Ref: Ro J Neurol. 2024;23(2)
DOI: 10.37897/RJN.2024.2.13

Association of MTHFR C677T, MTHFR A1298C polymorphism in relation to patients with autism in Thi-Qar Governorate

Roaa M. Nashee[1], Lamees M. Al-Janabi[2], Mohammed A. Altahan[3]

[1]Department of Chemistry, College of Science, University of Thi-Qar, Thi-Qar, Iraq
[2]Department of Biochemistry, College of Medicine, University Thi-Qar, Thi-Qar, Iraq
[3]College of Health and Medical Technologies, Al-Ayen Iraqi University, Thi-Qar, Iraq

ABSTRACT

Background. Recent data suggests that environmental factors may account for up to 40-50% of the variability in the risk of developing autism spectrum disorder (ASD).

Aim. The purpose of this study was to examine the correlation between MTHFR polymorphism and the heightened susceptibility to autism in children with autism in Thi-Qar.

Material and method. A comparison case-control study of blood samples collected from a cohort of 100 individuals diagnosed with autism, along with 100 healthy individuals serving as a control group. The participants were divided into two groups. The first group, known as the control group, consisted of one hundred healthy people aged between 3 and 14 years. The second group consisted of one hundred autistic patients, ranging in age from 3 to 14 years old.

Results. The findings demonstrated a noteworthy elevation in the gene expression of MTHFR C677T and MTHFR A1298C in all groups of patients, in comparison to the control group. The current study showed a significant difference at p-value < 0.05 was recorded the highest genotype was CT in autism patients 85% and in control group 1.43%, while the lowest genotype was TT 5% in autism patients, and in the control group 1.43%. Within the haplotype, a non-significant difference was noted at p-value <0.05, showing that the highest allele was C in the control group at 97.18%, and in autism patients at 51.35%. According to the odds ratio, it showed a significant gene frequency in autism patients than in the control group. The current study showed that a significant difference at p-value <0.05 was recorded in the highest AC genotype in autism patients at 85%, and in the control group 2.86%, while the lowest genotype was CC 4% in autism patients, and in the control group 0%. Within the genotype, a significant different at p-value <0.05 was noted, showing that the highest allele was A in the control group at 97.22%, and T in autism patients at 51.89%.

Conclusion. The study data indicates a higher likelihood of ASD in individuals with the MTHFR C677T CT/CC and MTHFR A1298C AC/CC polymorphisms, suggesting a potential involvement of abnormalities in the folate/methylation cycle in autism.

Keywords: autism spectrum disorder, autism, (ASD), gene, MTHFR C677T, MTHFR A1298C

INTRODUCTION

Autism, often known as autism spectrum disorder (ASD), is currently recognized as one of the most prevalent neurodevelopmental disorders [1]. These encompass issues related to communication, social interaction, and conduct. Currently, there remains a significant portion of the population who lack a clear understanding of the concept of ASD. Consequently, ASD is frequently perceived in a negative light, with some individuals even classifying it as an illness. They frequently exhibit aberrant conduct [2,3]. MTHFR is an enzyme that has been linked to various intricate psychiatric mental health disorders. The enzyme converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and is involved in the conversion of folate and homocysteine, which is linked to DNA methylation [4-6]. Multiple variations of the MTHFR

Corresponding author:
Roaa M. Nashee
E-mail: roaa.moh.ch@sci.utq.iq

Article History:
Received: 2 April 2024
Accepted: 28 June 2024

gene have been identified [7, 8]. Two frequent polymorphisms, C677T and A1298C, have been proven to decrease enzyme activity. The MTHFR C677T mutation is caused by a substitution of cytosine with thymine at position 677 of Exon 4. This substitution leads to the replacement of alanine with valine, resulting in an enzyme that is sensitive to changes in temperature and has decreased activity [9-12]. For example, the presence of the homozygous C677T (TT) condition is linked to elevated levels of Hcy and reduced levels of folate. The MTHFR A1298C mutation is caused by a substitution of adenine for cytosine at position 1298 of exon 7. This alteration occurs in the substitution of glutamic acid with alanine, leading to a significant decrease in activity in individuals with the homozygous 1298CC disease [13].

MATERIAL AND METHOD

Patients

This study was a comparison between cases with autism and normal subjects conducted in the Autism Center in Nasiriyah, Thi-Qar Governorate, by the Biochemistry Laboratory in the College of Science. The study took place from December 2022 to June 2023. The study consisted of 200 cases, 100 control subjects, and 100 patients. Children with autism and controlswere diagnosed by a pediatrician (Dr. Naama J. Gazar), the director of the Autism Center in Nasiriyah, Thi-Qar Governorate, Mohammed Al Mousawi Children's Hospital.

This study comprised a total of 100 male and female volunteers, both control and patients with trace elements, who were between the ages of 3 and 14 and had autism spectrum disorder (ASD). The data comes from our work by conducting genetic analysis through the DNA of autistic and healthy children who were diagnosed by the pediatrician (Dr. Nima Jazzar), Director of the Autism Center in Nasiriyah, Thi-Qar Governorate, and Muhammad Al-Mousawi Children's Hospital. Until recently, experts talked about different types of autism, such as autistic disorder, Asperger's syndrome, pervasive developmental disorder not otherwise specified (PDD-NOS). But now they are all called "autism spectrum disorders".

They split into two factions as follows:

Control: The study consisted of 100 participants who were in good health and ranged in age from 3 to 14 years old.

Autistic patients: The study included a total of 100 patient volunteers aged between three and 14 years old.

Based on the College of Science's approval of the research project form submitted by the researcher, the form was presented to the expert committee and the ethical committee in the Thi-Qar Health Department, approving the research project in its advanced form and with the approval of the families of the children from whom samples were taken.

Collection of blood samples

Gene sequencing and alignment: The PCR results were subjected to amplification and subsequently sequenced using the Sanger sequencing method using dideoxynucleosides from a company in South Korea. The sequencing findings were analyzed using bioinformatics analysis tools, such as Bio edits, after receiving them from the firm. This analy-

TABLE 1. Genotype and allele frequency of MTHFR C677T and MTHFR A1298C gene, patient with autism and controls

Gene	Genotype	Patients		Control		OR	CI95%	p. value
		No.	%	No.	%			
MTHFR C677T	CC	10	10.0	68	97.14	0.003	0.001-0.013	< 0.001
	CT	85	85.0	1	1.43	5.61	72.5-4335	< 0.001
	TT	5	5.0	1	1.43	5.21	0.59-45.4	0.097
	Allele	Patients		Control		OR	CI95%	p. value
MTHFR C677T	C	95	51.35	69	97.18	0.032	0.010-0.180	< 0.001
	T	90	48.65	2	2.82	31.0	9.22-104.5	< 0.001
Gene	Genotype	Patients		Control		OR	CI95%	p. value
		No.	%	No.	%			
MTHFR A1298C	AA	11	11.0	68	97.14	0.004	0.001-0.014	< 0.001
	AC	85	85.0	2	2.86	183.2	51.2-654.6	< 0.001
	CC	4	4.0	0	0.00	2.04	1.77-2.35	0.043
MTHFR A1298C	Allele	Patients		Control		OR	CI95%	p. value
	A	96	51.89	70	97.22	0.034	0.010-0.113	< 0.001
	C	89	48.11	2	2.78	29.8	8.86-100.4	< 0.001

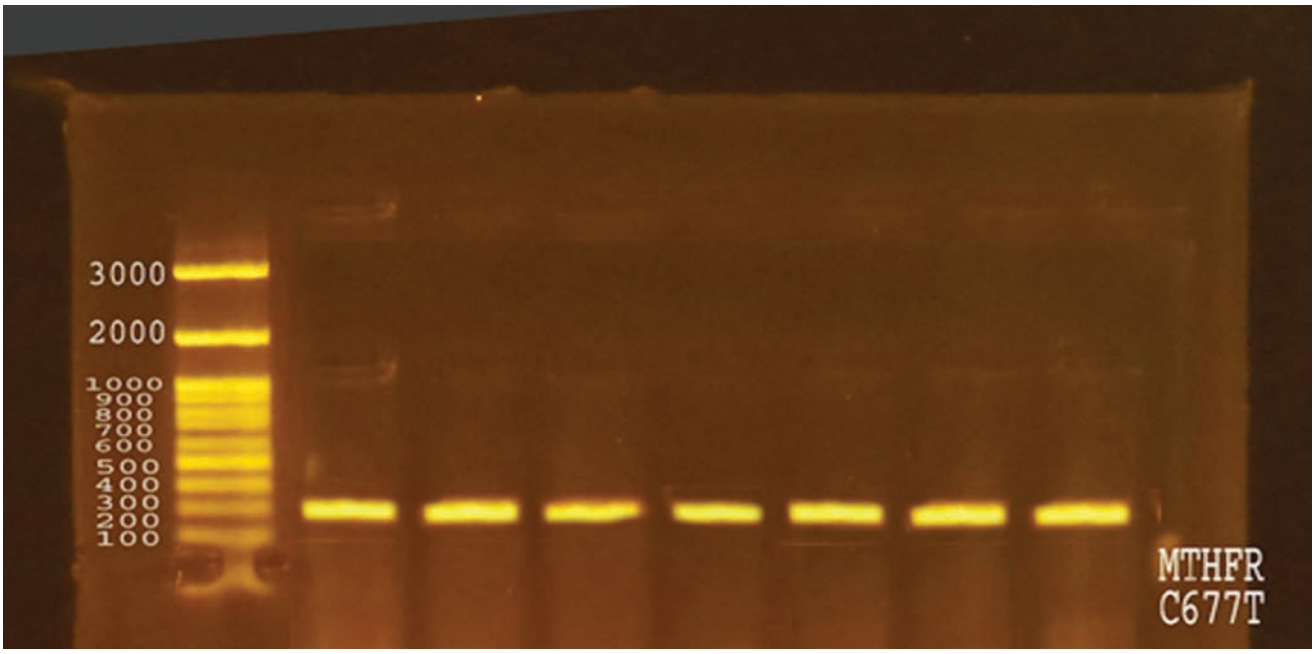

FIGURE 1. Electrophoresis by Gel Agarose 1.5% for MTHFR Gene " C677T SNP product size (198 bp)

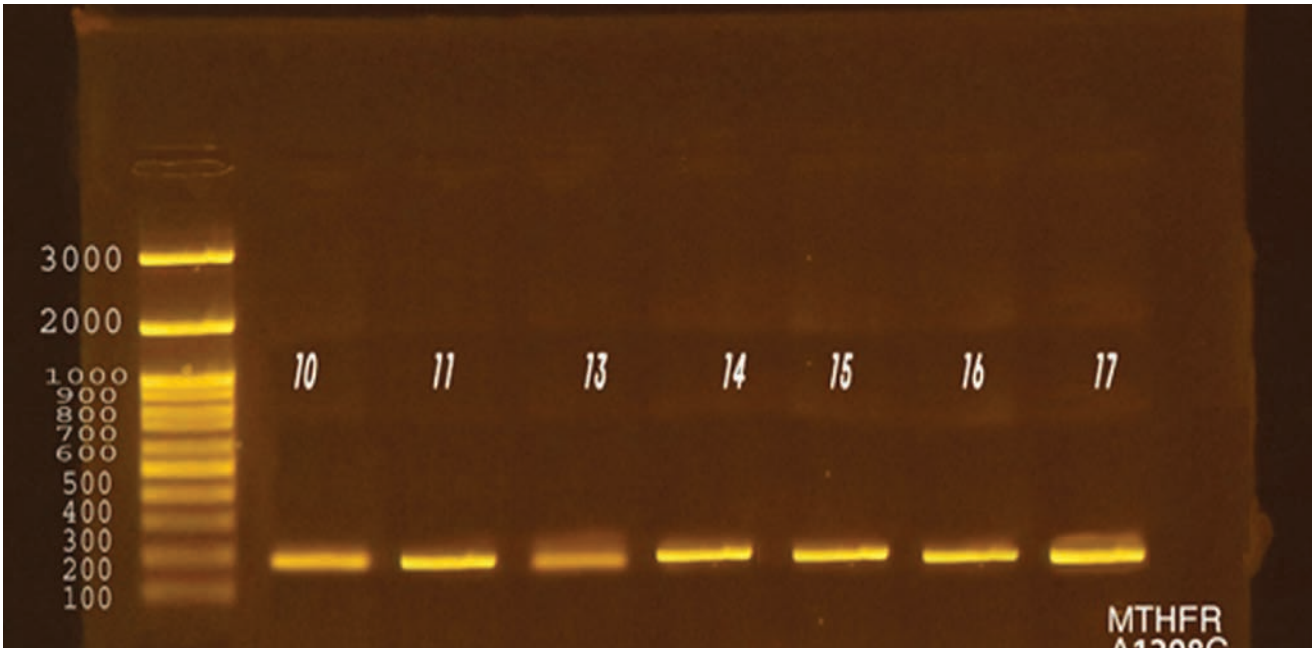

FIGURE 2. Electrophoresis by Gel Agarose 1.5% for MTHFR " A1298C SNP product size is (163 bp)

sis revealed the polymorphisms of the investigated SNP.

Statistical analysis

The data of this study was statistically analyzed by using SPSS version 26, based in using Chi-square and Odds ratio at p-value <0.05. All statistical analysis was performed by using Microsoft Excel 2010 and SPSS version 23 for Windows. Data were expressed as mean±S.D. The significance of the difference in the mean of normality distributed variables was assessed by one-way ANOVA, also using T test to compare Toxo and CMV groups, and odds ratio for the genetic study. Pearson correlation coefficient analysis was used to assess the correlation between continuous variables. Also, for the significance of the difference between variables of groups, Post Hoc (LSD) comparisons were used. P-values were less than or equal to 0.05 ($p \leq 0.05$) is considered significant.

RESULTS

The data presented in Table 1 indicate a statistically significant difference ($p < 0.05$) between the genotypes observed in autism patients and the control group. The greatest genotype observed in both groups was CT, with a prevalence of 85% in autism patients

and 1.43% in the control group. Conversely, the lowest genotype observed was TT, with a prevalence of 5% in autism patients and 1.43% in the control group. A non-significant difference was seen within the haplotype, with a p-value of less than 0.05. The control group had the highest allele frequency of C at 51.35%, whereas the autism patients had a frequency of T at 48.65%. The odds ratio analysis revealed that there was no statistically significant difference in the gene frequency between autism patients and the control group.

DISCUSSION

This study showed a significant difference at p-value <0.05, which recorded the highest CT genotype in autism patients at 85% and in the control group at 1.43%, while the lowest genotype was TT 5% in autism patients, and in the control group 1.43%. Within the haplotype, a non-significant difference was noted at p-value <0.05, showing that the highest allele was C in the control group at 97.18%, and in autism patients at 51.35%. According to the research, odds ratio showed a more significant gene frequency in autism patients than in control group patients.

This study showed a significant difference at p. value < 0.05 was recorded in the highest AC genotype in autism patients at 85%, and in the control group 2.86%, while the lowest genotype was CC 4% in autism patients, and in control group 0%. Within the genotype, a significant difference at p-value <0.05 was noted, showing that

the highest allele was A in control group 97.22%, and T in autism patients 51.89%. According to research odds ratio, a more significant difference of gene frequency was noted in autism patients than in the control group (Table 1). The findings of several studies [1,14-20] indicated a substantial correlation between the C677T gene variant and autism spectrum disorder (ASD). ASD, or autism spectrum disorder, is a chronic neurological condition that results in difficulties with social relationships, deficits in communication, and repetitive activities [21]. Research has shown that ASD has a strong hereditary component, with both rare and common genetic variations contributing to the chance of developing the disorder [22]. Consequently, the genetic inclination towards ASD can vary among individuals. The precise etiology of ASD remains unknown, and certain hypotheses, such as the involvement of both rare and common single nucleotide polymorphisms (SNPs), have generated debates [23].

Table 1 presents the findings of the study, which examined the presence of a significant difference at a p-value of less than 0.05. The results indicate that the greatest genotype observed in both autism patients and the control group was AC, with a prevalence of 85% and 54%, respectively. On the other hand, the lowest genotype observed was CC, with a prevalence of 4% in autism patients and 0% in the control group. A significant difference was seen within the haplotype, with a p-value of less than 0.05. The control group had the highest allele, C, with a frequency of 97.22%, whereas the autism patients had the highest allele, A, at a frequency of 48.11%. The odds ratio analysis revealed that there was no statistically significant difference in gene frequency between autism patients and the control group.

The primary aim of this study is to examine the correlation between MTHFR polymorphism and the heightened susceptibility to autism in children residing in Thi-Qar. This study is the first genetic investigation in Thi-Qar to examine such a connection. Autism is a complex disorder influenced by multiple factors, including environmental, epigenetic, and genetic factors. These factors contribute to the frequency and severity of autistic symptoms [24,25]. Epigenetic pathways exert a significant influence on the manifestation of autism phenotypes. Furthermore, these systems are subject to modulation by drugs and nutrition. A study conducted by Meguid et al. found a correlation between the MTHFR 1298 AC/CC SNP and disruptions in the folate/methylation cycle, which in turn increased the risk of autism [26,27].

The limitations of the study are small sample size, short time and lack of facilities.

CONCLUSION

This data supports an increased risk for ASD in association with MTHFR C677T CT/CC, MTHFR A1298C AC/CC polymorphism and hence the role of folate/methylation cycle disturbances is confirmed in autism.

Conflicts of interest: none declared
Financial support: none declared

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