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UBE3A T485A mutation screening in Egyptian Children with Autism Spectrum Disorder.

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ABSTRACT

Ubiquitin E3 ligase (UBE3A) is a protein with twofold functions as a steroid hormone receptor transcriptional coactivator and as an E3 ubiquitin ligase. UBE3A excess through gene duplication or triplication or gain-of-function mutation increases the risk for autism spectrum disorders (ASD). Our aims were, first, to screen for UBE3A T485A mutation in a cohort of Egyptian children with ASD. Second, to evaluate any associations between UBE3A T485A mutation with behavioral and psychological data of children with ASD. UBE3A T485A mutation analysis was done by real-time PCR in fifty children with ASD and fifty healthy controls. Eighteen patients with ASD (36%) have homozygous mutations, 16 patients (32%) have heterozygous mutations and 16 patients have no mutation. Our study suggested that UBE3A T485A missense mutation, may contribute to autism pathology. Future large studies will be needed on T485A point mutation that hyperactivates UBE3A and the relation of this mutation to the clinical and psychological characteristics of ASD patients.

1. INTRODUCTION

Autism spectrum disorder (ASD) is a genetically heterogeneous neurodevelopmental syndrome; it begins before the age of 3 years and is characterized by deficits in social communication and interaction (social-emotional reciprocity, nonverbal communication, and developing, maintaining, and understanding relationships) in addition to restricted, repetitive patterns of behavior, interests, or activities. ASD develops through a complex set of causes, that involve genetic, environmental, and immunological factors [1- 3]. The interplay between genetic and other factors has become a subject of intensified research in the last decade [3], [4]. No known etiologies have been identified to explain ASD, which can be attributed to complex behavioral

phenotypes as well as the involvement of several genetic and environmental factors [1], [3], [4].

Ubiquitin E3 ligase (UBE3A) is a protein with twofold functions as a steroid hormone receptor transcriptional coactivator and as an E3 ubiquitin ligase. UBE3A is crucial to mammalian neural circuit development. It is expressed ubiquitously in tissues and has significant importance as a regulator of activity-dependent synapse development and plasticity. It is expressed entirely from the maternal allele [3], [5]. While UBE3A has a broad variety of targets in many tissues, it is highly important in the human brain as it regulates different aspects of neuronal function, growth, and repair. Thus, loss of UBE3A is the leading cause of a debilitating neurodevelopmental disorder Angelman syndrome (AS) and UBE3A excess through gene duplication or triplication or gain-of-function mutation, increases the risk for autism [3], [5]. The pathological features of the two neurological disorders include behavioral dysfunctions in learning, sociability, and motor skills [3], [5]. [3] reported that protein kinase A phosphorylates UBE3A in the region of the catalytic domain at residue T485 and suppresses UBE3A activity. An autism-linked mutation interrupts this phosphorylation site, leading to increased UBE3A activity, increased substrate turnover, and excessive dendritic spine development in the brain. The same study [3] reported that protein kinase A is a regulator of UBE3A activity and shows that a mutation linked to ASD interrupts the phosphorylation control. Furthermore, excessive UBE3A activity and synaptic dysfunction may be related to ASD pathogenesis [3].

Since the data on UBE3A T485A mutation in children with ASD are generally limited and entirely absent studies in Egypt, our aims were, first, to screen for UBE3A T485A mutation by real-time PCR in a cohort of children with ASD in the largest tertiary care center in Upper Egypt. Second, to evaluate any associations between UBE3A T485A mutation with behavioral and psychological data of children with ASD.

2. Patients and Methods

This study was approved by the Ethical Scientific Committee in Assiut University Hospital, Assiut, Egypt, and was conducted in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. in 2000. Participants' caregivers were informed of the intervention composition, the safety profile, and the study requirements. All caregivers of all participants have given their informed written consent in accordance with Ethical Committee guidelines (Assiut University, Egypt). The study was conducted in Assiut University Hospitals, Assiut city, Egypt from June 2018 to May 2021.

2.1 Study design

This was a case-controlled study undertaken in Assiut University hospitals Assiut city, Egypt.

2.2 Participants

The inclusion criteria included a confirmed diagnosis of ASD. Subjects were 50 children from 47 families, selected from a sample of 77 autistic children. 27 children with ASD were excluded from the study, 23 patients did not meet the inclusion criteria and/or have one or more of the exclusion criteria (summarized in table 1) and 4 families declined to participate in the study. All patients were recruited from the neuropediatric clinics of Assiut University hospitals and 3 private centers in Assiut, Egypt. The study included 50 age and sex-matched healthy children from the siblings of the same families of the patient group, as a control group. All controls are free from any psychiatric disorders and other exclusion criteria (Table1).

2.3 Methods

2.3.1 Clinical and psychiatric assessment

Detailed medical history and physical examination were done for all patients, including a family history of

consanguinity, similar conditions in the family, social activities, self-care, and time of diagnosis of ASD.

Diagnosis of autism was established by two senior psychiatrists before patients were recruited for the study. The diagnosis of ASD was confirmed using the Diagnostic and Statistical Manual of Mental Disorders 5th edition [6] and the "Autism Diagnostic Interview-Revised" (ADI-R) [7]. Two parent interviews of at least one hour, the first session probed for ASD diagnosis, later on, the other session was conducted to evaluate ASD symptom severity using the "Childhood Autism Rating Scale" (CARS) [8] and the "Aberrant Behavior Checklist" (ABC) [9]. CARS assesses behavior in 14 domains that are affected by autism, in addition to one parameter of the general impression of autism. The maximum CARS score is 60, while the cut-off value for ASD is 30. A score of less than 30 is considered non-autistic. Scores of 30 to 37 are rated as mild-moderate ASD, and 37. 5 to 60 as severe ASD [8]. ABC is a 58-item behavior rating scale used to evaluate behavior problems in autistic patients across 5 subscales (hyperactivity, social withdrawal, irritability, inappropriate speech, and stereotypic behavior). Subscales are rated on a 4-point scale {ranging from zero (not at all a problem) to three (severe problem) [9].

2.4 Molecular Analysis

2.4.1 DNA extraction

Genomic DNA was extracted from peripheral blood samples using a QIAamp DNA Mini Kit, Germany, on a QIAcube extractor and stored at –70ºC until tested.

2.4.2 UBE3A T485A mutation

For UBE3A T485A mutation analysis, we added 2 µl (100 ng) of DNA, 10 µl VeriQuest Fast Probe qPCR Master Mix (Affymetrix, USA) 1.0 µl (10µM) of forward primer 5'CCACCTGATCTGACCACTTTC-3' and 1.0 µl (10µM) of reverse primer 5' GTAAAACGACGGCCAGTTGTTCCTATCTCCCATTTACTGC -3', 0.5 µl (10µM) of TaqMan probes (Affymetrix, USA) and water up to a volume of 20µl. The PCR consisted of an initial hold step at 50 °C for 2 minutes followed by another hold step at 95°C for 5 minutes followed by 35 cycles at 95°C for 3 seconds, 60°C for 30 seconds, and the final extension step was at 72°C for 10 minutes. PCR products were analyzed on 7500 Fast Real Time PCR.

2.5 Statistical analysis

Data analysis was done by the statistical package for social sciences (SPSS), version 16. All data were expressed as the mean ± standard error of the mean (SEM). differences between the groups were examined for statistical significance using the Mann-Whitney U test. A p-value of ≤ 0.05 denoted the presence of a statistically significant difference.

3. Results

Table 2 shows patients' demographics, including age, gender, age at diagnosis of ASD, CARS scores and classification of autism, and ABC scores and mutations results. Subjects were 3.5-6 years old (mean age of 3.8 years (SEM 2.4). The study included 39 males (78%) with a male to female ratio of 3.5:1. 34 children with ASD (68 %) were diagnosed before the age of 3 years, while 16 patients (32%) were diagnosed after three years. As regards the baseline CARS scores, they ranged from 30.5 to 53.7 (mean 36.2 (SEM 3.9). 56% of patients in our study had mild/moderate autism while 44% had severe autism. The mean ABC scores for the ASD children were irritability (25.2 ± 3.7), hyperactivity (25.6 ± 11.5), lethargy/social withdrawal (18.9 ± 4.8), inappropriate speech (6.2 ± 1.5), and stereotypic behavior (6.7 ± 2.7) (Table 2).

Regarding autism probands harboring the phospho-mutant T485A missense mutation (UBE3A T485A), 18 patients with ASD (36%) have homozygous mutation, 16 patients (32%) have heterozygous mutation and 16

patients have negative results for mutation (Table 2). All healthy controls were negative for mutation. Table 3 shows some psychological characteristics of all studied patients. We noticed that autistic patients with positive phospho-mutant T485A missense mutation (UBE3A T485A) had significantly higher scores in CARS (total and verbal communication scores) than the negative group (table 3). The homozygous group had a significantly higher number of children with severe ASD in comparison with heterozygous and negative mutation groups. The full-scale IQ of autistic children with positive mutation groups was significantly lower than the negative ASD group (Table 3). Regarding the ABC and ADI-R scores, all scores are comparable between patient groups except ABC- inappropriate speech and ADI-R communication which were significantly higher in positive mutation groups.

4. Discussion

In our study, we analyzed UBE3A T485A mutation in a cohort of Egyptian children with ASD and its correlation with the behavioral and psychological data of these patients. We found UBE3A T485A mutation in 70% of our cohort. 18 (36%) patients have homozygous mutation, while 16 (32%) patients have heterozygous mutation. The gene of UBE3A is found at chromosome 15 and encodes the E3 ubiquitin ligase proteins [3], [5], [10]. Loss or excess of UBE3A has been associated with some neurodevelopmental disorders. UBE3A duplication of maternal origin is associated with autistic traits [5], [10]. Numerous genome-wide researches [11- 14] from patients with ASD recognized UBE3A as an autism-risk gene. UBE3A catalyzes the proteasomal degradation that targets several substrate proteins, including itself. UBE3A may have a role in the regulation of the equilibrium of protein synthesis and degradation (proteostasis) at the synapses where cell-to-cell transmission fall in [15]. Control of proteostasis is crucial for the synapses to adjust and accommodate with time in response to experience, which is called synaptic plasticity. Synaptic plasticity is important for memory and learning [15]. In addition, UBE3A acts as a transcriptional coactivator for the nuclear hormone receptor superfamily of transcription factors [3], [10]. In neurons, UBE3A concentrates in the nucleus and cytosol, as well as dendrite and pre-and post-synaptic partitions to regulate dendritic morphology [16], [17]. In mouse brains, maternal deficiency of UBE3A showed defects in dendritic spine development in the cerebellum, hippocampus, and cortex. Also, reduced UBE3A in cultured neurons of mouse brains resulted in defects of dendritic polarization in pyramidal neurons [16]. We found that autistic patients with positive UBE3A T485A mutation had significantly lower IQ scores than the negative ASD group (table 3).

Only one report [13] with an autistic child with near-normal IQ, and had A: G substitution at chromosome 15:25,615,808; with T485A missense mutation. We reported for the first time the correlation between the type of T485A missense mutations and the psychological characteristics of ASD patients. Patients with positive mutation have significantly higher scores in CARS, ABC- inappropriate speech, and ADI-R communication scores when compared to ASD children with negative mutation (Table 3). Unfortunately, in all the previous studies with UBE3A T485A in autistic patients, there were no details about the clinical and psychological characteristics of the patients. A previous study [3] reported that mutation of UBE3A T485A disrupts UBE3A phosphorylation control and abnormally increases UBE3A level, and this leads to enhanced degradation of UBE3A itself and other substrates. They suggested that the overactive UBE3A T485A mutant promotes long-lasting structural changes in the brain with an increase in dendritic spine density [3]. In addition, they reported that UBE3A phosphorylation increased in the first 7 days of life in a mouse brain, which corresponded to the developmental window while synaptic plasticity is driven primarily by the signaling of protein kinase A (PKA) [18]. PKA might have a role in UBE3A regulation in the first week of life to control the progression of synapse development [3]. Finally, a decrease in the activity of PKA, which diminishes T485 phosphorylation and increases UBE3A activity, might contribute to ASD pathology.

5. Conclusions

In light of our findings and the previous studies, our study suggested that UBE3A T485A missense mutation may contribute to autism pathology. Future large studies will be needed on T485A point mutation that hyperactivates UBE3A and the relation of this mutation to the clinical and psychological characteristics of ASD patients. Moreover, we need clinical therapeutic trials with the treatment of neurons with pharmacological agents that stimulate PKA, this can in turn down UBE3A activity. This may be a hope for a possible cure for ASD patients.

Competing interests:
All authors do not have a potential conflict of interest,

6. References

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Table 1: Exclusion criteria

• Other neurological and genetic disorders, e.g., cerebral palsy, phenylketonuria, tuberous sclerosis, neurofibromatosis, seizure disorders.
• History of severe head trauma or stroke.
• Prematurity.
• Subjects who had associated autoimmune disorders.
• Children with known endocrine, cardiovascular, pulmonary, and liver or kidney disease

Table 2: Demographic data, ABC scores, classification of autism and UBE3A T485A mutation of the studied group

	ASD children (N = 50)	**Control (N = 50)**
Age (years)		
Range	3.5-6	3-7
Mean ± SEM	3.8 ± 2.4	3.5 ± 1.9
Gender [Number (%)]		
Males	39 (78)	15 (75)
Females	11 (22)	5 (25)
Age at diagnosis [Number(%)]		
Before 3 years	34 (68)	-
After 3 years	16 (32)	-
Childhood Autism Rating Scale (CARS)		
Severe (≥37)	22 (44)	-
Mild/moderate (≤36.5)	28 (56)	-
Range	30.5 to 53.7	-
Mean ± SEM	36.2± 3.9	-
Aberrant Behavior Checklist (ABC) scores (Mean ± SEM)		

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Irritability	25.2 ± 3.7	-
Hyperactivity	25.6 ± 11.5	-
Lethargy/social withdrawal	18.9 ± 4.8	-
Inappropriate speech	5.7 ± 1.5	-
Stereotypic behavior	6.7 ± 2.7	-
UBE3A T485A mutation [Number (%)]		
Homozygous	18 (36)	-
Heterozygous	16 (32)	-
Absent	16 (32)	50 (100)

Table 3: Some psychological characteristics of studied patients

Parameter	**Homozygous group (N=18)**	**Heterozygous group (N=16)**	**No mutation group (N=16)**	**P1 value**	**P2 value**	**P3 value**
Childhood Autism Rating Scale (CARS)						
Severe (≥37) (N/%)	**13 (72.2)**	**5 (31.3)**	**4 (25)**	**0.031***	**0.021***	**NS**
Mild/moderate (≤36.5) (N/%)	**5 (27.8)**	**11 (68.7)**	**12 (75)**	**0.029***	**0.023***	**NS**
Total scores (Mean ± SEM)	**38.2 ± 10.2**	**37.9 ± 6.2**	**33.7 ± 5.2**	**NS**	**0.015***	**0.013***
FSIQ (Mean ± SEM)	**79 ± 7.2**	**80 ± 9.2**	**88 ± 11.2**	**NS**	**0.011***	**0.016***
Aberrant Behavior Checklist (ABC) scores (Mean ± SEM)						
Irritability	**24.7 ± 2.7**	**25.1 ± 4.2**	**25.3 ± 1.9**	**NS**	**NS**	**NS**
Hyperactivity	**26.1 ± 10.5**	**25.3 ± 8.3**	**25.8 ± 9.1**	**NS**	**NS**	**NS**
Lethargy/social withdrawal	**17.4 ± 3.2**	**17.7 ± 5.1**	**16.8 ± 4.4**	**NS**	**NS**	**NS**
Inappropriate speech	**6.9 ± 2.4**	**5.8 ± 3.7**	**4.3 ± 1.9**	**0.043***	**0.009***	**0.026***
Stereotypic behavior	**6.4 ± 0.8**	**6.5 ± 1.2**	**6.3 ± 1.3**	**NS**	**NS**	**NS**
Autism Diagnostic Interview-Revised" (ADI-R)						
ADI-R Communication	**18.2 ± 6.2**	**17.6 ± 7.1**	**15.8 ± 8.3**	**NS**	**0.010***	**0.027***
ADI-R Stereotyped behavior	**3.5 ± 1.7**	**3.4 ± 2.0**	**3.3 ± 1.2**	**NS**	**NS**	**NS**
ADI-R Social interaction	**12.4 ± 5.3**	**12.3 ± 4.1**	**12.7 ± 6.9**	**NS**	**NS**	**NS**
ADI-R Play	**5.8 ± 2.1**	**6.1 ± 2.7**	**5.7 ± 1.8**	**NS**	**NS**	**NS**

Mann-Whitney Test. Data represented as means ± SEM, NS= Non-significant, FSIQ= full-scale IQ
P1 between Homozygous group and heterozygous group
P2 between Homozygous group and non-mutation group
P3 between Heterozygous group and non-mutation group