Original Article

Study of SLC25A12 Gene in Pediatric Age Group with Autism Spectrum Disorder Aged 3-13 Years in Thi-Qar Center of Autism

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ABSTRACT

Objective: To find out the mutation in gene SLC25A12, rs2292813 (T>C) single nucleotide polymorphic is a cause of autism spectrum disorder.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Thi-Qar Autistic Center in Nasiriya city in Thi-Qar Governorate from 15st November 2022 to 15th of June 2023.

Methods: Ninety six patients were diagnosed as cases of autism spectrum disorder by pediatric psychiatry were enrolled and age range was 3 to 13 years. The apparent healthy control group is consisting 96 of children with ages range was 3-13 years. The polymerase chain reaction-tetra-ARMS method was conducted using a commercially available polymerase chain reaction master mix according to the instructions and special primers were designed to detect rs2292813 (T>C) single nucleotide polymorphic mutation.

Results: The heterozygous genotype (CT) is observed in 10 (10.4%) of samples and homozygous genotype (CC) is observed in 84 (87.5%) of samples while homozygous genotype (TT) is observed in 2 (2.08%) of samples. For control group the heterozygous genotype (CT) is observed in of samples 26 (27.1%) and homozygous genotype (CC) is observed in 69 (71.8%) of samples while homozygous genotype (TT) is observed in 1 (1.04%)

Conclusion: There is statistical association between SLC25A12 single nucleotide polymorphism and autism spectrum disorders.

Key Words: Autism spectrum disorders, SLC25A12, Polymerase chain reaction, Single nucleotide polymorphism

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INTRODUCTION

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders that affect social interaction and communication with repetitive and stereotyped behaviour.[1] Most ASD effective genes are recognized to participate in development of fetal nervous system especially in synaptic generation , neuronal generation and differentiation of nervous cells and migration of neurons.[2] Many of researches on twins and relatives show high heritability with a concordance degree for monozygotic twins of 60-95% compared 0-30% for dizygotic twins for ASD.[1]

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These genetic factors include sex chromosomal aneuploidies which are present in 2-5% of autistic patients. High levels of autistic features are found in Klinefelter syndrome (47, XXY), and Turner syndrome (45, X).[3] Males with Y chromosome aneuploidy (47XYY and 48XXYY) are 20 times more likely to have a diagnosis of ASD than males in the general population.[4]

Copy number variations (CNVs) are molecular phenomenon proposed that the sequences of the genetic material are repeated, and the quantity of repeats differs between individuals of the same species.[5] Duplications, deletions, translocations, and inversions are examples of the submicroscopic structural abnormality which known CNVs.[6] About 5-10% of ASD conditions carry CNVs and most CNVs condition rise spontaneously or de novo, although they can also be hereditary in families, and at least 90 pathogenic CNVs have been informed for ASD.[7]

Monogenic Autism, is recognized genetic causes and it is existing in the general population at a frequency of ≤1% and it is categorized by dysmorphic traits and an equal male to female rate.[8] Fragile X syndrome is an instance of monogenic autism who is caused by magnification number of repeats of triplet CGG in

promoter region of FMR1gene causing lack of transcription and production of protein of the gene[9] and 21-50% of affected persons have ASD.[10]

Single nucleotide polymorphism (SNPs) area genomic abnormality at a single base position in the DNA which comprise exchanges, deletions or insertions. These variants are significant since they modify the action of the gene production. Polymorphisms, is changes with a slight allele frequency of at least 1% in population.[11] The common of ASD-associated SNPs are located within the non-coding components of the genome in regulatory elements that modify gene express.[12] For instances is the mutations in the SHANK3gene of chromosome 22 in Phelan-McDermid syndrome in which autism is a projecting features.

Epigenetic mechanisms are the molecular factors that produce complexes at controlling areas of DNA to effect genetic action without altering the prime DNA sequence. Such factors are typically hereditary.[13] Epigenetic alteration act by dysregulation of DNA or histone methylation, DNA acetylation, chromatin transformation or RNA-based mechanisms[14], mutations in HIST1 H1A gene which encoding H1 histone linker protein and this mutation perform lesser protein expression and can cause ASD or other social difficulties.[15]

METHODS

This study was performed in Thi-Qar Autistic Center in Nasiriya city in Thi-Qar Governorate. A total of 96 patients were diagnosed as cases of ASD by pediatric psychiatry were enrolled. The ages range was 3 to 13 years. The control group is consisting of 96 apparently healthy children with ages range was 3-13 years. The newly diagnosed cases of ASD before start intake treatment and the control group were apparently healthy children and meet the same age range were included. Children with schizophrenia, cerebral palsy, children with cardiac disease, children with asthma, infection or inflammatory condition (increase ferritin), and patient with iron supplement were excluded.

DNA extraction from blood is done by use special kit and protocol by AddBio company -south Korea .The amounts of double-stranded DNA (dsDNA) in a purified samples are measured by QuantusFluorometer System that contains a fluorescent DNA-binding dye that can sensitively quantitate small sample amount of dsDNA and it is sensitive in a range of 0.2-400 ng/µl of dsDNA.[16]

The most effective way of separating DNA fragments of varying sizes ranging from 100 bp to 25 kb.[17] Agarose polymers form a network of bundles whose pore sizes determine a gel's molecular sieving properties. Figures 1 and 2 showed DNA agarose gel electrophoresis image for patients group and controls group.

Primers were shipped in a lyophilized state. The units of a lyophilized primer were given as a mass in Picomoles. To create a stock of primers, one would reconstitute the primer in free nuclease deionized water. The company supplied the amount of free nuclease deionized water to be added to each primer to obtain master stock that would be used again to obtain a working stock.A specific mutation was selected from the National Center for Biotechnology Information (NCBI) that shows the loci of the mutation as in the following:

SLC25A12 SNP: (rs2292813) (T>C)
Position in chromosome: chr2:171787719
(GRCh38.p14)

For the purpose of detection this mutation, a specific Polymerase chain reaction (PCR) technique known as Tetra-ARMS PCR was carried out as a simple, highly precise and efficient more cost-effective choice that requires fewer reagents and less time. This was achieved by designing specific primers targeting the homozygous and heterozygous genotype (Table 1). QuantusFluorometer System that contains a fluorescent DNA-binding dye was used for measurements the concentration of dsDNA for patient s group that mean was 9.4 ng/uL with a range (3.69-16 ng/ul) and for control group was 10.2 ng/uL with a range (5-16 ng/uL). The lower accepted concentration of dsDNA is 0.2 ng/uL.

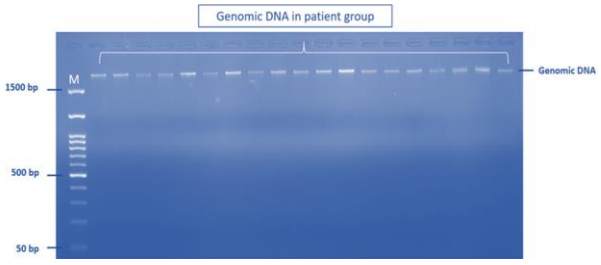

Figure No. 1: Agarose gel electrophoresis image (1.5%) shows the extracted genomic DNA from patient group. M is molecular marker from Gene Direx (South Korea)

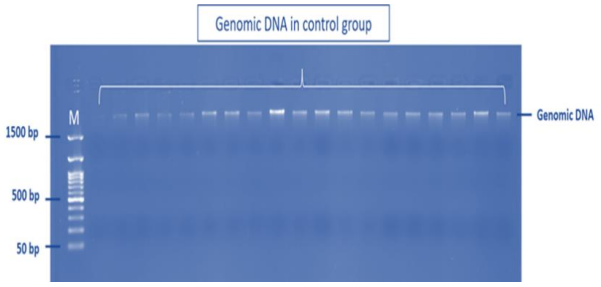

Figure No. 2: Agarose gel electrophoresis image (1.5 %) shows the extracted genomic DNA from control group. M is molecular marker from Gene Direx (South Korea)

The data was analyzed using Software Package for Social Science (SPSS-22 version). t- test have been

used to determine the significant difference between the groups.. Utilizing the odd ratio and Chi-square ($\chi 2$) test, genetic analysis was carried out. Significant difference $p<0.05$, very significant $p<0.01$ and high significant $p<0.001$.

RESULTS

The mean age was 7±2.65 years of ASD group and the patients were divided into male and female subgroup and also divided into two age subgroups G1 (3-8 years) subgroup and G2 (9-13 years) subgroup. The mean was 6.5±2.55 years of control group. There were no significant differences in age ($P>0.05$) between patient with autism and apparent healthy control groups. There were no patients seen below 3 years in this study. The number of patients who visit the center with age 3 years as a first visit from about 9.6% of patients (10/96) while the most frequent age of visit was 6 years which form about 19.2% (20/96) of total patients. There were no patient's age seen above 13 years (Table 2).

Twenty one patients were females (22%) of total patients and 75 were males (78%) of total patients and male to female ratio was 3.54:1 (Fig. 3).

Table No. 1: The primer used for SLC25A12 gene mutation (rs2292813) and their loci, properties and the expected size for genotyping

Primer	Sequence '5->3'	Start	End	Tm C	Genotype	Size
Forward outer	CATAAGTGACCAAGGT-AACACCAAACTGG	111,665	111,693	67°C	In both	160
Reverse outer	ACGACATACAGTGGTG-TCATCGACTGTT	111,434	111,461	67°C		
Inner forward	TTGAAACCAGGACAAA-TGTGGTAAATAC	111,588	111,612	64°C	C-allele	180
Inner Reverse	CGTCCTCCCCTGTGACTC-AGTGGATA	111,560	111,582	70°C	T-allele	134

SLC25A12 genotyping: Conventional PCR was used for amplification of target DNA of SNP rs2292813, (T>C) mutation by using four designed primers in single PCR reaction to amplify the target DNA region. The two outer primers amplify a larger regions that include the SNP or mutation while to inner primers are designed to specifically amplify the mutant or wild (normal) alleles .The PCR product is illustrated in figure 4 which represent the SLC25A12 gene polymorphic site bands that can be visualized by using agarose-gel electrophoresis, these bands were 260 for both C and T- alleles and 180 bp for C-allele and 134 bp for T-alleles (Fig. 4). The heterozygous genotype (TC) is observed in 10 (10.4%) of samples and homozygous genotype (CC) is observed in 84(87.5%) of samples while homozygous genotype (TT) is observed in 2 (2.08%) of samples. For control group the heterozygous genotype (CT) is observed in 26 (27.1%) of samples and homozygous genotype (CC) is observed in 69 (71.8%) of samples while homozygous genotype (TT) is observed in 1 (1.04%) with statistical summary for patients and control groups (Tables 3-6).

Table No. 2: Comparison of age according to patients and control groups

Group	Age (years)	P value
Patients	7±2.65	>0.05
Controls	6.5±2.55	

Table No. 3: Genotyping and alleles frequency of SLC25A12 gene polymorphism

Group	Genotype				Allele frequency	
	No.	TT	TC	CC	T allele	C allele
Patients	96	2(2.08%)	10(10.4%)	84(87.5%)	14(7.3%)	178(92.7%)
Control	96	1 (1.04%)	26 (27.1%)	69(71.8%)	28(14.5%)	164(85.5%)

Table No. 4: SLC25A12 gene polymorphism characterization in patients and control groups genotype

Genotype	Patients	Control	$\chi 2$ P- value	Odd Ratio	CI (95%)	P-value
TT	2	1	8.91 (P<0.05)	0.82	0.11-5.98	P>0.05
TC	10	26		2.6	0.32-21.04	P>0.05
CC	84	69		1.0 reference		

Table No. 5: SLC25A12 gene polymorphism characterization in patients and control Alleles frequency

Alleles frequency	Patients	Control	$\chi 2$ P- value	Odd Ratio	CI (95%)	P-value
T alleles	14	28	8.240 (P<0.05)	0.46	0.23-0.90	P<0.05
C alleles	178	164		1 reference		

Table No. 6: SLC25A12 gene polymorphism characterization in patients and control in inheritance dominant and recessive

Alleles frequency	Patients	Control	χ2 P-value	Odd Ratio	CI (95%)	P-value
Dominant						
TT	2(2.08%)	1(1.04%)	8.339	2.0	0.18-22.67	P>0.05
CT & CC	94(97.92%)	95(98.98)	(P>0.05)	1 reference		
Recessive						
TT	84(87.5%)	69(71.8%)	8.752	3.19	1.44-7.70	P<0.05
CT & CC	12(12.5%)	27(28.12%)	(P<0.05)	1 reference		

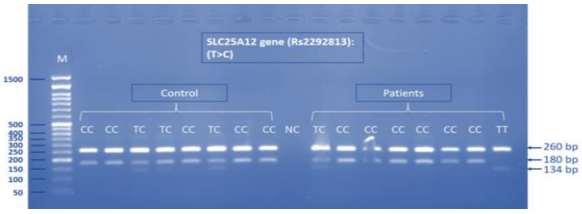

Figure No. 4: Agarose gel electrophoresis image (1.5%) shows genotypes of SLC25A12 gene (rs2292813) (T>C). 260 for both C and T-alleles and 180 bpfor C-allele and 134 bp for T-alleles. NC is referred to negative control in which similar PCR conditions were used except H_2O was added instead of DNA. M is molecular marker from Gene Direx (South Korea)

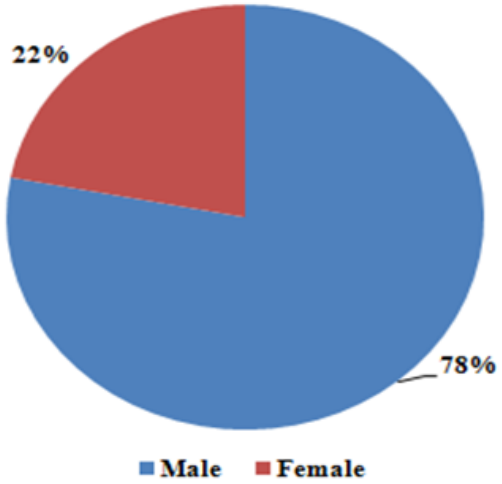

Figure No. 3: The percentage of male and female in autism spectrum disorder

DISCUSSION

The cause of division of patients and control groups into two age subgroups is that the age subgroup G2 (9-13 yrs) represent the age of beginning of hormonal changes of puberty[18] that may effect on autism development. There were no significant differences in age (P>0.05) between patients with autism and apparent healthy control group (Table 1). This ages matching helps to eliminate in the variation of results which may originate from the variation in ages.

Although the clinical picture of disease may start in 2yrs or below.[19] There were no patients seen below 3 years in this study which may be attributed to low awareness of parents to the disease especially in low socioeconomic population or hope of parents to spontaneous improvement of their children with increase age. In this study the number of patients who visit the center with age 3 years as a first visit from about 9.6% of patients (10/96) because in this age the symptoms of disease become prominent while the most frequent age of visit was 6 years which form about 19.2% (20/96) of total patients and this may be attributed to the 6 years is the age of school attendance so the parents aware that the child is unfit for teaching so start medical consultation also the routine medical examination for students to acceptance in school may refer some cases to the autistic center. There were no patients age seen above 13 years this may be due to this group of patients consult adult psychiatrist instead of pediatric psychiatrist.[21]

The male number is higher than female in multiple times and the possible explanation of this is a result of intrauterine life fetal exposure of testosterone can causes sex difference in autistics features[20] and another study[21] explain the high male to female ratio as a result of higher threshold of genetic liability is required for females or higher genetic load was required in females to reach the threshold for a diagnosis as compared with males; thus, this is known as the “female protective model” or as result the clinical presentation of disease is different between males and females and a portion of girls with higher cognitive and language abilities are at risk if not being identified until later in life[22] and male to female ratio was 3.54:1. The M:F ratio was 4:1 in studies of Catherine et al[23] in Global study. While in study of Antonio et al[23] they found a range of M: F ratio from 2:1 to 5:1 in different autistic centers and another study the ratio was 3:1 by Rachel et al.[24] In all above studies the male is higher than female incidence of ASD.

There were statically significant association of the rs2292813 (T>C) SNP of SLC25A12 gene with ASD (p<0.05) [Tables 3,4] also there is statistically significant (p<0.05) with high frequency of (C) alleles in patients group in compare with the control group while low frequency of (T) alleles in patient group in compare with control group (Table 5). It can be seen the model of penetrate if recessive or dominant, and it was recessive type of inheritance (P<0.01).

Aspartate Glutamate Carrier (AGC 1) protein, is encoded by SLC25A12 gene, which is involved in mitochondrial function, since the physiological function of neurons depends greatly on energy supply, any alteration in mitochondrial function or the level of ATP in the cell could lead to corresponding changes in special neurons and can cause autism.[25] SLC25A12 gene in have a role in myelination of nerves. Demyelination causes decreased connectivity, a phenomenon that might account for some of the behavioral observations in ASD and participate in pathophysiology of ASD.[26] Although (rs2292813) is intronic polymorphism, SLC25A12 gene presented in significant statistical association with ASD risk, so It can be suggest that these non-coding SNPs may directly modulate SLC25A12 expression or splicing leading to alternated coding mRNA or abnormal levels of RNA expression.[27] This result of significant association between SLC25A12 gene mutation and ASD agree with studies of Jun et al[25] and studies of Nicolas et al[28] who found significant association between autism and mutation in the gene rs2292813 (T>C) SNP of SLC25A12 but disagree with study of Raquel et al[29] and studies of Wei-Hsien et al[30] in which there was no significant association between this SNP and ASD.

CONCLUSION

There is statistical association between SLC25A12 single nucleotide polymorphism and autism spectrum disorders.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Naeem Salih Yaser
Drafting or Revising Critically:	Maha Fadhil Semaism, Rebee Mohsin Hasani
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

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REFERENCES

1. Montanari M, Martella G, Bonsi P, Mirangolo M. Autism spectrum disorder, focus on glutamatergic neurotransmission. Int J MolSci 2022;23: 3861.
2. Doi M, Li M, Usui N, Shimada S. Genomic Strategies for Understanding the pathophysiology of Autism Spectrum Disorder. Frontiers Molecular Neurosci 2022; 15.
3. Freitag CM, Haslinger D, Yousaf A, Waltes R. Genomic strategies for understanding the pathophysiology of autism spectrum disorder. Frontiers Molecular Neurosci 2022;15.
4. Tartaglia NR, Wilson R, Miller JC, Rafalk J, Cordeiro L, Davis S. Autism spectrum disorder in males with sex chromosome aneuploidy: XXY/Klinefelter syndrome, XYY, and XXYY. J Dev Behav Pediatr 2017; 38(3): 197-207.
5. Pos O, Radvanszky JB, Buglyoe G, Pos Z, Rusnakova D, Nagye B, et al. DNA copy number variation: Main characteristics, evolutionary significance, and pathological aspects. Biomed J 2021;44:548-59.
6. Rylaarsdam L, Guemez-Gamboa A. Genetic causes and modifiers of autism spectrum disorder. Frontiers Cellular Neurosci 2019; 13.
7. Drakulic D, Djurovic S, Syed YA, Trattaro S, Caporale N, Falk A, et al. Copy number variants (CNVs): a powerful tool for iPSC-based modelling of ASD. Molecular Autism 2020;11:42
8. Yoo H. Genetics of autism spectrum disorder: current status and possible clinical applications. Exp Neurobiol 2015;24(4):257-72.
9. Lisik MZ. Molecular aspects of autism spectrum disorders. Psychiatr Pol 2014;48(4):689-700.
10. Kliegman RM. St Geme JW, Blum NJ, Shah SS. Nelson Textbook of Pediatrics 21st ed. Elsever Saunders. Philadelphia; 2018.p.1725-49.
11. Tamargo-Gómez I, Fernández AF, Mariño G. Pathogenic single nucleotide polymorphisms on autophagy-related genes. Int J MolSci 2020; 21: 8196.
12. Golovina E, Fadason T, Lints TJ, Walker C, Vickers MH, O'Sullivan JM. Understanding the impact of SNPs associated with autism spectrum disorder on biological pathways in the human fetal and adult cortex. Scientific Reports 2021; 11:15867.
13. Loke YJ, Hannan AJ, Craig JM. The role of epigenetic change in autism spectrum disorders. Frontiers Neurol 2015; 6.
14. Ng R, Kalinousky A, Harris J. Epigenetics of cognition and behavior: insights from Mendelian disorders of epigenetic machinery. J Neuro-developmental Disorders 2023; 15:16.
15. Barbara W, Beata A. Genetics and epigenetics of autism spectrum disorder - current evidence in the field. J Appl Genet 2019; 60:37-47.
16. Sasson NJ, Pinkham AE, Kimberly LH. Belger A. The benefit of directly comparing autism and schizophrenia for revealing mechanisms of social cognitive impairment. J Neurodevelop Disord 2011;3: 87-100.
17. HavdahlA, Niarchou M, Starnawska A, Uddin A, van der Merweand C, Warrier G. Genetic

contributions to autism spectrum disorder. Psychological Med 2021; 51:2260–73.
18. Esfarjani SV, Jamalzade NG, Hakim AA, Hesam S. Puberty signs and its relationship with lifestyle in 8to10yearold girls: A descriptiveanalytical study. J Fam Med Primary Care 2022; 11(8).
19. Qiu S, Qiu Y, Li Y. Xianling C. Genetics of autism spectrum disorder: an umbrella review of systematic reviews and meta-analyses. Translat Psychiatr 2022; 12:249.
20. Baron-Cohen S. The extreme male brain theory of autism. Trends Cognitive Sci 2002; l6(6).
21. Zhang Y, Li N, Li C, Zhang Z, Teng H, Wang Y, et al. Genetic evidence of gender difference in autism spectrum disorder supports the female-protective effect. Translat Psychiatr 2020;10:4.
22. Yousif MG, Al-Amran FG, Rawaf S, Al-Jumeily D. Effect of Vitamin D3 Levels on Varicella-Zoster Virus Infection and IFN-Gamma Expression in Children: A Cross-Sectional Study in Iraq. Advancements Life Sci 2023;10:35-40.
23. Antonio N, Schiavi S, La Rosa P, Rossi-Espagnet MC, Petrillo S, et al. Sex Differences in Autism Spectrum Disorder: Diagnostic Neurobiological and Behavioral Features. Frontiers Psychiatr 2022;13.
24. Rachel L, Hull L, Polmear W, Mandy L. What Is the male-to-female ratio in autism spectrum disorder? a systematic review and meta-analysis. J Am Acad Child Adolescent Psychiatr 2017; 56(6).
25. Alsaadi JH, Yousif SY. HIF-1α and ENPP2 as Biomarkers in Chemotherapy Resistance of Children with Neuroblastoma in south Iraq. Advancements in Life Sci 2023;10(S1):79-84..
26. Hashem S, Nisar S, Bhat AA, Yadav SK, et al. Genetics of structural and functional brain changes in autism spectrum disorder. Translational Psychiatr 2020;10:229.
27. Prakashand A, Banerjee M. Genomic selection signatures in autism spectrum disorder identifies cognitive genomic trade off and its relevance in paradoxical phenotypes of deficits versus potentialities. Scientific Reports 2021;11:10245.
28. Nicolas R, Reichert JG, Smith CJ, et al. Linkage and Association of the Mitochondrial Aspartate/ Glutamate Carrier SLC25A12 Gene With Autism. Am J Psychiatr 2004;161:662-9.
29. Khan MU, Rehman R, Kaul H, Mahmood S, Ammar A. Mutational analysis of CYP1B1 gene in Pakistani pediatric patients affected with Primary Congenital Glaucoma. Advancements Life Sci 2019;7(1):32-7..
30. Wei-Hsien C, Samir S. YY, GauGF, Huang YS, Soong TS, et al. Association study of the SLC25A12 gene and autism in Han Chinese in Taiwan. Progress Neuropsychopharmacol Biol Psychiatr 2010;34:189-92..