CHAPTER1

Motivation and Outline of the Thesis

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1.1. Motivation for the current study

Autism Spectrum Disorder (ASD) is a neurological condition identified by deficits in social interactions, including verbal and nonverbal communication abilities, repetitive or restricted behavioral traits, and atypical actions regarding sensory stimuli [1]. The ASD encompasses a spectrum of disorders, including Asperger syndrome, Autistic disorder, Rett disorder, and pervasive developmental disorder [2, 3].

The estimated incidence in accordance to the Autism and Developmental Disabilities Monitoring (ADDM) Network constructed by the Centers for Disease Control and Prevention (CDC) in the United States of America, the reported incidence of ASD amongst kids aged 8 was 90/10,000 in 2006 [4], increasing to 145/10,000 in 2012 [5]. The latest report, published in 2018, indicates that ASD impacts approximately 230 out of every 10,000 children [6], signifying a 243% increase in prevalence since the initial ADDM Network study conducted in 2000 [6]. Prior systematic reviews have indicated that the observed variations in prevalence estimates over time are likely not attributed to a genuine elevation in prevalence. Instead, they are linked to alterations and enhancements in diagnostic criteria, methodological approaches, research quality, and improved accessibility to diagnostic and intervention services. Furthermore, heightened awareness of ASD within professional and non-professional spheres, along with recognition of ASD's potential co-occurrence with other developmental disorders, contributes to these discrepancies [7-10].

The latest studies on global prevalence present some variability. For instance, Zeidan et al. [11] conducted a systematic review [9] examining 71 studies from 34 countries spanning 2012 to 2021, revealing a median prevalence of 100 per 10,000 children. Whilst Salari et al. [12] performed a meta-analysis encompassing 74 studies from 2008 to 2021, which yielded a pooled prevalence of 60 per 10,000.

Apart from temporal variations, additional factors contribute to heterogeneity, including geographic location, national economic status, research methodology employed, diagnostic criteria applied, age distribution within the sampled cohort, and various socio-demographic parameters [11-13]. The intricate interplay of these factors poses significant challenges in establishing a consistent and accurate global prevalence

estimate. Nonetheless, the compilation of such pooled prevalence data holds substantial importance in assessing the public health impact of the disorder. It facilitates resource allocation for ASD within nations lacking prevalence studies or are in the process of conducting them. Furthermore, it helps identify shortcomings in ASD identification and diagnostic practices in regions where prevalence rates fall below the global average. It sheds light on potential environmental risk factors in specific geographical areas [14]. The ASD patients necessitate substantial care, demanding considerable financial commitments. The combined direct and indirect expenditures for supporting individuals with ASD in the United States in 2015 were assessed at $268.3 billion, surpassing the costs associated with conditions such as hypertension and stroke. ASD places a significant economic strain on society and affects individuals' families [15]. In total, the annual expenses for education, healthcare, and other lifelong favors for an ASD patient range between $ 1.4 million and $ 2.4 million [16].

To evaluate the incidence of ASD in India, Arora et al. employed the INCLEN Diagnostic Tool for Autism Spectrum Disorder (INDT-ASD) screening tool, finding that approximately 1 in 100 children under the age of 10 in India exhibit autism [17]. Another investigation focused on determining the autism prevalence among a specific group of schoolchildren in Kolkata, India. The estimated prevalence was 0.23%. Nevertheless, considering that approximately 20% of children in this area are not attending school, the reported estimate of ASD prevalence is likely lower than the true prevalence [18].

Numerous investigations have been studied ASD, linking its etiology to genetic, immunological, perinatal, biochemical, environmental, and factors [19]. Despite ongoing research efforts to advance our comprehension of potential causative mechanisms in ASD, a singular underlying cause has yet to be definitively identified [20]. Genetic agents contribute to the susceptibility of ASD, evidenced by the heightened risk of diagnosis among siblings of ASD patients more significant, albeit not absolute, concordance of autism diagnosis in monozygotic twins [21].

ASD encompasses diverse mechanisms and neurodevelopmental pathways. The most significant and crucial pathophysiological trajectories associated with ASD are synaptopathies and neuroinflammation [22-24]. A key gene implicated in synaptopathy is SHANK3, which holds a central position in governing synaptic construction and activity within excitatory neurons [25].

The recognition of pathological mutations associated with ASD within the N-terminal domains of SHANK3 underscores the significance of this region, emphasizing the necessity to elucidate its underlying mechanisms for comprehending the etiology of ASD. Earlier investigations and documented findings have indicated that stability and interactions between the SPN and ARR domains N-terminal are pivotal in shaping SHANK3 characteristics [26]. Nevertheless, the precise molecular intricacies governing remained an unmet challenge [26].

Additionally, recent findings in both animal models and humans supported a pivotal role of the neuroimmunity system in ASD pathogenesis [27, 28]. Prior research has demonstrated an association between maternal immune activation (MIA) during pregnancy and neurodevelopmental phenotypes, including autistic-like behaviors in newborns [29, 30]. Although these studies provide evidence for the involvement of immune genes in ASD, it is crucial to reveal the complexity of immune system genetics and the diverse pathways of immune genes [31].

Moreover, genetic alterations in synaptic proteins may activate the brain's immune system, a phenomenon termed "immune-synaptopathy." Dysfunctional synapses or circuits can communicate with immune-responsive brain cells like microglia and astrocytes, activating the immune system through signaling mechanisms. The possibility that this activation represents an adaptive response to address defective synapses or circuits is an intriguing avenue for investigation [32]. Hence, previous investigations have experimentally examined the effects of point mutations in the N-terminal region of SHANK3 on post-synaptic function in ASD. Additionally, they have explored the involvement of neuroinflammation in the pathogenesis of ASD. However, the identification of high-risk genes in the brain and accessible tissues for detecting reliable and potential biomarkers for early diagnosis remains an unresolved challenge.

Therefore, employing computational analysis to explore these two significant pathomechanisms involved in ASD offers an alternative method for understanding the atomic-level interactions that are challenging to discern through experimental approaches. Recent research emphasizing synaptopathies and neuroinflammation has inspired my focus on investigating the involvement of SHANK3 variations and neuroimmune dysfunction in ASD pathogenesis, thereby establishing the theme of my thesis as a computational investigation into the role of SHANK3 in ASD and the

identification of potential biomarkers for early diagnosis. Thus, this thesis endeavors to address two broad aspects concerning this specific topic: (1) To study the four point mutations in the SHANK3 gene and (2) To investigate the role of the neuroinflammation pathway and detect reliable biomarkers for early detection of ASD. To conduct this work, Molecular Dynamics (MD) simulation with the AMBER 14 software package and R programming with DESeq2 and Clusterprofiler packages have been our primary methodologies.

1.2. Outline of the Thesis:

Chapter 2 The chapter elucidates the manifestations of autism spectrum disorder and its etiology, with a specific focus on genetic factors, and highlights significant pathomechanisms implicated in ASD, including synaptopathy and neuroinflammation. Notably, the SHANK3 gene assumes a pivotal role in post-synaptic density, particularly concerning N-terminal mutations that influence ASD development. Furthermore, it depicts the role of neuroinflammation, the interactions between microglial and astrocytic cells, and cytokine production.

Chapter 3 provides a comprehensive literature review, offering an overview of pertinent research endeavors in the field. The chapter elucidates the functional implications of point mutations occurring in the N-terminal region of SHANK3, which are closely associated with ASD. Additionally, it examines the involvement of neuroinflammation and various cytokines in the context of ASD.

Chapter 4 details the methods utilized to accomplish the computational work, as well as the analytical trajectory employed to investigate the roles of point mutations in N -the terminal. Furthermore, describes the steps to define the differentially expressed genes in two different types of tissues.

Chapter 5 is dedicated to the impact of E71S mutation on SHANK3 conformational dynamics at the SPN-ARR Interface, as well as its effect on the interactions with two protein partners, αCaMKII and α-Fodrin.

Chapter 6 highlights the effect of N52R mutation at the SPN-ARR interface on the stability, compactness, and intramolecular interactions between the SPN-ARR tandem.

Chapter 7 depicts the results of the two point mutations (L270M, P141A) found in ASD patients on the conformational dynamics of N-terminal SHANK3.

Chapter 8 presents the findings of significant differentially expressed genes in two types of tissues, besides the common genes as promising biomarkers for early diagnosis of ASD.

Chapter 9 presents a collective conclusion of the significant results, as well as future prospects of the research.

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