominantly positioned at the spine head in close proximity to the presynaptic active area. Comprising an array of proteins such as scaffolds, receptors, and signal transmitter molecules, the PSD orchestrates synaptic transmission by ensuring the alignment of neurotransmitter receptors opposite the active zones. This organizational mechanism facilitates the efficient release of neurotransmitters into the synaptic transmission via vesicle exocytosis from clusters of synaptic vesicles attached to the active area in the presynaptic [59-61]. The PSD is depicted under microscopy as an electrons-dense thickened in the postsynaptic cytoplasmic membrane. However, new research employing super-resolution imaging techniques have explored the molecular structure of synapses and found that the key postsynaptic proteins are arranged into nanoclusters in the PSD [62-64]. One pivotal protein that plays a role within the postsynaptic density of excitatory glutamatergic synapses is the SHANK3 gene [65], as illustrated in **Figure 2.4**.

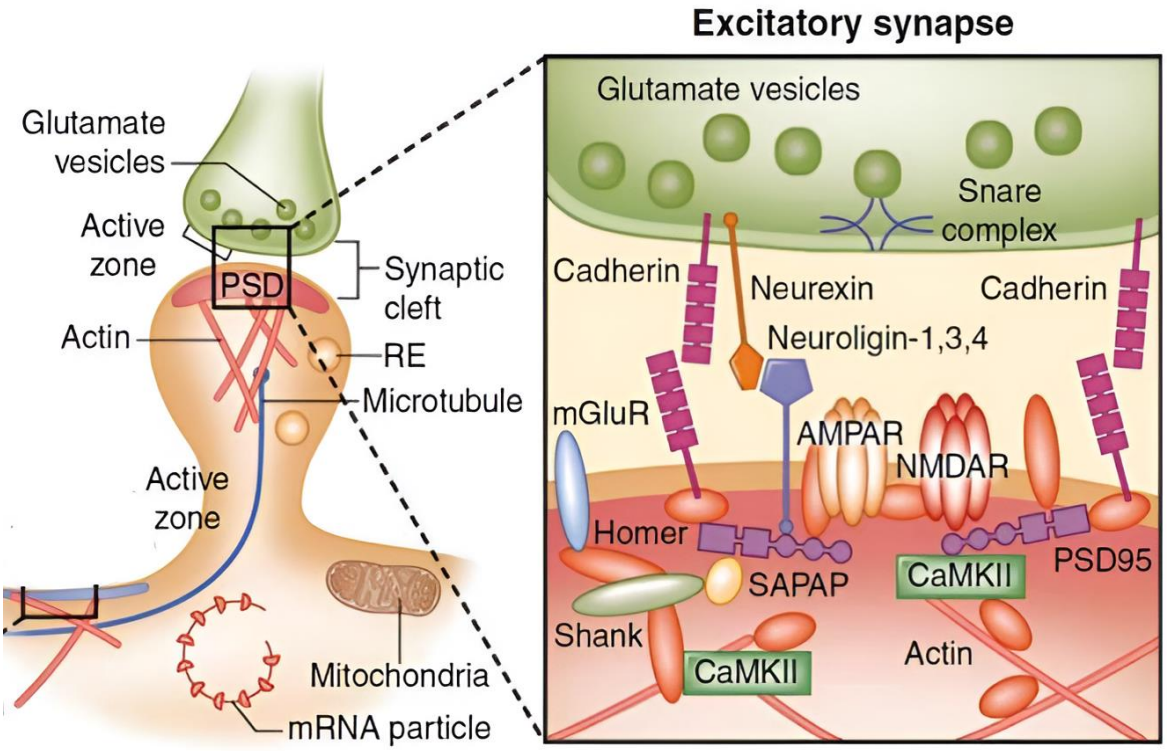

Figure 2.4 *The morphology of the post-synaptic density in excitatory synapses (Taken from [61])*

2.5. Shankopathies

One of the extensively investigated scaffolds of excitatory synapses is the family of SHANK gene, which has engaged in the etiology of diverse neurodevelopmental and psychiatric conditions, including ASD. The SHANK1, SHANK2, and SHANK3 genes encode SHANK proteins [65]. Although primarily associated with the central nervous system (CNS), SHANK expression extends beyond the CNS. Specifically, the SHANK3 gene demonstrates ubiquitous expression across various human tissues [66]. In glutamatergic synapses, SHANK3 is a vital element of the pallial layer within the PSD, where it forms subsynaptic clusters or nanoregions [62].

The deficiency of SHANK3, resulting in reduced function of synapses and impaired inter-neuronal communication, may be implicated in developmental delay, intellectual disability, and the absence or severe delay of speech [67]. Mutations in the SHANK3 gene have also been identified in individuals diagnosed with ASD. Many of these mutations lead to a decrease in SHANK3 protein levels or alter its function, thereby

disrupting neuronal communication. This dysregulation is believed to play a significant role in the development of ASD [68].

Moreover, SHANK3 has been implicated in synaptic dysfunction. Prior research indicates that synaptic malfunction can trigger microglial activation, potentially influencing neuronal communication [69, 70]. Excessive microglial activation may induce chronic neuroinflammation, which could impact various physiological processes, including angiogenesis [71]. Previous studies have observed signs of neuroinflammation and deficits in angiogenesis in several instances of ASD [72, 73]. Therefore, these pathological disturbances may significantly contribute to the pathogenesis of ASD [17]. While these observations highlight correlations, it is essential to note the dynamic nature of the field, necessitating ongoing research to elucidate the precise pathways and processes linking mutations in SHANK3, synaptic malfunction, and neuroinflammation in ASD. The interaction between genetic and environmental agents is intricate, and forthcoming investigations are likely to offer more comprehensive insights into this association.

2.5.1 The structure of the SHANK3 gene

SHANK3 is located within the crucial region of 22q13.3 [74]. SHANK3 is structured into several distinct domains along with a proline-rich segment, housing binding sites for various proteins such as homer1 and cortactin. These domains encompass a SHANK/ProSAP N-terminal (SPN) domain, an ankyrin repeat region (ARR), a Src homology 3 (SH3) domain, succeeded by a PSD-95/Discs large/ZO-1 domain (PDZ), and a C-terminal sterile alpha motif (SAM) [65]. The SH3 and PDZ domains of SHANK3 have been demonstrated to link with the C-terminal segment of the voltage-gated L-type Ca^{2+} channel Cav1.3. This interaction has been established as essential and adequate for the synaptic clustering of Cav1.3 [75]. The PDZ domain of SHANK3 has been found to interact with additional binding partners, such as SAPAP1 and the GluA1 subunit of AMPARs [76]. Furthermore, the proline-rich stretch, situated between the PDZ and SAM domains and containing binding sites for homer1 and cortactin, functions as a crucial connector to the spinous F-actin cytoskeleton and other scaffolds within the PSD [77]. Ultimately, the SAM domain plays a vital role in directing the localization of SHANK3 to dendritic spines and facilitating activity-dependent oligomerization in the PSD [78, 79], as shown in **Figure 2.5**.

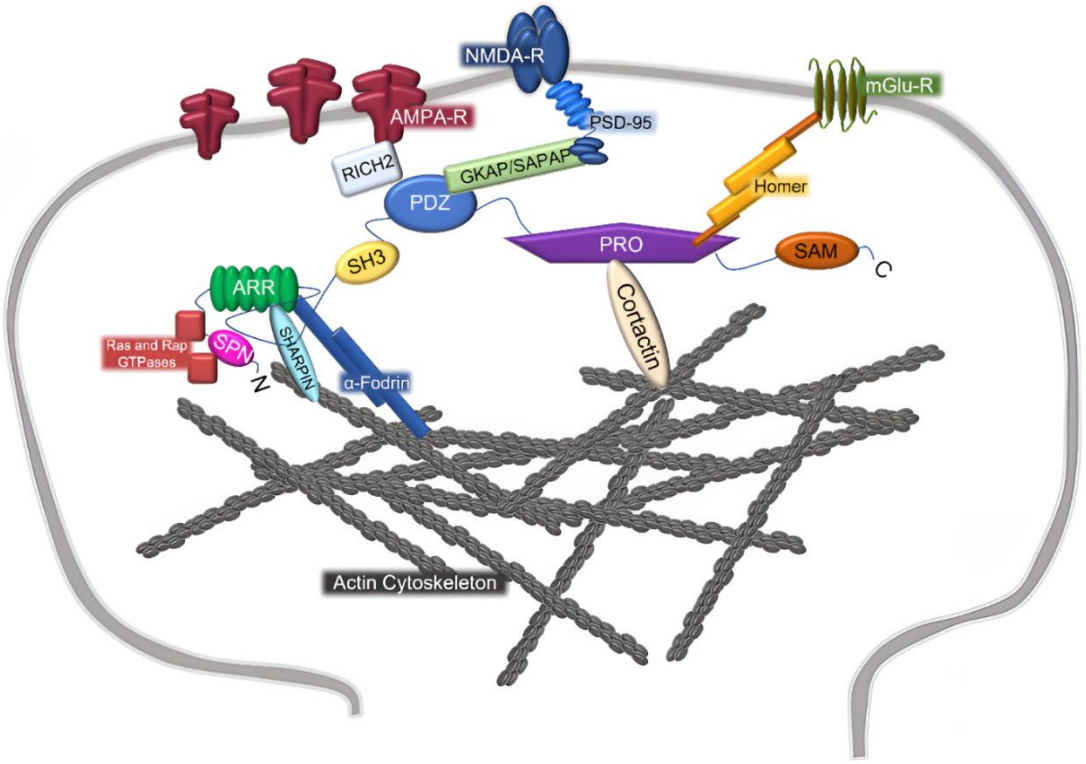

Figure 2.5 *Schematic illustrates the structure of the SHANK3 gene and the protein partners (Taken from [80]).*

2.5.2 The structure and function of the SHANK3 N-terminus

Recently, the structure of an N-terminal SHANK3 fragment comprising the residues 2 - 347 (PDB: 5G4X), including the SPN and ARR domains, has been solved and provided insights into the potential role of point mutations located in this region [66]. The SPN domain constitutes one of the essential domains of SHANK3 and adopts a conformation resembling a ubiquitin-like (Ubl) domain analogous to the F0 domain of the Talin domain. The SPN interacts with signaling proteins relevant to the regulation of F-actin structure and dynamics, such as R-Ras, H-Ras or Rap1, all belonging to the Ras superfamily of small GTPases [66, 81]. This interaction was shown to inhibit integrin activation by sequestration of active Rap1 and R-Ras [66]. In contrast, the ARR domain interacts with cytoskeletal proteins such as α-Fodrin, the cell adhesion protein δ-catenin or sharpin (a cytosolic signaling protein), a component of the linear ubiquitin chain assembly complex [82]. In the three-dimensional structure, the seven ankyrin repeats of SHANK3 are linked (by a 19 amino acid long linker region) to the 90 amino acid conserved SPN domain. This structure confirms biochemical data indicating that the

SHANK3 SPN domain is engaged in an intramolecular interaction with ARR and forms a large interface with this region [83].

2.5.3. The Intra-Domain Interaction between the ARR and SPN Domains

Previous investigations have shown that the ARR and SPN domains interact, and this interaction is critical to a possible autoregulatory mechanism between the domains [83]. It was previously identified that at the interface of the SPN and ARR domains, there are several polar and charged residues that oppose one another, that could form electrostatic interactions between the two domains [66], as shown in **Figure 2.6**. The linker region between the ARR and SPN domains is also likely to be closely associated with the two domains. This flexible linker region would allow for a dynamic interaction between the domains, acting like a hinge in the 'open' conformation, further supporting this as a regulatory mechanism [83, 84].

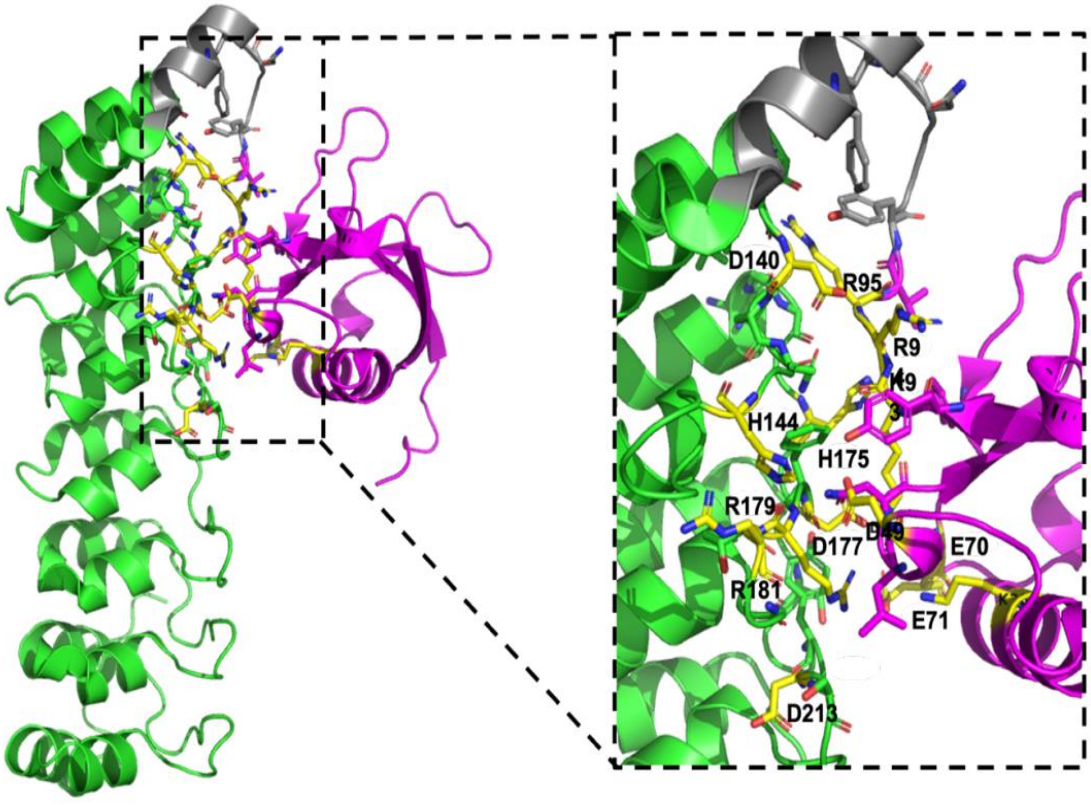

Figure 2.6 *The electrostatic interactions between the SPN (pink) and ARR (green) domains. Polar and charged side chain residues at the interface are colored as yellow sticks. The linker region is depicted in grey.*

2.5.4. SHANK3 N-terminus protein partners

Numerous interaction partners link to SPN, ARR domains, or even the loop structure between SPN and ARR, which serves as a specific site for the interaction with protein mediators. Recent reports have highlighted the involvement of the linker domain connecting both regions, along with a segment of the SPN, in establishing a binding surface for Ca2+/calmodulin-dependent kinase IIα (αCaMKII) which is a crucial element in synaptic plasticity and learning processes, plays a pivotal role in decoding synaptic Ca^{2+} oscillations, regulate calcium concentrations, PSD assembly, and dendritic spine morphology. This binding occurs in its inactive state, non-phosphorylated, and requires a closed configuration of the SPN-ARR tandem [85, 86]. Furthermore, the SPN domain has been proven to have a strong affinity for various Ras group G-proteins, including Rap1a and Rap1b, with two binding sites for Rap1 situated on the N-terminus SHANK3. The interaction between signalling proteins and SPN is crucial in regulating the structure of F-actin [66, 81].

On the other hand, α-Fodrin, sharpin, and δ-catenin have established interactors with the ARR domain. An emergent association involves δ-Catenin as a novel interacting partner of Shank3, specifically binding to the ARR domain [82]. SHANK3 assumes a pivotal role in the postsynaptic localization and stabilization of Catenin proteins, wherein δ-Catenin-mediated regulation of spine density necessitates interactions facilitated by SHANK3 [87]. δ-catenin is expressed in excitatory synapses and functions as an anchor for the glutamatergic AMPA receptor (AMPAR) GluA2 subunit in the postsynaptic density [88]. Conversely, the absence of δ-Catenin results in a reduction in both overall excitatory synapse density and active synapses expressing the GluA subunit of the AMPA receptors [89, 90]. Notably, CTNND2 is a δ-Catenin gene, and its deletion is associated with the cri-du-chat syndrome (CDCS), characterized by severe cognitive and language impairments, motor delays, and behavioral challenges [91]. Moreover, CTNND2 has emerged as a candidate ASD gene, as loss-of-function mutations in this gene, such as deletions and unbalanced translocations, are linked to severe ASD [92, 93]. Furthermore, earlier research indicated that intramolecular interaction prevents α-Fodrin from accessing its location on the ARR domain in SHANK3 [83] since the α-Fodrin protein provides another link to the actin cytoskeleton by direct interaction with the ARR SHANK3 [94].

2.6. The interactions between genetic alterations in synaptic proteins and brain immune activation

The genetic modifications occurring in synaptic proteins may stimulate the brain's immune system, leading to an activated state known as "immune-synaptopathy." In this context, dysfunctional synapses or circuits may emit signaling cues to other brain cells possessing immune functions, such as microglia and astrocytes, through molecular signaling, thereby triggering immune system activation. The potential interpretation of this immune system activation as a compensatory response to address impaired synapses or circuits presents an interesting area that is worthy of further investigation.

2.4.2. Neuroinflammation

Neuroinflammation is an intricate process that occurs when the brain reacts to various stimuli. It is a significant hallmark of numerous clinical disorders [95]. Microglia are a primary responders to any insult to the brain parenchyma, translating the signals into diverse molecules. These microglia-derived molecules can regulate the stimuli-dependent reactivity of astrocytes. Once activated, astrocytes, in turn, can control microglia phenotypes [96]. Recent evidence indicates that the crosstalk between these glial cells plays an important role in delaying or accelerating neuroinflammation and overall disease progression [96-98]. Persistent neuroinflammation plays a pivotal role in the progression of various neurodevelopmental conditions by instigating an excessive release of proinflammatory cytokines, which in turn triggers significant pathological alterations and neurobehavioral complications [99]. Increasing evidence suggests that neuroinflammation serves as a fundamental contributor across a spectrum of conditions, spanning from neurodevelopmental disorders like ASD to neuropsychiatric conditions such as schizophrenia. While the accurate pathophysiological mechanisms underlying ASD remain incompletely understood, growing research supports the involvement of neuroinflammation in the etiology of ASD disorder [100-102]. This is evidenced by sustained activation of microglia and astrocytes observed in postmortem brains [103-105], along with elevated levels of cytokines and chemokines detected in cerebrospinal fluids (CSF) [106-108], as illustrated in **Figure 2.7**.

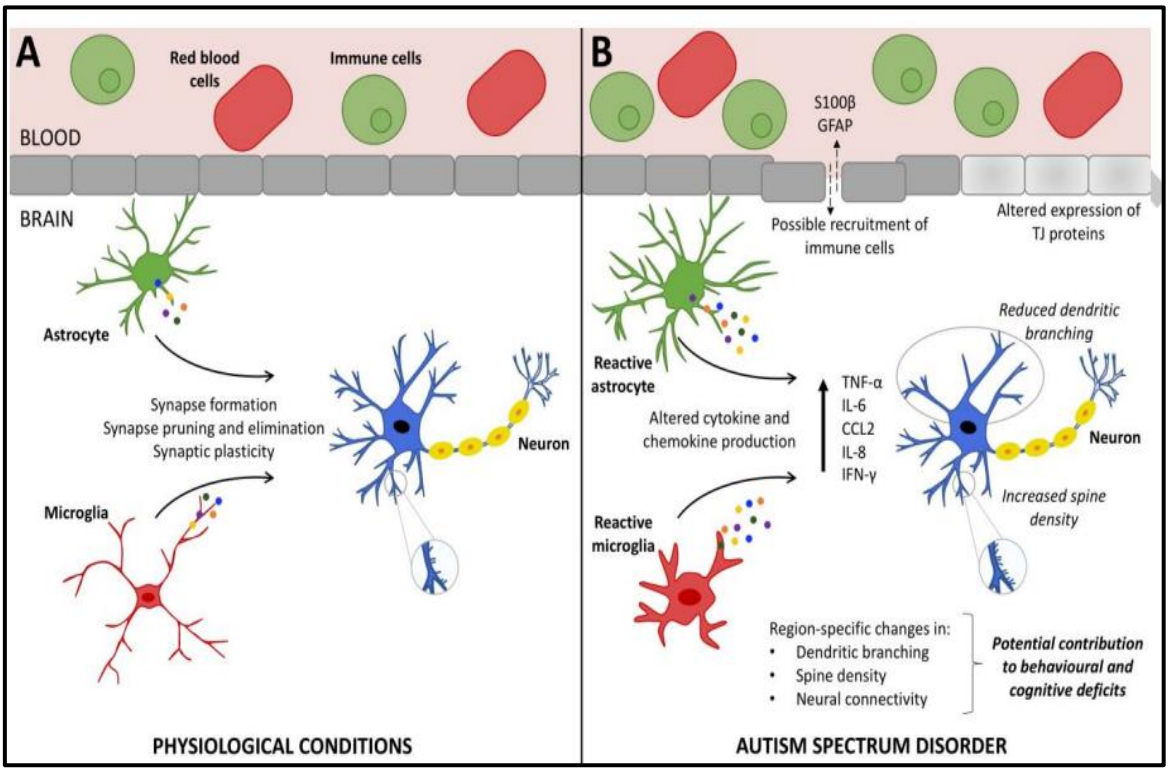

Figure 2.7 *Diagram showing the potential impacts of reactive astrocytes and microglia in the brain of an ASD patient* ***(A)*** *Under normal physiological conditions.* ***(B)*** *In the case of ASD condition. BBB: Blood-brain barrier (Taken from [73]).*

2.4.2.1. Microglia

Microglia, the first type of glial cells, originate in the brain from the embryonic yolk sac [109, 110] and migrate along the course of the fibers of the corpus callosum to all parts of the brain [111]. Microglia act as the primary immune cell in the CNS and form the first line of defence by regulating immunological and inflammatory responses [112]. The activation of microglia is a phenotypically and functionally diverse process that depends on the type of stimulus and cellular contexts [113]. Microglia can be induced into two distinct types of activation, M1 (classical activation state) and M2 (alternative activation state), which might lead to different phenotypic characteristics and secretion profiles from those of macrophages [114] M1-like microglia (so-called pro-inflammatory microglia) and M2-like microglia (so-called anti-inflammatory microglia) exert respectively detrimental and beneficial effects, depending on their intrinsic properties and the interaction with cellular microenvironments [115] M1-like microglia generate a detrimental microenvironment for neurons by producing inflammatory cytokines and reactive oxygen species (ROS) [116], while M2-like microglia produce a supportive

microenvironment for neurons by producing anti-inflammatory cytokines and neurotrophic factors [117, 118]. Although this type of dichotomic classification provides a framework for exploring the diverse functions of microglia, this classification appears to oversimplify the activation status of microglia [119]. Emerging studies indicate the activation of microglia as a dynamic process, which occurs in the form of a continuum across the M1 and M2 phenotypes [120]. Microglia perform a wide range of physiological functions in the healthy brain, involving the development of synaptic networks [121, 122], stimulation of developmental apoptotic[123], positioning of neurons within the developing cortex and secretion of growth factors for neuronal survival [124]. The biological significance of microglia engagement can be either neuroprotective or neurotoxic, depending on the balance between their anti-inflammatory versus pro-inflammatory activities [125], leading to containment or aggravation of disease progression [113]. Consistent with this variety of functional roles, microglia can take on a range of phenotypes, including ramified, primed, reactive and amoeboid microglia [126]. The morphological changes indicate that the microglia have detected a change in homeostasis, but they do not specify a particular response state or type of activity in any given disease [121]. Increasing evidence highlighted the significant involvement of microglia in the pathophysiology of neuroinflammation especially through their ability to interact with mast cells, neurons, and other glia [127]. Although astrocytes also have some of these properties [128]. Astrocytes and microglia are, however, highly dynamic and in addition to regulating neuroinflammation they are involved in homeostatic processes such as synapse formation, pruning and plasticity [73]. Besides, the activation of microglia has been implicated in the pathogenesis of various disorders, ranging from neuropsychiatric disorders such as schizophrenia to neurodevelopmental disorders such as ASD [129].

2.4.2.2. Astrocyte

Astrocytes are critical in maintaining physiological homeostasis within the CNS, with important roles in supporting neuronal function, glial transmission and signalling via Ca^{2+} release and uptake [130]. For example, astrocytic glutamate release can affect both pre- and post-synaptic neuronal activity. The idea that astrocytes are integral to pre- and post-synaptic function has led to the concept of a ‘tripartite’ synapse and places astrocytes amongst the central pathways of neuronal function [131]. Cytokines released

during the initiation of the immune response stimulate astrocytes to undergo reactive gliosis characterized by upregulation of astrocyte intermediate filament proteins, most notably glial fibrillary acidic protein (GFAP) [132]. Similar to microglia, reactive astrocytes undergo morphological changes and proliferate to mount an immune response. In contrast with microglia, however, they extend rather than retract their processes and undergo hypertrophy to enable this process [133]. Astrocytes and microglia express different biochemical markers. Specifically, astrocytes express excitatory amino acid transporters (EAATs) 1-5, whereas microglia lack EAAT4 [134]. Moreover, astrocytes express a larger number of EAATs than microglia and as a result, glutamate uptake by astrocytes plays a significant role in maintaining extracellular glutamate levels [135]. A prominent role in glutamate uptake enables astrocytes to facilitate precise spatial and temporal transmission of glutamatergic neurotransmission, thus regulating the activity of surrounding synapses[136]. Astrocytes are also less morphologically reactive than microglia, potentially indicating a secondary role in responding to infiltrating stimuli. Indeed, it has been a point of controversy as to whether microglia or astrocytes are the initial responders to external threats to the immune system, with recent evidence suggesting that reactive microglia drive the induction of neuroprotective effects [137] or neurotoxic activity [138] in a subset of reactive astrocytes. Nonetheless, both microglia and astrocytic populations are also modulated via bidirectional communication [96].

2.4.2.3. The impact of cytokines and chemokines released through microglia-astrocytic communications

Bidirectional communication exists between microglia and astrocytes, and it modulates CNS inflammation through the inflammatory mediator and secretion of multiple cytokines. Consequently, the basis of neuronal function and dysfunction is microglia–astrocyte crosstalk [139]. Activated microglia secrete a variety of cytokines and chemokines that can induce astrocyte reactivity [140]. Indeed, the majority of cytokines, such as IL-1, IL-2, IL-6, Tumour Necrosis Factor alpha (TNF-α), and Interferon gamma (IFN-γ), have been reported to cause reacting astrogliosis in newborn mice when administered into stab wound regions via evaluated the presence of GFAP positivity [141]. Conversely, reactive astrocytes release adenosine triphosphate (ATP) to initiate microglial activation. Reactive microglia and astrocytes work together to form a glial scar at the location of an injury. This scar acts as a physical barrier to prevent additional

harm and induce the healing of the tissue [142]. Post CNS damage, astrocytes increase the production of chondroitin sulfate proteoglycans (CSPGs), which constitute a significant part of the glial scar [143]. CSPGs enhance the signaling pathways associated with the recruitment of reactive microglia and other immunological cells to the injury position, indicating a potential role of heightened astrocyte activity in microglial recruitment post-injury [144] suggesting that increased astrocyte activity can signal to further recruit microglia following injury. The ongoing interaction between reactive microglia and astrocytes, facilitated by bidirectional communication, leads to the prolonged release of pro-inflammatory molecules and reactive ROS that can induce vascular-endothelial malfunction and structural harm within the surrounding CNS tissue environment [145]. These agents collectively contribute to heightened permeability of BBB through various mechanisms, including modulation of expression and reorganization of cytoskeletal and tight junction proteins constituting BBB [146]. Peripheral immune cell infiltration is promoted by the production of chemokines on endothelial cells, such as CXCL12, which interact with leukocyte receptors to stimulate adhesion and transmigration across the blood-brain barrier [147]. Increased pro-inflammatory stimuli cause microglia to coordinate the flow of immunological cells from the blood vessels, thereby escalating the CNS inflammatory response [113]. Astrocytes subsequently assume a protective function by releasing various regulatory factors, including glial-derived neurotrophic factor and transforming growth factor β, that connect with endothelial cells to prevent the influx of peripheral leukocytes and macrophages across the damaged BBB and promote its repair [148]. Consequently, the sustained activation of microglia-astrocyte interactions, often persisting for days or months, is considerable as a pathological characteristic of neuroinflammation.

Scope and aim of the Thesis:

Studying SHANK3 mutations in ASD is of paramount importance due to the critical role SHANK3 plays in synaptic function and neuronal communication. Mutations in the SHANK3 gene have been implicated in a subset of ASD cases, leading to alterations in synaptic structure and function. Understanding the impact of point mutations on the dynamics, stability, folded conformation, and interactions with protein partners of SHANK3 protein was an unmet challenge in experimental studies. Consequently, the computational approach is essential for elucidating the underlying pathophysiology of

SHANK3 point mutations in ASD. Furthermore, insights gained from studying SHANK3 mutations may pave the way for the development of targeted therapeutic interventions aimed at restoring synaptic function and ameliorating the core symptoms of ASD.

Moreover, Investigating biomarkers from various types of tissues in ASD is crucial for enabling early diagnosis and intervention. ASD is a complex neurodevelopmental disorder characterized by heterogeneous symptomatology, making early identification challenging. Prior research have been successfully predicted biomarkers from brain tissues. Nevertheless, the genes may not possess predictive value if they are not expressed or modified in accessible tissues. Conversely, many high-risk genes were detected in various obtainable tissues, such as blood, saliva, and placenta, without confirming their crucial roles in the brain of ASD patients. In order to address the defined aims of this study as stated above, the following objectives have been framed:

[1] To investigate the impact of E71S mutation on SHANK3 conformational dynamics at the SPN-ARR interface.

[2] To investigate the effect of N52R mutation at the SPN-ARR tandem on the conformational dynamics of SHANK3.

[3] To investigate the impact of two point mutations P141A and L270M in the N-terminal of SHANK3 in post-synaptic function in ASD.

[4] To investigate the ASD-susceptibility risk genes and their potential role in neuroinflammation and unraveling potential biomarkers for early detection.

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