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Assessment of SHANK3, UBE3A, Dopamine Levels, and *CACNA1H* Gene Expression in Autism Spectrum Disorder: A Case-Control Study.

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Abstract:

This study investigated dopamine levels and changes in UBE3A, SHANK3, and *CACNA1H* gene expression in children with autism spectrum disorder (ASD) to enhance understanding of the biological changes associated with the disorder. The study was conducted at Al-Zahra Teaching Hospital in Al-Kut, Iraq, involving 50 children with ASD (40 males and 10 females), aged 3–12 years, and 25 healthy controls. Blood samples were collected from all participants. Dopamine, SHANK3, and UBE3A levels were measured using ELISA, while *CACNA1H* gene expression was assessed by real-time PCR. Dopamine levels were significantly lower in ASD children (26.49 ng/L) than controls (44.5 ng/L; $P<0.05$), whereas SHANK3 levels were significantly higher (501.24 vs. 335.82 ng/L; $P<0.05$). UBE3A showed a non-significant increase (6.71 vs. 5.2 ng/ml). *CACNA1H* expression significantly decreased to 0.04-fold compared with controls (1.0).

Keywords: SHANK3, UBE3A, Dopamine, *CACNA1H* Gene, Autism Spectrum Disorder.

1.Introduction

Autism Spectrum Disorder (ASD) represents one of the most complex and heterogeneous neurodevelopmental disorders currently recognized in the field of psychiatry and neuroscience. Marked by impairments in social communication and the presence of restrictive and repetitive behaviors, ASD manifests across a broad spectrum of severity and presentation. The disorder's clinical, genetic, and neurobiological complexity continues to challenge both researchers and clinicians in their efforts to understand its etiology, improve diagnostic practices, and develop effective interventions [1].

Epidemiologically, ASD is one of the most prevalent neurodevelopmental conditions, with recent estimates suggesting a global prevalence of approximately 1 in 100 individuals [2]. In high-income countries, such as the United States and the United Kingdom, prevalence rates have risen dramatically [3-4]. Males are diagnosed more frequently than females, with an estimated ratio of 4:1 [5].

Diagnosis of ASD typically involves comprehensive behavioral assessments guided by criteria outlined in the DSM-5. Tools such as the Childhood Autism Rating Scale (CARS) assist clinicians in differentiating ASD from other developmental disorders [6-7].

Recent advancements in neurobiology and genetics have begun to unravel mechanisms underlying ASD. Neurotransmitters such as dopamine [8] and genetic mutations in SHANK3 and UBE3A, have been identified as relevant to ASD's pathogenesis [9-10]. Additionally, the *CACNA1H* gene encodes the α1H subunit of T-type calcium channels (Cav3.2), which are crucial for neuronal excitability and neurotransmission. Mutations in *CACNA1H* have been linked to altered calcium signaling and ASD risk [11-12].

2. Material and Methods

2.1. Study Sample Description

The study included 75 children aged 3–12 years, comprising 50 children with autism spectrum disorder (ASD) and 25 healthy controls. Most ASD cases were aged 5–8 years. The ASD group included 40 males (80%) and 10 females (20%) recruited from Al-Zahra Teaching Hospital, Al-Kut, Iraq.

2.2. Diagnosis of ASD

ASD diagnosis was confirmed by a consultant psychiatrist using clinical evaluation and the standardized Childhood Autism Rating Scale, Second Edition (CARS-2), which was also used to assess symptom severity.

2.1. Study Sample Description:

2.3. Methods

Serology study:

Three milliliters of venous blood were collected from each participant. Samples were allowed to clot at 37°C for 30 min, centrifuged at 5000 rpm for 5 min, and the separated serum was stored frozen until ELISA analysis [13].

Molecular study:

Total RNA was extracted using TRIzol® LS Reagent according to the manufacturer's protocol [14]. Primers for *CACNA1H* and the *GAPDH* reference gene were designed using Primer3 (v4.1.0), verified using UCSC, and synthesized by Alpha DNA Ltd. (Canada) Table (1). RNA was reverse-transcribed into cDNA using the Easyscript® Kit in a 20 µl reaction. Gene expression was quantified by qRT-PCR using TransStart® Top Green qPCR Super Mix (SYBR Green). *GAPDH* was used as the endogenous control for normalization.

2.4. Statistical Analysis:

Statistical analyses were conducted using SPSS 27 (2020). Data are presented as mean ± standard deviation. one-way ANOVA, independent T-test and Pearson correlation coefficient were used to assess significant differences and correlations among markers at $P \leq 0.05$.

Table (1): The Primers Sequence that Used in the Study [15].

Primer	Sequence (5′→3′ direction)	Primer size per base pair	Product size per base pair	Temperature in Celsius
Calcium Voltage-Gated Channel Subunit Alpha1 H Gene (*CACNA1H*)				
Forward	AACCTGGGCCTTCTTTTCAT	20	195	59.94 C°
Reverse	CTTCATGATCCCGTTCCAGT	20		59.93 C°
Glyceraldehyde 3-Phosphate Dehydrogenase Gene (*GAPDH*)				
Forward	GGTCTCCTCTGACTTCAACA	20	135	60.3 C°
Reverse	AGCCAAATTCGTTGTCATAC	20		61.6 C°

3. Results:

3.1. SHANK3, Dopamine, and UBE3A Levels

Table (2) shows the SHANK3 level (ng/L), Dopamine (ng/L), and UBE3A(ng/ml) in autism patients and healthy controls.

In study conducted by Delling and Boeckers, (2021) findings animal models with *SHANK3* deficiency exhibit behavioral, cognitive, and motor impairments similar to neurodevelopmental disorders like ASD

and Phelan-McDermid syndrome (PMDS) [16].

Table (2): SHANK3, Dopamine, and UBE3A Levels in Autism Patients and Healthy Controls. N: Number; †: Independent Samples T-Test; NS: Not Significant at *P≤0.05*; S: Significant at *P≤0.05*.

Parameters	Group	N	Mean ± Standard Deviation	P-value
SHANK3 (ng/L)	ASD Patients	50	501.24 ± 185.76	0.009 † S
	Control	25	335.82±133.53	
UBE3A (ng/ml)	ASD Patients	50	6.71 ± 2.32	0.155 † NS
	Control	25	5.2 ± 1.72	
Dopamine (ng/L)	ASD Patients	50	26.49 ± 10.01	0.001 † S
	Control	25	44.5 ± 13.07	

A study investigates how autism-associated *SHANK3* missense mutations affect protein structure, dynamics, and stability at synapses. Findings suggest that these mutations alter *SHANK3* conformational fluctuations, leading to changes in protein turnover, which may contribute to synaptic dysfunction in autism. In this research, the amount of SHANK3 protein decreased. These mutations affected the protein's conformational stability, leading to increased degradation and altered protein turnover at synapses [17].

Another study explores the relationship between the loss of SHANK3 protein and autism spectrum disorder/intellectual disability by examining the rate of brain protein synthesis in genetically modified mice lacking this gene. An increase in brain protein synthesis rates was observed in *SHANK3* knockout mice compared to wild-type mice. This increase was evident in several brain regions associated with cognition and neural communication. These findings suggest that the loss of *SHANK3* not only affects synaptic structure but also leads to dysregulated protein synthesis, which may be a key factor in neurological disorders such as autism and intellectual disability [18].

In 2021 study, A recurrent frameshift mutation in the *SHANK3* gene, known as p. Ala1227Glyfs*69, has been identified. This mutation occurs at codon 1227, where the amino acid alanine (Ala) is replaced by another amino acid, leading to a shift in the reading frame and the introduction of a stop codon after 69 amino acids. This mutation has been found in approximately 0.08% of individuals with autism spectrum disorder (ASD). This mutation leads to loss of protein function or abnormal protein production. The neurological effects of this mutation have been demonstrated through genetic

and functional studies. The findings support the role of SHANK3 as an important factor in ASD [19].

Kasherman *et al.,* (2020) focuses on the role of the Ubiquitin system as a major organizer of cellular functions, especially with regard to mental disabilities and ASD disorders. The Ubiquitin system is a major organizational axis of nervous development, and its disorders may be a major factor in the emergence of mental disabilities and autism [20].

The research examines the effect of the autism-linked *UBE3A* mutation when inherited from either the mother or father in mice. The *UBE3A* gene is important for brain function and is normally expressed from the maternal copy in neurons. research findings the mutation that increases UBE3A activity affects interneurons, cells that regulate the activity of neural circuits in the brain. When the mutation was inherited from either the mother or father, the mice exhibited behavioral changes, including autism-related behaviors such as anxiety and impaired social interaction. The effects were not limited to the maternal copy; they also occurred when inherited from the father, suggesting that increased UBE3A activity can disrupt brain function in unexpected ways [21].

A study concluded that *UBE3A* gene mutations may not be a major contributor to autism spectrum disorder in the Chinese Han population (samples consisted of 192 patients with autism according to the DSM-IV diagnostic criteria and 192 healthy controls), as no significant differences in allele frequencies were found between patients and controls [22].

Study focused on the effects of increased gene dosage of the *UBE3A* gene on autism and its impact on neural connectivity, behaviors, and transcriptome (gene expression) in mice. The research found that increased *UBE3A* gene dosage affects males and females differently. Males appear to be more susceptible to the negative effects of this disorder than females. The research found that increasing the dose of UBE3A affects neural connections in the brain, leading to changes in neural structure that in turn affect behavior. The effects on gene expression and their relationship to increasing *UBE3A* dosage were studied. The results showed changes in the gene expression of several genes that may contribute to symptoms associated with autism [23].

Mutations in *UBE3A* or its dysregulation are associated with autism symptoms. Abnormally high activity leads to synaptic dysfunction and the

stereotypical behaviors associated with autism. UBE3A could also be a biomarker for early diagnosis of autism. Analyzing its expression in blood or brain tissue may help understand symptom severity and the potential for therapeutic intervention [24].

Pavăl, (2017) proposed his "dopamine hypothesis of ASD". This hypothesis postulated that autistic behavior is caused by dysfunctions in the dopaminergic system of the midbrain. To be more specific, a malfunction of the mesocorticolimbic circuit is associated with social deficiencies, while a dysfunction of the nigrostriatal circuit is associated with stereotypical behaviors [25].

Researchers investigated dopamine (DA) neurotransmission in an autism spectrum disorder (ASD) mouse model with elevated expression of eukaryotic initiation factor 4E (eIF4E). Using an integrative approach that combined genetic manipulation, behavioral testing, synaptic physiology, and imaging techniques, they found that increased eIF4E expression leads to behavioral inflexibility and impaired striatal DA release. The impairment in DA signaling was linked to dysfunctional nicotinic acetylcholine receptor activity, which disrupts calcium dynamics in dopaminergic axons. These findings highlight a mechanistic pathway through which translational dysregulation affects DA neurotransmission and contributes to core behavioral symptoms of ASD [26].

A review employed a comprehensive literature analysis focusing on the role of dopamine dysfunction in autism spectrum disorder (ASD), with particular attention to partial D2/D3 receptor agonists such as aripiprazole and cariprazine. The authors synthesized findings from preclinical and clinical studies evaluating these agents' efficacy and safety profiles. Results indicated that aripiprazole effectively reduces irritability in ASD patients, while preliminary data on cariprazine suggest potential benefits in addressing core and associated symptoms. Both agents demonstrated favorable tolerability compared to traditional antipsychotics. The review concludes that D2/D3 partial agonists are promising therapeutic candidates [27].

Transgenic mice serve as valuable models for investigating the effects of autism spectrum disorder (ASD) risk genes on neuronal function, particularly within the dopamine system. Individuals with ASD may possess de novo missense mutations in the *SLC6A3* gene, which encodes the dopamine transporter, but such occurrences are few [28]. Research

on mouse models with ASD-associated mutations has shown compromised dopamine transmission in the striatum and modified social behaviors typical of ASD [29]. Mutations in the *SHANK3* gene are significantly linked to ASD, and Shank3 knockout mice, when introduced to a novel environment, demonstrate heightened dopamine release in the tail of the striatum along with various behavioral symptoms akin to ASD, such as repetitive behaviors [30].

3.2.2. *CACNA1H* Gene Data

Table (3) shows the *CACNA1H* gene folding in autism patients and healthy controls.

Table (3): *CACNA1H* Gene Folding in Autism Patients and Healthy Controls. N: Number; †: Independent Samples T-Test; S: Significant at *P≤0.05*.

Group	N	Mean ± Standard Deviation	P-value
ASD Patients	50	0.04 ± 0.01	0.009 † S
Control	25	1.0	

The study investigated the role of rare variants in voltage-gated calcium channel (VGCC) genes in autism spectrum disorder (ASD) by analyzing whole genome sequences from 105 families. Researchers identified 53 rare damaging inherited variants in VGCC genes in 40 families, with particular focus on biallelic missense variants in the *CACNA1H* gene, which encodes the Cav3.2 T-type calcium channel. Electrophysiological analysis revealed that three out of four *CACNA1H* variants slightly altered channel function, potentially disrupting calcium signaling in neurons. These findings support the involvement of *CACNA1H* variants in ASD, highlighting their potential contribution to ASD susceptibility [31].

A study found that *CACNA1H* knockout (KO) mice exhibited autistic-like behaviors, including impaired social novelty recognition, increased anxiety, and repetitive grooming. Although brain size and weight were unaffected, KO mice showed a significant reduction in hippocampal neuron numbers and increased dendritic spine density in the dentate gyrus, with no change in spine maturity. These findings suggest that *CACNA1H* gene deletion may contribute to autism-like phenotypes through altered hippocampal neuronal structure and connectivity [32].

Splawski *et al.,* (2006) performed case-control research with 461 patients and 480 controls, all of Caucasian descent, and identified four missense loss-of-function variants (R212C, R902W, W962C, and A1874V) in the *CACNA1H* gene among six of the 461 ASD cases.

These mutations were identified in the essential area of the protein and resulted in diminished currents that reduced current conductance, leading to lower channel function [11].

4. Conclusion:

This study identifies significant neurobiological alterations in autism spectrum disorder, including reduced dopamine levels, increased SHANK3, and downregulated *CACNA1H* expression, while UBE3A showed no significant change. These findings highlight disruptions in neurotransmission and synaptic regulation, suggesting that these biomarkers may improve understanding of ASD pathophysiology and support future diagnostic and therapeutic approaches.

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