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SHANK3 Gene Polymorphism Rs2146772569: A Replication in Iraqi Middle Euphrates Children

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1**. Abstract:** Autism spectrum disorder is a neurodevelopmental condition marked by persistent challenges in social communication and interaction, along with limited and repetitive behavior, hobbies, or activities. Neuroimaging and examinations have shown disturbances in the structure and functioning of the brain, namely in regions responsible for social communication and sensory processing. Multiple genes contribute to synapse function and plasticity. Shank3 is an example of a gene that has been linked to a higher risk to autism. It functions by controlling the organization and activity of synapses, which are vital for neuronal transmission. Several genetic variations in the shank3 gene have been extensively investigated. Numerous single nucleotide polymorphisms (SNPs) have been identified and found to have a deleterious impact on the function of this protein. This study examined the shank3 SNP (Rs2146772569) in Iraqi population. A total of forty healthy children and thirty children diagnosed with Autism Spectrum disease (ASD) have been selected to participate in this study. The aim of the study is to examine the relationship between shank3 SNP and autism spectrum disease in the Iraqi community. Genomic DNA was isolated from blood samples, followed by PCR amplification and subsequent preparation of the samples for sequencing.The statistical analysis was conducted using the SPSS software tool. The findings indicate that the frequency of the C—T allele (CC, CT, and TT genotypes) are 37.5%, 32.5%, and 30% in healthy persons, and 26.67%, 23.33%, and 50% in patients. The frequency of C→T mutations are 53.75% and 46.25% in healthy persons, and 38.33% and 61.67% in autistic children, respectively. The findings indicate that there is no notable correlation between this specific single nucleotide polymorphism (SNP) and autistic spectrum disorder in children from Iraq.

Keywords: Autism,Polymorphism,Iraq

2**. Introduction:** Autism spectrum disorder is a neurodevelopmental disorder characterized by persistent impairments in social communication and interaction, as well as restricted and repetitive patterns of behavior, interests, or activities. [1]

autism is affecting approximately 1 in 36 children in the US, with a higher prevalence rate in boys than girls.[2] globally, the prevalence of autism varies across different countries and regions, but it is estimated to affect about 1 in 160 children worldwide.[3] In the middle east ,the prevalence of autism has been reported to range from 1. to 29 per 1,000 children, depending on the study and population examined.[4]

the cause of autism is still not fully understood, but research suggests that a combination of genetic and environmental factors play a role in its development. Genetic factors contribute to the risk of developing autism, with studies indicating that heritability estimates range from 50-80%. twin studies have shown a higher concordance rate of autism in identical twins compared to fraternal twins, and even less concordance rates in siblings. further supporting the role of genetics.[5], [6]

environmental factors such as maternal prenatal factors, complications during pregnancy or birth, exposure to certain chemicals or toxins, and parental age have also been suggested to influence the risk of autism.[7]

many genes have been identified as potential risk factors for autism, including those involved in brain development, synaptic functioning, and immune response. Additionally, there is evidence of abnormalities in the structure and function of the brain in individuals with autism. SHANK3 gene mutations, for example, have been associated with a higher risk of autism.[8]

Furthermore, studies have shown that SHANK3 mutations are associated with a broader clinical presentation that includes intellectual disability, speech and language impairments, and motor coordination difficulties.[9] Research has also focused on potential therapeutic interventions targeting SHANK3 and its related pathways to ameliorate the impact of SHANK3 mutations in individuals with autism spectrum disorder.[10]

The SHANK3 gene encodes a scaffold protein that is important for the structure and function of synapses, the connections between nerve cells in the brain. It consists of several protein domains that play key roles in its function. These domains include the ankyrin repeat domain, the SH3 domain, the PDZ domain, and the proline-rich region. The ankyrin repeat domain is involved in protein-protein interactions, the SH3 domain is a protein interaction module, the PDZ domain is also involved in protein-protein interactions and plays a role in organizing protein complexes at the synapse, and the proline-rich region is important for linking SHANK3 to other proteins.[8] Mutations or variants in these domains can disrupt the function of SHANK3, leading to synaptic dysfunction and impaired communication between nerve cells.

The ankyrin repeat domain is a protein interaction module that is involved in mediating protein-protein interactions. In the context of autism, mutations or variations in the ankyrin repeat domain of the SHANK3 gene have been found to disrupt the normal functioning of synaptic connections in the brain. This disruption in synaptic function can contribute to the characteristic behavioral and cognitive symptoms observed in individuals with autism spectrum[11]

Furthermore, the ankyrin repeat domain of SHANK3 is essential for its localization and stability at the synaptic sites, where it plays a crucial role in the organization and function of excitatory synapses.[8] Dysregulation or loss of function of the ankyrin repeat domain can lead to impairments in synaptic transmission and plasticity, which are implicated in the pathophysiology of autism.

Ankyrin repeat domain mutations in SHANK3 have been of particular interest in the study of autism spectrum disorder. These mutations can disrupt the protein-protein interactions and cellular localization of SHANK3, leading to impaired synaptic function and neuronal communication. Several studies have investigated the impact of ankyrin repeat domain mutations in SHANK3 on autism risk and severity[12]

One study found that individuals with autism who had SHANK3 mutations, particularly those affecting the ankyrin repeat domain, exhibited more severe clinical phenotypes, including intellectual disability and language impairment. This suggests that specific mutations within the ankyrin repeat domain of SHANK3 may be associated with a more pronounced presentation of autism spectrum disorder. [12]

Furthermore, recent research by Monteiro and Feng has demonstrated the critical role of SHANK3 ankyrin repeat domain mutations in disrupting the balance of synaptic signaling pathways and contributing to the core features of autism, such as social and communication deficits, and repetitive behaviors.[8]

Overall, the literature indicates that SHANK3 ankyrin repeat domain mutations play a significant role in the pathogenesis of autism spectrum disorder, potentially influencing its clinical manifestations and severity.

many SHANK3 polymorphisms have been studied for their role in ASD. though (rs2146772569) hasn't been extensively studied for it's role in autism. it shows an important mutation in the ANK domain and potentially has a deleterious effect on it's function.

3. Method:

3.1: Sample Collection: 30 children diagnosed with ASD and undergoing treatments in ALSIBTEIN academic center for autism spectrum disorder have been recruited for this study(mean age 4±2 years). And 40 healthy children were collected from visitors to alzahraa Teaching Hospital (mean age 5±2).

All participants and their caregivers have been informed about the enrollement in this research. The ethics committee of alkufa university have permitted this research and it has been carried out by the declaration of Helsinki, the code for ethics established by world medical association.

3.2: DNA extraction and genotyping:

Blood samples have been obtained from both patients and healthy groups in EDTA tubes. DNA was extracted using (addprep genomic DNA extraction kit). Agarose gel electrophoresis using

0.7% agarose gel was performed to determine DNA quality. The primers used for PCR are 5´CTGGGCCTGGTGTGGATAC as the forward primer and 5´ATGTGCAGGACACACAGTC as the reverse primer and the product size is 470bp. 25µl of master mix and 4µl of each primer and 13µl of deionized water and 4µl of DNA was used to prepare the PCR solution. PCR conditions were: 1 cycle of initial denaturation at 94℃ for 5 minutes, 35 cycles each 2 minutes of (denaturation at 94℃, annealing at 56℃, extension at 72℃), last, 1 cycle of final extension at 72℃ for 5 minutes. PCR product was purified using wizard SV gel and PCR cleaning system(Promega)[13]. and sent to be sequenced using Sanger sequencing method at Macrogen, Korea. All DNA sequences were compared to a reference genome sequence of gene SHANK3 (accession number: NG_070230.1) using BLASTn server:(https://blast.ncbi.nlm.nih.gov/Blast.cgi) .Final comparison of the nucleotides and amino acids were applied using ClustalW server (https://www.ebi.ac.uk/Tools/msa/clustalo/).

4. **Statistical Analysis:** SPSS V26.0 program was used. Genotype characteristics were compared between the two groups, t rest and ANNOVA test were performed. Chi square was calculated to determine the significance of this polymorphism.

5.Results: DNA sequencing data analysis

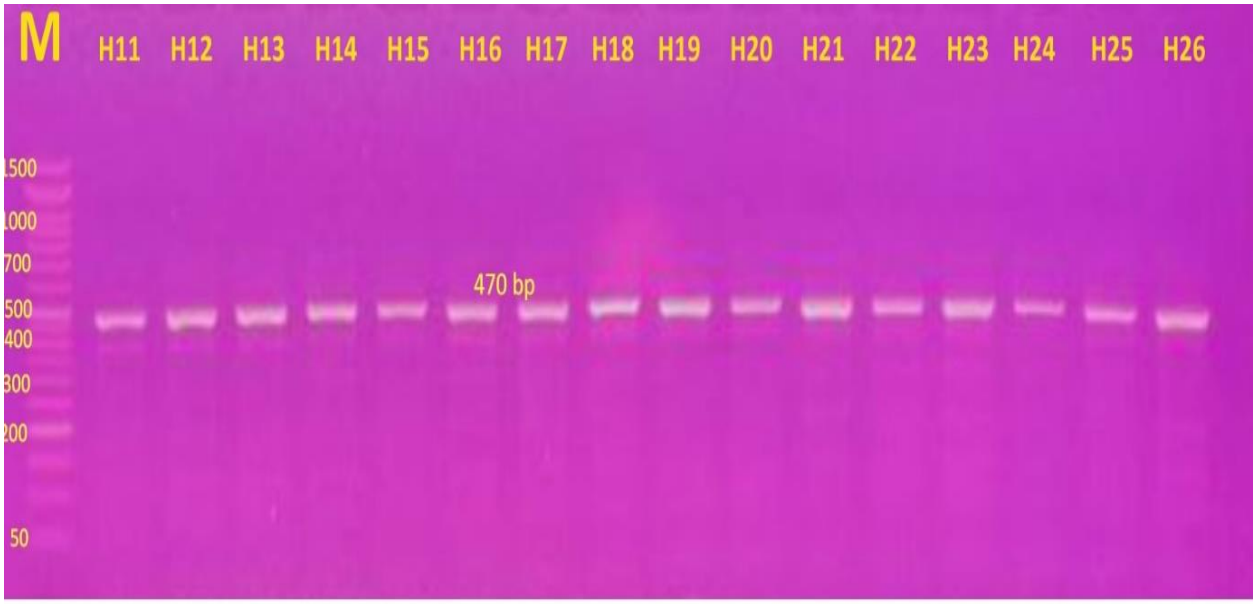

Figure (1): PCR Amplification for Healthy Group of Partial Region of Gene SHANK3 in *homosapiens* for Detection of SNP

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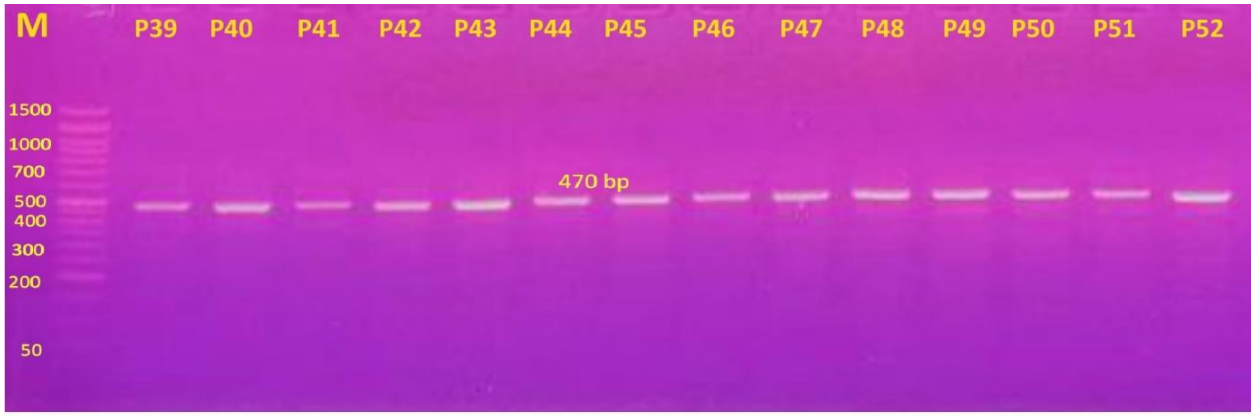

Figure (2): PCR amplification for patient group of partial region of gene SHANK3 in homosapiens for detection of SNP

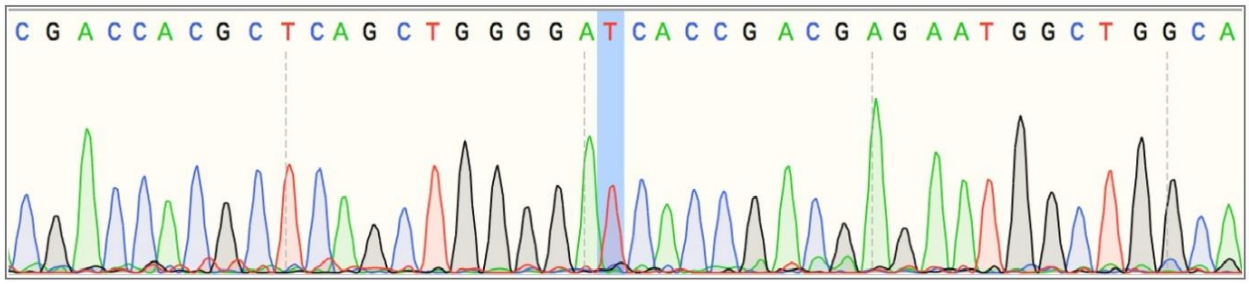

Figure (3): Chromatogram of DNA sequencing of sample GMH75 that showed the wild-type allele TT of SNP rs2146772569 (highlighted in a blue box).

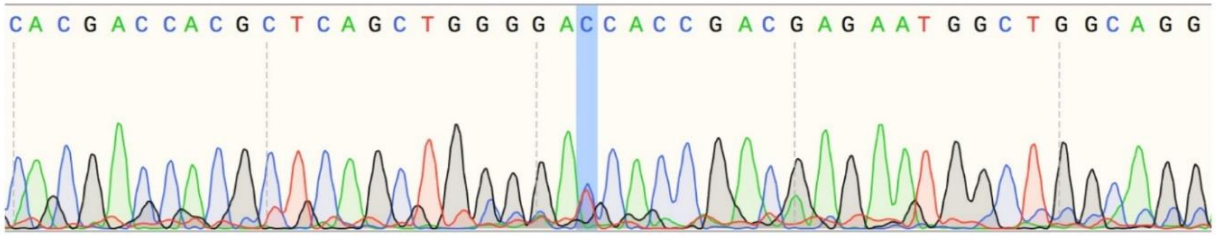

Figure (4): Chromatogram of DNA sequencing of sample GMH70 that showed the heterogenotype allele TC of SNP rs2146772569 (highlighted in a blue box).

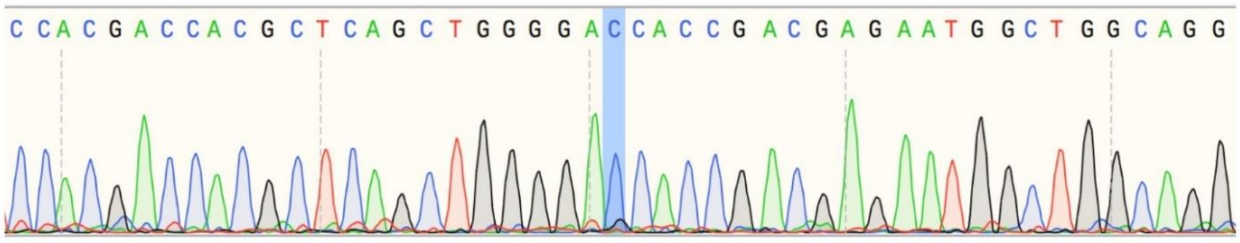

Figure (5): Chromatogram of DNA sequencing of sample GMH66 that showed the homogenotype allele CC of SNP rs2146772569 (highlighted in a blue box).

Genotyping results showed that 8 patients and 15 healthy had the wild genotype CC,and 7 patients and 13 healthy had the heterozygous genotype CT (OR=1.01, P=0.98), and 15 patients and 12 healthy had the homozygous genotype TT (OR=2.3, P=0.15) . all data listed in table 1.

According to hardy Weinberg equilibrium, both patient and healthy group results are not consistent with HWE (table2)

Table (1): Genotype *SHANK3* gene polymorphism SNP rs2146772569 C>T in the studied pathological group

Snp (C>T)	Normal Kids N=40	Autism Kids N= 30	Or (95% Ci)	P Value
Co-Dominant				
Cc	**15**	**8**	**-**	
Ct	**13**	**7**	**1.01 (0.287-3.55)**	**0.98**
Tt	**12**	**15**	**2.3 (0.7453-7.37)**	**0.15**
Dominant				
Ct+Tt	**25**	**22**	**1.6 (0.588-4.63)**	**0.34**
Recessive				
Cc+Ct	**28**	**15**	**-**	
Tt	**12**	**15**	**2.3 (0.872-6.246)**	**0.09**
Over Dominant				
Cc+Tt	**27**	**23**	**-**	
Ct	**13**	**7**	**0.6 (0.216-1.85)**	**0.4**
Additive				
C Allele	**43**	**23**	**-**	
T Allele	**37**	**37**	**1.8 (0.946-3.695)**	**0.07**

Table (2): Hardy Weinberg Equilibrium for SNP rs2146772569 C>T normal and autism kids' groups, both are not consistent with HWE

Genotype	Normal Kids (Frequency)	Expected	Autism Kids (Frequency)	Expected	P For HWE Normal Kids	*P* For HWE Autism Kids
CC	**15**	**11.56**	**8**	**4.41**		
CT	**13**	**19.89**	**7**	**14.18**		
TT	**12**	**8.55**	**15**	**11.41**	**0.03**	**0.005**
C%		**53.75**		**38.33**		
T%		**46.25**		**61.67**		

6. Discussion: Autism spectrum disorder is a disease that has a strong genetic component, though the full picture of autism genetics is yet to be understood, many studies have advanced in trying to explain the etiology of ASD with genetics as the culprit. So far more than 400 genes have shown association with ASD and each gene has many areas of research.Many studies have focused on shank3 gene as a culprit in ASD, many de novo and inherited variants have been reported to disrupt the function of scaffolding protein at the synaptic level in addition to its role in spine neuronal development(14) Current study focused on a single nucleotide polymorphism with unknown significance in ASD, protein analysis have shown that this SNP is likely to be benign and this is supported by our statistical analysis results of allele frequencies. According to NCBI , this SNP alternative allele frequency is 0.000008, though our data shows a much higher frequency in Iraqi population. A clear limitation of our research is the small sample size that may decrease the statistical significance of our results. Our results suggests that polymorphisms in different ethnicities can have a different impact, highlighting the need to replicate studies for most polymorphisms in the Iraqi population.

7.Conclusion And Future Recommendations: Our study results showed that SNP rs2146772569 is likely to be associated with ASD development in Iraqi children.

However the sample size is rather small and a more diverse demographic sample is required in future research to support these results.

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