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

Roaa M. Nashee¹, Lamees M. Al-Janabi², Mohammed A. Altahan³

¹Department of Chemistry, College of Science, University of Thi-Qar, Thi-Qar, Iraq
²Department of Biochemistry, College of Medicine, University Thi-Qar, Thi-Qar, Iraq
³College 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.

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
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 comprised of 200 cases, 100 control subjects, and 100 patients. Children with autism and controls were diagnosed by a pediatrician (Dr. Nama J. Jazzar), the director of the Autism Center in Nasiriyah, Thi-Qar Governorate, Mohammed 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 Bioedit, after receiving them from the firm. This analy-

<table>
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<th>Genotype</th>
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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 ≤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.

**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)
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 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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**REFERENCES**


