Hot Spots Copy Number Variations, 15q Methylation, and *SHANK3* Single Nucleotide Polymorphisms Sudy in a Group of Egyptian Autistic Children

Original Article

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ABSTRACT

Background: Autism spectrum disorder (ASD) is a common, highly heritable and heterogeneous neurodevelopmental disorder. The etiology of ASD remains to be clarified, yet, it is postulated that both genetic and environmental factors play a pivotal role in the risk of ASD development. The work aimed to investigate the rate of CNVs of the ASD hot spots at ***SHANK2***, 16p11.2, and 15q13.3 as well as to investigate the imprinting defects involving 15q11.2-q13 region, using the MLPA assay and to investigate the association between the ***SHANK3*** SNPs rs9616915 and rs76224556 and ASD in a group of ASD Egyptian patients.

Results: De Novo CNVs were detected in 2/40 patients (5%) in ***SHANK2***, 2/40 patients (5%) in the 16p11.2 region, and 2/40 patients (5%) in the 15q13 region. However, no CNVs were detected in the 15q11 region which represents the area for Prader-Willi/Angelman region. Moreover, all patients showed no maternal duplication at the 15q11-q13 region. Moreover, we reported a significant association between ***SHANK3*** SNP rs9616915 and ASD, whereas the rs76224556 genotypes were not significantly associated with ASD.

Conclusions: MLPA is a cost-effective and rapid assay to detect CNV imbalances in large groups of patients and should be the first-tier test for genetic screening in ASD. The resulting data fortifies the understanding of the genotype and phenotype correlation of CNVs in patients with autism. However, further studies on larger sample sizes are still needed to evaluate the association between SNPs in the ***SHANK3*** gene and ASD.

Key Words: 16p11.2, Copy Number Variations, Maternal duplication, *MLPA, SHANK*.

Received: 18 August 2024, **Accepted:** 31 August 2024.

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ISSN: 2090-8571, 2023

INTRODUCTION

Autism spectrum disorder (ASD) is a common, highly heritable, and heterogeneous neurodevelopmental disorder with social, and communicative deficits and confined repetitive behavioral patterns that have underlying cognitive features and commonly co-occur with other conditions. According to the World Health Organization (WHO), approximately 1 in 100 children worldwide has ASD (**Lord *et al.,* 2020; Meguid *et al.,* 2020; Abdelrahman *et al.,* 2021**). The The estimated prevalence of ASD has increased over the last two decades (**CDC, 2018; Wang *et al.,* 2023**). The data concerning ASD prevalence in developing Arab countries are lacking. This is attributed to differences in participants' age, study methodologies, and case recognition approaches, in addition to the applied diagnostic techniques as until now there is no standardized screening tool for ASD, which represents a great challenge. Owing to the same causes, studies in Egypt reported diverse prevalence of ASD varying from 5.4/1000 to 33.6% (**Metwally *et al.,* 2023; Apte and Kumar, 2023**). The etiology of ASD still needs to be clarified, yet it is presumed that genetic and environmental factors are major players in ASD development (**Abdelrahman *et al.,* 2021**). The ASD heritability is estimated from 70 to 90% with hundreds of risk genes and lots of genetic syndromes (**Genovese and Butler, 2023; Jiang *et al.,* 2023**).

About 50% of ASD individuals have either chromosomal deletions/duplications, single gene disorders, or a known syndrome (**Genovese and Butler, 2023**). For instance, microscopic chromosomal abnormalities have been found in around five percent of instances of ASD. Moreover, de novo or copy number variations (CNVs) are

 DOI: 10.21608/mxe.2024.394589

found in 5–10% of patients with idiopathic ASD (iASD), the majority of which are monogenic and typically account for 5% of cases of ASD. A submicroscopic structural alteration in aberrant transcripts, deletions, or duplications in DNA regions larger than 1000 base pairs is referred to as CNV. Several of these abnormalities involve genes that are extremely active throughout brain development (**Meguid *et al.,* 2018; Genovese and Butler, 2023**).

SHANK gene mutations are linked to other mental disorders such as autism spectrum disorder and intellectual disability (IDs), schizophrenia, and Phelan-Mcdermid Syndrome **(Choi *et al.,* 2015; Schmeisser, 2015**). ***SHANK*** genes (***SHANK1,2,3***) encode SH3 and various ankyrin repeat transcription proteins, which are postsynaptic scaffolding proteins for excitatory synapses (**Monteiro and Feng, 2017**). More than 80 and 30 ASD-associated mutations have been identified in ***SHANK3*** and ***SHANK2,*** respectively. ***SHANK2*** is a scaffolding protein that exhibits postsynaptic distribution in excitatory neurons. ***SHANK2*** has been linked to ASD, particularly social difficulties, as numerous groups have extensively reported (**Lee *et al.,* 2020; Wan *et al.,* 2022; Choi and Kaang, 2023**). The ***SHANK2*** gene spans 621.8 kb and is located on chromosome 11q13.3. With its one-stop codon, three promoters, and 25 exons, it can produce four distinct isoforms. The first gene whose function in Phelan-Mcdermid syndrome (PMS) was thoroughly investigated was the ***SHANK3*** gene. But ***SHANK1*** and ***SHANK2*** have also been connected to a number of neuropsychiatric disorders (**Wan *et al.,* 2022; Sungur, 2017**). The 16p11.2 gene locus is an approximately 500–600 kb region containing 27–29 genes located on the proximal short arm of chromosome 16 (**Rein and Yan, 2020**). Deletions and duplications of 16p11.2 have highly pleiotropic phenotypic effects, which are strongly linked to ASD, ID, motor/developmental delay (DD), dysmorphic features (DFs), and epilepsy/seizures.16p11.2 deletions have been reported at rates of 0.028–0.043%, while duplications have been reported between 0.035% and 0.053% in the general population (**Green *et al.,* 2016; Vysotskiy *et al.,* 2021**).

Microdeletions and microduplications affecting 15q13.3 have been recorded to have very different phenotypes, from normal to neurodegenerative (**Budisteanu *et al.,* 2021**). It has been suggested that the gene ***CHRNA7*** (cholinergic receptor nicotinic alpha 7 subunit) is a candidate gene for neuropsychiatric phenotypes (**Gillentine *et al.,* 2018**). Description of various patients with 15q13.3 interstitial deletion exhibiting a range of neuropsychiatric symptoms, such as speech issues and ID/DD, epilepsy, and autism spectrum disorders (**Whitney *et al.* 2021**). Although the clinical presentation of patients with a heterozygous deletion at 15q13.3 varies greatly, even among affected individuals from the same family, patients with a homozygous deletion are more affected and present with epilepsy, hypotonia, and growth failure (**Budisteanu *et al.,* 2021**).

Small microduplications at 15q13.3, often associated with ***CHRNA7***, are frequently detected in microarray studies. ***CHRNA7*** duplications are associated with various neurodevelopmental disorders, including ID/DD, developmental delay, autism spectrum disorder (ASD), mood disorder, obsessive-compulsive disorder, childhood schizophrenia (COS), Tourette syndrome (TS), attention deficit hyperactivity disorder (ADHD), and epilepsy (**Sinkus *et al.,* 2015**). A study investigated the clinical and behavioral changes, the researchers phenotyped 18 children with ***CHRNA7*** duplications and noted that these mutations exhibited abnormal expression and incomplete penetrance (35% of CNV recipients from parents were unaffected; the same competition was observed in healthy siblings). While the range of neuropsychiatric diseases associated with 15q13.3 microdeletions and microduplications is the same, microduplications typically exhibit greater variability in expression and penetration, hence necessitating diagnostic and clinical interpretation (**Gillentine *et al.,* 2017**).

15q11.2-q13 region includes a bunch of methylated, imprinted genes with SNRPN and UBE3A, which are either paternally or maternally expressed, respectively. Maternal duplications (maternal uniparental disomy (mUPD) or imprinting defects involving the 15q11.2-q13 region have been associated with autism (1–3%). Despite the few reports, paternal duplications (disomy) do not appear alone to be totally penetrant for autism (**Dawson *et al.,* 2015; Ryan and Heron, 2023**). So, it was suggested that ASD patients should be routinely screened for 15q genomic abnormalities and their methylation status.

Moreover, the genetic risk of ASD has been attributed to single nucleotide polymorphisms (SNPs) and CNVs, rather than certain single-gene mutations (**Rodriguez-Gomez *et al.,* 2021**). Among the ***SHANK*** genes, ***SHANK3***, on 22q.13.3, is proposed as a key player in ASD pathogenesis and its loss causes synaptic function disruption (**Mashayekhi *et al.,* 2021; Siddiqua *et al.,* 2022**). Single nucleotide polymorphism researches provide insight into the function of ***SHANK3***, and the role ofits small-effect variants in the risk of ASD (**Manning *et al.,* 2021; Siddiqua *et al.,* 2022**). A non-synonymous single nucleotide polymorphism (nsSNP) of the ***SHANK3*** gene, reported as rs9616915 (T>C) in exon 6, directly affects the ***SHANK3*** role in the regulation of splicing and damages the structure of ***SHANK3*** protein (**Mashayekhi *et al.,* 2021**). Additionally, ***SHANK3*** SNP, rs76224556; where a c.1304+48C>T transition in intron 10, affects a methylated cytosine in a CpG dinucleotide in one of the four intragenic regulatory elements. Previous studies reported that the region encompassing this CpGisland appeared to be critical for ***SHANK3*** isoform regulation, and its loss in mice leads to significant synaptic disorganization in addition to ASD-like features (**Oberman *et al.,* 2015**).

Taking into account these considerations and for a better understanding of ASD etiology that might deliver guidance for yet-to-come treatment and management of ASD, our study aimed to explore the rate of CNVs of the ASD hot spots at ***SHANK2***, 16p11.2, 15q13.3 as well as to investigate the imprinting defects involving 15q11.2-q13 region in a group of ASD Egyptian patients, using the MLPA assay. In addition, we aimed to investigate the association between the ***SHANK3*** SNPs rs9616915 and rs76224556 and ASD among them.

SUBJECTS AND METHODS

Forty patients with ASD from the "Autistic Disorders Clinic", Medical Research Center of Excellence, at the National Research Centre in Giza, Egypt, were involved in our study as well as 40 age- and sex-matched control subjects. Their ages ranged between 3 to 5 years. Informed written approval was obtained from parents/guardians of patients by the code of ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects. The study protocol was accepted by the Institutional Ethical Committees of the National Research Centre, Egypt (Registration number 16373).

Clinical evaluation

ASD diagnosis was carried out using the Diagnostic and Statistical Manual of Mental Disorders (5th ed.; DSM-5) criteria (**American Psychiatric Association, 2013**) and Autism Diagnostic Interview-Revised (ADI-R) (**Rutter *et al.,* 2003**). The Childhood Autism Rating Scale (CARS) was used for assessing autism severity (**Schopler *et al.,* 1980**).

The Short Sensory Profile (SSP) was done and intended to rate a variety of sensory impairments. SSP gives clues to ASD children's sensory processing skills and facilitates in evaluating and planning the required interventions for these children (**Dunn, 1999**).

Laboratory investigation

According to the manufacturer's instructions, the QIAamp DNA Mini Kit was used to extract DNA from 3 milliliters of peripheral blood drawn from each of the 40 patients and the reference samples (one reference for every seven patient samples, with a minimum of three references per test). The NanoDrop spectrophotometer was used to measure the DNA samples' the quantity and quality.

MLPA assay for CNVs detection

CNVs evaluation for ***SHANK2***, 16p11.2 region, and 15q13 region was carried out using P396 and P343 SALSA MLPA probemixes, according to the manufacturer's instruction (MRC-Holland) (https://www.mrcholland.com).

MS-MLPA assay for maternal duplication detection

Maternal duplication of the proximal region of 15q11.2 was investigated using MS-MLPA probemix ME034-B1 Multi-locus Imprinting to evaluate its methylation status, detecting its parental origin. The procedure was carried out according to the manufacturer's instruction (MRC-Holland) (https://www.mrcholland.com).

SNP genotyping

Assessment of ***SHANK3*** gene polymorphisms was determined using rs9616915 and rs76224556 assays by TaqMan SNP genotyping assay (C_11891599 and C_102796602, Thermo-Fisher Scientific, Germany) through qPCR technique. We prepared the proper volume from the PCR reaction mix with a total volume of 20µL/well. The reaction mix of each sample contained 10µL of TaqMan Genotyping Master Mix, 1µL of TaqMan assay (10X), and 2-4µL RNase-free water. The protocol of thermal cycling was adjusted as follows; 95°C for 10min, followed by 40 cycles at 95°C for 15s, and 60°C for 1min. The qPCR was implemented on the Step One Real-Time system (Applied Biosystems, Thermo-Fisher, Germany).

Statistical analyses

Were performed using SPSS version 21.0 (SPSS Inc, Chicago, Illinois, USA). Data were analyzed; frequencies for qualitative data, mean and standard deviation for quantitative data. Fisher's exact test and Chi-Square test were applied for qualitative data analysis. The quantitative variables were compared, and a *t*-test was used for parametric data. The difference between groups was considered statistically significant if $p < 0.05$.

RESULTS

Eighty participants whose ages ranged between 3 and 5 years were enrolled in the present study, including 40 autistic cases and 40 age- and sex-matched healthy controls. The mean age of autistic subjects was 4.1±0.82 years, while that of controls was 4±0.8 years, with an insignificant statistical difference between both groups ($p= 0.54$, $t= 0.62$). The patients' group comprised 27(67.5%) males and 13(32.5%) females, while the control group included 25(62.5%) males and 15(37.5%) females, with no statistically significant difference between them ($p= 0.64$). The autistic cases' history and clinical data are illustrated in Table (1).

The studied patients displayed multimodal symptoms mainly under the responsive/seeks sensation domain then tactile sensitivity, and lastly the hypo-activity (low energy/weak domain). CARS score and total SSP score were carried out to correlate the severity of autistic symptoms and sensory processing. Negative correlation with the total SSP score was detected ($r= -0.839$, $p < 0.001$) and this demonstrated that children with more severe autism had more sensory symptoms.

Of the 40 individuals, only nine (22.5%) with ASD had CNVs in the ***SHANK3*** and/or its surrounding area

(3 patients, 7.5%) (Previously shown) (**Meguid *et al.*, 2020**), in ***SHANK2*** (2 patients, 5%), in the 16p11.2 region (2 patients, 5%) and in the 15q13 region (2 patients, 5%) Figure (1). However, no CNVs were detected in 15q11 region which represents the area for the Prader-Willi/ Angelman region. Moreover, these children's parents did not have these CNVs. Moreover, all patients showed normal methylation status of the 15q11.2 region which indicates that no patients have maternally inherited duplications at the 15q11-q13 region.

The 1st 3 patients were presented previously having CNVs in the ***SHANK3*** and/or its flanking region (**Meguid *et al.*, 2020**). The 2nd 2 patients were unrelated boy and girl having ***SHANK2*** duplication at one exon. The boy was 3 years old with moderate to severe non-verbal autism, intellectual disability (ID) with comorbid ADHD, and a positive family history of abortion and suffered sleep problems in the form of insomnia. MLPA showed de novo duplication at ***SHANK2***, exon 2. As regards sensory assessment, the SSP total score was clearly different from normal. The girl was 5 years old with mild verbal autism, mild intellectual disability (ID), positive family history of abortion and intrauterine fetal death, and short status. MLPA showed de novo duplication at ***SHANK2***, exon 17. As regards sensory assessment, SSP's total score fell into the category of typical performance.

The 3rd 2 patients were siblings, a boy and a girl having de novo 16p11.2 duplication, at *MAZ*, exon 6. The boy was 10 years old. The girl was 3 years old. Their father was diagnosed on the schizophrenic spectrum and their uncle with intellectual disability. Both siblings have severe non-verbal autism, and intellectual disability (ID). As regards sensory assessment, both SSP total scores was clearly different from normal.

The 4th 2 patients were two boys having de novo CNVs at the 15q13 region, one was duplication at one exon and the other was deletion at one intron. One of them was 5 years old with severe non-verbal autism, intellectual disability (ID), and asymmetry. His maternal uncle had brain atrophy, and the son of his mother's cousin was autistic. His MLPA showed de novo 15q13 duplication at ***CHRNA7***, exon 4. The other boy was also 5 years old with moderate to severe non-verbal autism, and intellectual disability (ID). His MLPA showed de novo 15q13 deletion *TJP1* Intron 1. As regards sensory assessment, both patients' SSP total scores was clearly different from normal. The main clinical data of MLPA-positive patients are described in Table (2).

The genotype distribution and allelic frequencies of ***SHANK3*** gene SNPs rs9616915 and rs76224556 among autistic cases and controls are presented in Tables (3, 4), respectively.

Regarding **SNP rs9616915**, the frequency of the TT genotype was 2.5% in cases and 20% in controls, whereas the TC genotype was observed in 92.5% of cases and 77.5% of controls. The mutant CC genotype was detected in 5% of cases and 2.5% of controls. A statistically significant difference in rs9616915 genotypes was found between both groups (p= 0.029). However, there was no significant difference in allele distribution between autistic cases and controls (p= 0.2) (Table 3).

On studying the genotypic and allelic frequencies of the **SNP rs76224556**, no significant differences were detected in comparing cases to controls (P >0.05). Although the CC and TT genotypes showed higher frequencies among cases (82.5% and 7.5%, respectively) versus controls (67.5% and 2.5%, respectively) (Table 4).

Table 1: History and clinical data of the autistic cases:

Variable	Cases (*N*=40)			
	Positive		Negative	
	N	%	*N*	%
Consanguinity	25	62.5	15	37.5
Family history	30	75	10	25
Clinical data				
Short stature	1	2.5	39	97.5
Obesity	2	5	38	95
Febrile convulsions	2	5	38	95
Epilepsy/EEG	6	15	34	85
Dysmorphism	6	15	34	85
ADHD	5	12.5	35	87.5
Hypotonia	5	12.5	35	87.5
Sleep disorders	3	7.5	37	92.5
Movement problems	1	2.5	39	97.5
GIT problems	4	10	36	90
CARS				
Mild to moderate autism	24	60		
Severe autism	16	40		

EEG: Electroencephalography; ADHD: Attention Deficit Hyperactivity Disorder; GIT: Tract; CARS: Childhood Autism Rating Scale.

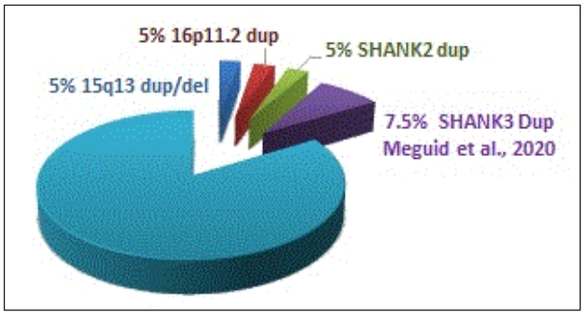

Figure 1: Pie chart of the percentage of cases with CNVs.

Table 2: The main clinical data of MLPA positive patients:

	CNVs	Severity of Autism	ADI-R B domain	Total SSP	ADHD	ID	Epilepsy/EEG changes	Insomnia
1	SHANK2 -2 dup	moderate to severe	nonverbal	definite difference	+ve	+ve	-ve	+ve
2	SHANK2 -17 dup	mild to moderate	verbal	typical	-ve	+ve	-ve	-ve
3	16p11.2 dup, MAZ6	moderate to severe	nonverbal	definite difference	-ve	+ve	-ve	-ve
4	16p11.2 dup, MAZ6	moderate to severe	nonverbal	definite difference	-ve	+ve	-ve	-ve
5	15q13 dup, ***CHRNA7-4***	moderate to severe	nonverbal	definite difference	-ve	+ve	left frontotemporal activity	-ve
6	15q13 del, TJP1-Intr.1	moderate to severe	nonverbal	definite difference	-ve	+ve	-ve	-ve

CNV: Copy Number Variations; ADI-R: Autism Diagnostic Interview-Revised; ADHD: Attention Deficit Hyperactivity Disorder; ID: Intellectual Disability; MLPA: Multiplex Ligation-dependent Probe Amplification; EEG: Electroencephalography; SSP: The Short Sensory Profile.

Table 3: Genotype distribution and allele frequency of the SNP rs9616915 in cases and controls:

Genotype/ Allele	Cases (*n*=40)		Controls (*n*=40)		*P* value
	n	%	*n*	%	
TT	1	2.5	8	20	0.029*
TC	37	92.5	31	77.5	
CC	2	5	1	2.5	
T	39	48.75	47	58.75	0.2
C	41	51.25	33	41.25	

Fisher's exact test and Chi square test - *P* value is considered significant if <0.05; *: Significant *P* value.

Table 4: Genotype distribution and allele frequency of the SNP rs76224556 in cases and controls:

Genotype/ Allele	Cases (*n*=40)		Controls (*n*=40)		*P* value
	n	%	*n*	%	
CC	33	82.5	27	67.5	0.058
CT	4	10	12	30	
TT	3	7.5	1	2.5	
C	70	87.5	66	82.5	0.38
T	10	12.5	14	17.5	

Fisher's exact test and Chi square test - *P* value is considered significant if <0.05; *: Significant *P* value.

DISCUSSION

ASD is a worldwide rising neurobiological disorder at a disconcerting rate. It is highly heritable, with genetic contributions accounting for around 80% of its risk (**Abdelrahman *et al.,* 2021; Siddiqua *et al.,* 2022; Wan *et al.,* 2022**). In this study, of 40 patients only nine ASD cases (22.5%) had CNVs in the ***SHANK3*** and/or its flanking region; 3 patients (**Meguid *et al.,* 2020**), ***SHANK2*** (2 patients), 16p11.2 region (2 patients) and 15q13region (2 patients). However, no CNVs were detected in the 15q11 region which represents the area for Prader-Willi/ Angelman region. Moreover, none of these children's parents had these CNVs. Furthermore, no patients showed maternally inherited duplications at the 15q11-q13 region.

The ***SHANK*** family consists of ***SHANK1***, ***SHANK2*** and ***SHANK3***. ***SHANKs*** have been proposed as synaptic scaffold regulators (**Sala *et al.,* 2015**). All ***SHANKs*** have been associated with mental disorders such as autism spectrum disorder (ASD) and schizophrenia (**Guilmatree *et al.,* 2014**). Moreover, mutations in the ***SHANK*** genes have been associated with changes in the severity of cognitive impairment in ASD. Given the low frequency of deleterious mutations in ***SHANK1*** and ***SHANK2***, the clinical significance of these genes has not yet been determined. In contrast, the frequency and prevalence of ***SHANK3*** mutations in individuals with ASD and intellectual disability (more than 1 in 50) have been considered for screening in clinical practice (**Leblond *et al.,* 2014**). Disruption of ***SHANK2*** can be devastating and important for treating patients. De novo loss-of-function ***SHANK2*** alleles are associated with ASD, mild to moderate intellectual disability, and/or language impairment (***Hassani et al., 2022***). The association between CNVs/ mutations in ***SHANK2*** and ASD risk was first revealed in 2010 when they found CNVs and mutations in the PDZ domain in ASD subjects (**Berkel *et al.,* 2010**). Here we report 2 unrelated patients a boy and a girl having ***SHANK2*** duplication at one exon. The boy showed de novo duplication at ***SHANK2***, exon 2. While the girl's MLPA also showed de novo duplication at ***SHANK2***, exon 17. The sensory evaluation of the boy with moderate to severe nonverbal autism was classified as a definite difference from normal. For the girl with mild verbal autism, the SSP total score fell into the category of typical performance. Again, this is consistent with all findings from our Autism Spectrum Disorders Research Group, where the severity of psychiatric symptoms is associated with autism severity. Research on ***SHANK2*** dysfunction in animal models (***SHANK2*** knock-out, KO) mice and its relation to sensory issues in autism has been investigated (**Ko *et al.,* 2016**). Tactile hyposensitivity is described in autistic patients

(**Moore, 2015**). Also, it is a domain measured in SSP. Tactile sensitivity was the second most affected domain in our sample (underresponsive/seeks sensation was the most affected domain followed by tactile sensitivity). **Ko *et al.*, (2016)** work demonstrated that ***SHANK2*** KO mice have impaired basal tactile sensitivity and acute pain response. The authors clarified that this reduced sensitivity could be caused by defects in the central or peripheral nervous system. ***SHANK2*** is expressed in both peripheral and central neurons. More study on how ***SHANK2*** could cause pain deficits in ASD is needed.

Speech and/or language problems have been frequently observed in 16p deletion and duplication carriers in previous cases (**Weiss *et al.*, 2008; Bijlsma *et al.*, 2009; Fernandez *et al.*, 2010; Fetit *et al.*, 2022; Zuffery *et al.*, 2012**). The importance of tremor as another action of the 16p11.2 duplication phenotype is surprising and has been documented in two previous studies (**Posar and Visconti, 2020**). Mutations in 16p11.2 have previously been associated with epilepsy (**Olson *et al.*, 2014**), and mutations in the PR gene region are associated with benign infantile epilepsy syndrome (**Scheffer *et al.*, 2012**). **Weiss *et al.*, (2008)** indicated that duplication of the 16p11.2 region co-segregated with autism in two families (six of six affected offspring) and occurred as a de novo event in a third family. Duplication in this region was also observed in four clinical samples referred for diagnosis of developmental delay, although no autistic features were reported in three of these subjects, which matches our findings of 2 siblings, a boy and girl, having de novo 16p11.2 duplication, at *MAZ*, exon 6. Both were diagnosed with severe autism. Their SSP total score fell into the category of definite difference from normal. Both patients scored a definite difference from normal in all SSP domains. Sensory processing deficits in patients with 16p11.2 deletions and duplications have been a topic of interest recently. In a study by **Smith *et al.*, (2022)** on 38 children with a 16p11.2 deletion and 31 children with a 16p11.2 duplication, sensory processing deficits were obvious in both 16p11.2 deletion and 16p11.2 duplication. Sensory assessment in both cohorts indicated the impact of sensory behavior was more severe when compared to neurotypical children, with levels being similar to autistic patients. Another study by **Osório *et al.* (2021)** on 17 children with 16p11.2 deletion in comparison with 121 autistic children. They found that on the parent-reported Sensory Processing Measure, children with a 16p11.2 deletion showed changes in vision, hearing, body awareness, and balance motion, but no alterations in touch, taste, or smell. The children with 16p11.2 deletion showed no much differences in the touch, taste, or smell domains from the autistic children, who performed better on all tests in all sensory domains. Understanding the changes in sensory processing in 16p11.2 deletion and duplication may help in better genotyping and phenotyping of a group of autism patients.

It is commonly known that genetic rearrangements around 15q11–13 are linked to neurological and neuropsychiatric disorders (**Lowther *et al.*, 2015; Chilakamarri and Mellin-Sanchez, 2022**). This is in line with the 15q13.3 duplication phenotype reported in earlier research (**van Bon *et al.*, 2009; Szafranski *et al.*, 2010; Gillentine and Schaaf, 2015**). Chromosome 15q13.3 microdeletions are linked to a number of neurological and neuropsychiatric disorders. The group of patients with idiopathic generalized epilepsy (IGE), intellectual disability, and schizophrenia showed the biggest increase. It has also been found in patients with schizophrenia. Reciprocal microduplications of 15q13.3 are more difficult to interpret because microdeletions have been detected in the same type of neuropsychiatric disease but have different expression and decreased sensitivity compared to deletions and are often acquired de novo (**Budisteanu *et al.*, 2021**).

CHRNA7 at 15q13 is the gene that is thought to contribute to the neurological phenotype in patients with 15q13.3 CNV but given the complexity of the genome and the nearly equal number of partial copies, analysis of the coding region is only limited. Patients carrying this gene have microdeletions with clinically specific phenotypes. The gene is thought to contribute to the neurological phenotype in patients with 15q13.3 CNV but given the complexity of the genome and the nearly equal number of partial copies, analysis of the coding region is only limited. Patients carrying this gene have microdeletions with clinically specific phenotypes (**Masurel-Paulet *et al.*, 2010; Bacchelli *et al.*, 2015**). However, the association of ASD and epilepsy with ***CHRNA7*** duplications in probands may indicate that microduplications containing ***CHRNA7*** may have the same role as deletions in ASD/seizure susceptibility, albeit with reduced penetrance. Consistent with this hypothesis, ***CHRNA7*** transcript levels have recently been shown to be reduced in neuronal cells (**Meguro-Horike *et al.*, 2011; Bacchelli *et al.*, 2015**), or brain samples with parental 15q duplications. According to gene copy number expectations, it is exactly the opposite. Further evidence of ***CHRNA7*** dose sensitivity was provided by showing the association of ***CHRNA7*** triploidy with neuropsychiatric and cognitive phenotypes in a three-generation family (**Soler-Alfonso *et al.*, 2014; Özaltun *et al.*, 2021**). Of our nine cases, 2 boy patients showed de novo15q13 CNVs, one of them had duplication at ***CHRNA7***, exon 4 and the other had de novo 15q13 deletion *TJP1* Intron 1. The patient with 15q13 duplication had severe non-verbal autism. The other boy who showed de novo 15q13 deletion had moderate to severe non-verbal autism. As regards sensory assessment, both patients' SSP total scores fell into the category of definite difference from normal. Studies on sensory processing phenotypes concerning genes implicated in autism are still scarce. To our knowledge, research on genetic rearrangements in chromosome 15q13.3 and its association with specific

neurological and neuropsychiatric phenotypes such as sensory processing deficits is still growing.

Abnormalities of the 15q11-q13 region were reported in ASD patients. Moreover, maternal interstitial 15q11-q13 duplications were seen in 1-3% of ASD patients. Although paternal duplications of this region remain phenotypically silent in a large percentage of cases, they may cause developmental delay and ASD, suggesting that paternally expressed genes in this region can contribute to ASD with lower penetrance than maternal duplications (**Ryan and Heron, 2023**). However, our results showed normal methylation status of the 15q11-q13 region, which means no maternal duplications of this region for all the studied patients.

SHANK3 is considered a master synaptic risk gene with extensive studies that elucidated the pathogenesis of ASD (**Siddiqua *et al.,* 2022**). In the present, we investigated the association between the ***SHANK3*** gene SNPs rs9616915 and rs76224556 and ASD among 40 autistic Egyptian children, and 40 age- and sex-matched healthy controls.

Our results revealed a significant association between SNP rs9616915 genotypes and ASD cases (p= 0.029), where TT, TC, and CC genotypes were detected in 2.5%, 92.5%, and 5% of cases, respectively. However, no significant difference was revealed in the allele distribution between the studied groups (p= 0.2). Our results were concordant with those of an Iranian study conducted by 32. **Mashayekhi *et al.,* (2021)**, who revealed a significant association between ***SHANK3*** SNP rs9616915 and risk of ASD, but no significant difference was found in allele distribution. Whereas a Chinese study by **Shao *et al.,* (2014)** reported a significant association between rs9616915 SNP TT genotype and a higher risk of ASD. However, **Qiu *et al.,* (2018)** study, did not report any significant correlation between rs9616915 polymorphism and ASD. In addition, several previous studies on Bangladeshi, Swedish, and Japanese people came up with the same conclusion (**Waga *et al.,* 2011; Jonsson *et al.,* 2014; Siddiqua *et al.,* 2022**).

Regarding the genotypic and allelic frequencies of the SNP rs76224556, no significant difference was detected between autistic cases and controls (P >0.05). These findings were consistent with two research by **Boccuto *et al.,* (2013); Qiu *et al.* (2018)**.

The diversity between several reports about ***SHANK3*** gene polymorphisms and ASD might be explained by ethnic and racial variations in genotype frequency and sample sizes (**Qin *et al.,* 2009; Manning *et al.,* 2021; Siddiqua *et al.,* 2022**).

CONCLUSION

The clinical and molecular description of the positive cases may fortify the genotype and phenotype correlation of copy number variations (CNVs) at the autism spectrum disorder (ASD) hotspots which may help in better understanding the autism spectrum disorder (ASD) pathogenesis. However, further studies are needed including larger sample sizes. Moreover, the contribution of other autism spectrum disorder (ASD)-associated genes such as *NRXN1, NLGN1*, and *PTEN* should be considered for further study of the genetic basis of sensory phenotypes. Our data emphasize that the multiplex ligation-dependent probe amplification assay (MLPA), as it is a cost-effective and rapid assay, should be the first-tier test for genetic screening in large groups of autism spectrum disorder (ASD) patients to detect copy number variation (CNV) imbalances. Moreover, we reported a significant association between ***SHANK3*** Single-nucleotide polymorphism (SNP) rs9616915 and autism spectrum disorder (ASD), whereas the rs76224556 genotypes were not significantly associated with autism spectrum disorder (ASD). More studies on larger sample sizes are needed to evaluate the relationship between single-nucleotide polymorphisms (SNPs) in the ***SHANK3*** gene and autism spectrum disorder (ASD).

ACKNOWLEDGEMENTS

We declare that all authors of this manuscript have made substantial contributions. The authors are grateful to The National Research Centre (NRC) for providing necessary facilities. We did not exclude any author who substantially contributed to this manuscript. We have followed our ethical norms established by our respective institutions.

FUNDING

This research was financially supported by Research Project grants from the National Research Centre, Egypt (11010161). We would also like to express our gratitude to the funding agent, for giving us the chance to accomplish this study with the help of its updated equipment and instrumentation.

AUTHORS' CONTRIBUTIONS

RA: participated in conducting the laboratory work, writing the manuscript, and preparing the paper for submission. **OE:** Provided the idea of this research, participated in preparing the design of the research, conducting the laboratory work, interpreting the data, writing the manuscript, and preparing the paper for submission and final approval of the version to be published. **AA:** participated in preparing the design of the research, participated in conducting laboratory work, and statistical analysis, and prepared the paper for submission. **MF:** participated in conducting the laboratory work and preparing the paper for submission. **RM:** participated in conducting the laboratory work and preparing the paper for submission. **SA:** participated in preparing the design of the research, participated in conducting laboratory work, and

statistical analysis, and prepared the paper for submission. **AM:** participated in conducting the laboratory work and preparing the paper for submission. **FH:** participated in the clinical evaluation of the patients and prepared the paper for submission. **ME:** participated in performing the laboratory work, and preparing the paper for submission. **NM:** participated in the clinical evaluation of the patients and prepared the paper for submission. All authors have read and approved the manuscript.

DECLARATIONS

Ethics approval and consent to participate

The study was approved by the ethical committee of the National Research Centre, Egypt (16-373), which follows the ethical standards of the Declaration of Helsinki. All participants gave informed written consent before their inclusion in the study.

CONFLICT OF INTEREST

There are no conflicts of interest.

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