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An Investigation of the Correlation Between the CNTNAP2 rs7794745 gene Polymorphism and Autism in Children from the Middle Euphrates Area of Iraq

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Abstract

Background: Autism is an incurable condition that may be attributed to several factors, such as genetic predisposition and environmental influences. Multiple studies have discovered that a substantial number of genes linked to autism serve as constituents of signaling pathways that regulate the plasticity of synapses and development, hence exerting a notable impact on the origins of the illness. CNTNAP2 is increased during the initial phases of neural tube development. Given the increasing worldwide occurrence of autism, there is a growing demand for effective teaching methods and educational programs.

Methods: A case-control study recorded 90 samples, comprising 50 autistic individuals (males and females, mean age 4.5 ± 2 years) and 40 healthy youngsters (5 ± 2 years). PCR and restriction enzymes are used in polymerase chain reaction-restriction to amplify and analyze DNA sequences. PCR-RFLP genotyped CNTNAP2 at rs7794745. Genomic DNA was isolated from peripheral blood cells of healthy children while buccal cells were swapped from patients to obtain and genotype their DNA.

Results: Our results revealed that the low-frequency distribution (p-value > 0.05) of the rs7794745 SNP is statistically non-significant in ASD patients compared to healthy children.

Conclusion: Our case-control study suggests that rs7794745 polymorphism is unrelated to ASD.

دراسة العلاقة بين تعدد الأشكال الجيني CNTNAP2 rs7794745 والتوحد لدى أطفال منطقة الفرات الأوسط في العراق

صفاء الصفار، حيدر الزبيدي، رؤى حميد العويض

الملخص

التوحد هو حالة غير قابلة للعلاج يمكن أن تُعزى إلى عدة عوامل، مثل الاستعداد الوراثي والتأثيرات البيئية. اكتشفت دراسات متعددة أن عددًا كبيرًا من الجينات المرتبطة بالتوحد تشكل مكونات مسارات الإشارات التي تنظم مرونة وتطور المشابك العصبية، مما يؤثر بشكل كبير على أصل المرض. يزيد مستوى CNTNAP2 خلال المراحل الأولية من تطور الأنبوب العصبي. نظرًا لزيادة انتشار التوحد في جميع أنحاء العالم، هناك طلب متزايد على أساليب تعليمية فعالة وبرامج تعليمية.

الطريقة:

تم تسجيل دراسة حالة-شاهد لـ 90 عينة، تضم 50 فردًا مصابًا بالتوحد (ذكور وإناث، متوسط العمر 4.5 ± 2 سنوات) و40 طفلًا صحيًا (5 ± 2 سنوات). استخدمت تقنية تفاعل البوليميراز المتسلسل والإنزيمات القاطعة (PCR-RFLP) لتضخيم وتحليل تسلسلات الحمض النووي. تم تحديد النمط الجيني لـ CNTNAP2عند الموضع .rs7794745 تم عزل الحمض النووي الجينومي من خلايا الدم المحيطية للأطفال الأصحاء، بينما تم أخذ خلايا من اللعاب للمرضى للحصول على النمط الجيني للحمض النووي الخاص بهم.

النتائج:

كشفت نتائجنا أن التوزيع ذو التردد المنخفض (قيمة الاحتمال > 0.05) للنمط الجيني rs7794745 غير ذي دلالة إحصائية في مرضى اضطراب طيف التوحد مقارنة بالأطفال الأصحاء

الاستنتاج:

تشير دراستنا الحالة-شاهد إلى أن تعدد الأشكال rs7794745 غير مرتبط باضطراب طيف التوحد

1. Introduction

Disruptions in social interaction and limited or repetitive behavior are hallmarks of autism spectrum disorder (ASD), a neurodevelopmental medical condition (Abrahams & Geschwind, 2008). ASD consists of a range of disorders, including autistic disorder, Rett syndrome, Asperger syndrome, and pervasive developmental disorder (Bölte et al., 2019; Taylor et al., 2020). Patients with ASD have difficulties with both verbal and nonverbal forms of social communication in addition to problems with cognitive and physical abilities. These patients also have unusual reactions to sensory experiences, unique passion, and repetitive behaviors (van 't Hof et al., 2021).

A substantial rise in the prevalence of autism has occurred during the last two decades, with an estimated global prevalence of 0.62% (Elsabbagh et al., 2012). Males have about fourfold higher risk of developing the disorder than females (sex ratio 4.2:1) (Fombonne, 2009). Despite experts' best efforts, no one etiological factor has been found. However, some published findings imply that several sets of causal elements, including genetic, environmental, and neurobiological aspects, may contribute to the development of ASD by affecting the developing brain (Hodges et al., 2020). The fact that the frequency of autism spectrum disorder is higher among autistic siblings and the concordance rate is higher in monozygotic twins than in dizygotic twins lends credence to the hypothesis that around 80–90% of autism spectrum disorder may be linked to genetics (Castelbaum et al., 2020).

The formation and functioning of synapses require several vital genes. It has been established that several genes, including CNTNAP2 and NLGN3, NLGN4X, and NRXN1, as well as others, have been associated with the process of adhesion between neurons and glia (Abrahams & Geschwind, 2008). In areas of the brain that are linked with autism spectrum disorder (ASD), the contactin-associated protein-like two gene (CNTNAP2) is expressed. This gene has been investigated for its possible role in the development of ASD (Abrahams et al., 2007; Alarcón et al., 2008). One of the most considerable mammalian genes, CNTNAP2, is a member of a family known as the neurexin superfamily. This family consists of 24 exons, occupies 2.3 megabytes on chromosome 7q, and is one of the most significant genes in mammals (St George-Hyslop et al., 2023).

One gene highly expressed in the growing brain and spinal cord is CNTNAP2, which encodes contactin-associated protein-like 2 (Caspr2) (Zare et al., 2017). The CNTNAP2 protein plays a crucial role in the language impairment that is associated with autism spectrum disorder (ASD) as well as other language-related issues. Early childhood autism spectrum disorder (ASD) children experience difficulties with speech development as a result of reduced CNTNAP2 expression (Rodenas-Cuadrado et al., 2018). Another study revealed that rare and common CNTNAP2 gene variants are associated with ASDs, seizures, and intellectual disability. The CNTNAP2 SNPs rs7794745 and rs2710102 are two frequently occurring non-coding variants located in the 2nd and 13th introns of chromosome 7q, respectively (Uddin et al., 2021).

The results show that A/T in rs7794745 has a more substantial effect on the decrease in the brain's response to sensing human voices(Koeda et al., 2015) . Additionally, it was reported that the A/T genotype in rs7794745 increases the risk of ASD in the Chinese Han group (Lia et al., 2010). The goal of this study was to find out if there was a link between

a widespread change in the rs7794745 of the CNTNAP2 gene and the risk of autism in the Iraqi (middle Euphrates) community.

Aim of the Study: To examine the association of CNTNAP2 gene single nucleotide polymorphism and the risk of autism occurrence in Middle Euphrates Iraqi children.

2. Materials, patients and Methods:

2.1.Patients

Fifty children with autism (males and females, mean age 4.5 ± 2 years) were recruited from the Al-Sibtein Academic Center for autism spectrum disorder, and forty healthy individuals (control group) (mean age 5 ± 2 years) collected from visitors to Al-Zahraa Teaching Hospital were incorporated in our study. The diagnosis was done by well-trained psychiatrists based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria, relying on historical information from interviews and clinical records. All participants and their families were informed about this research. The Ethical Committee Approval: The study protocol received approval from the Ethical Committee (in the Faculty of Pharmacy /Kufa University). Fifty children with autism (males and females, mean age 4.5 ± 2 years) were recruited from the Al-Sibtein Academic Center for autism spectrum disorder, and forty healthy individuals (control group) (mean age 5 ± 2 years) collected from visitors to Al-Zahraa Teaching Hospital were incorporated in our study. The diagnosis was done by well-trained psychiatrists based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria, relying on historical information from interviews and clinical records. All participants and their families were informed about this research. The Ethical Committee Approval: The study protocol received approval from the Ethical Committee (in the Faculty of Pharmacy /Kufa University).

2.2.DNA Extraction and Genotyping

Buccal swaps were obtained from ASD patients due to the difficulties in obtaining blood samples from them; they were preserved in a tube with normal saline. Peripheral blood cells were collected from healthy children. A single nucleotide polymorphism (SNP) in the CNTNAP2 (rs7794745) was targeted and chosen from the National Center for Biotechnology Information SNP database. PCR is a popular method for analyzing genomic variations, and single nucleotide polymorphisms (SNPs) in DNA can serve as genomic character markers in diseases or treatment responses, emphasizing the growing acknowledgment of genomic variations in disease etiology (Mubarak et al., 2020). Genomic DNA was extracted from buccal swaps using (the AddPrep Genomic DNA Extraction Kit). As the manufacturer's instructions stated, the SNP was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

The PCR reaction was performed to amplify a 315 bp fragment containing the aimed SNP using a specified primer. By using a PCR-RFLP, the variants of CNTNAP2 were genotyped. A 315-bp fragment containing the loci was amplified using the following primers: 5' AATACGGACCAAGATACCAAC is the F primer, and 5' TTCACACACAGTGCCTT is the R primer. 50 µl of the following ingredients were added to each reaction:

- 25 µl of the PCR master mix
- 4 µl of each primer
- 13 µl deionized water
- 4 µl of DNA

2.3.The PCR Reaction

The PCR conditions were Initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 1 minute, and the final extension at 72°C for 5 minutes. Finally, the PCR products were separated on 1% agarose gel and stained with 5 µl of Safe-Green 100 bp Opti-DNA marker added to the gel's first line pores to measure the PCR products' size. Then, the gel was transferred into the UVP system to visualize the PCR products under a 320nm UV light source. MlucI (ew England Biolabs) restriction enzyme digestion was used to cleave the wild-type sequence into a 315-bp fragment. This A- o-T base pair mutation in the CNTNAP2 gene provides a restriction site. A 1X su table restriction buffer and ten units of the required restriction enzyme were used to digest 1-3 µg of PCR product. The dig sts were then incubated for 3 hours at 37°C (or enzyme-appropriate temperature) before being subjected to electrophoresis to visualize the results. The dig station products were visualized after electrophoresis on a 1% agarose gel stained with a Safe-Green marker.

3. Statistical Analyses

Using (SPSS.v.26.0 software) SPSS Inc. Chicago, IL, the mean levels of each characteristic via genotype were compared using the student t-test and ANOVA. The chi-square test was also used to examine categorical data (alleles and genotypes. A P value could be considered statistically significant if it was less than 0.05)

4. Results

During our research, we looked at a total of ninety participants, the control group consisting of forty healthy children and fifty children diagnosed with autism. According to the findings of the MlucI enzyme, patients and control individuals who do not have a mutation (A→T) in the CNTNAP2 gene (genotype AA) have a fragment of 315 base pairs in size in their PCR products. On the other hand, people with the heterozygous genotype (AT) have three bands in their PCR product: 95 base pairs, 315 base pairs, and 220 base pairs. Furthermore, it is worth noting that persons who are both healthy and patient, who have homozygous genotypes (TT), and who have a mutation (A→T) have two pieces, which are 95 and 220 base pairs Fig.1.

Table 1: Contains all the genotype and allele frequencies data and related ORs (95% CI) for controls and autistic individuals. The frequencies of the genotypes of the rs7794745 A→T are illustrated in Table 1. Thus, a significant association was obtained from genotype distributions of rs7794745 CNTNAP2 gene polymorphism between the cases

of autism and control (95% OR, CI). The ca s' A and T allele frequencies were 48% and 52%, and the healthy group was 39% and 41%, each in order (p = 0.9). There was no significant difference in the frequencies of alleles between the groups.

Table 1*: The Frequency of Each Allele and Genotype of The Rs7794745 Polymorphism in Patients and Controls.

Alleles	**Controls (n = 40)**	**Autism cases (n= 50)**	**OR (95% CI)**	**P**
Alleles (A→T) A	39	48	1.0	0.9
T	41	52	1.03	
Genotypes (A→T) AA	11	12	1.0	0.6
AT	17	24	1.3	
TT	12	12	1.1	
*OR odds ratio; 95% Cl 95% confidence interval; p < 0.05 is statistically significant.				

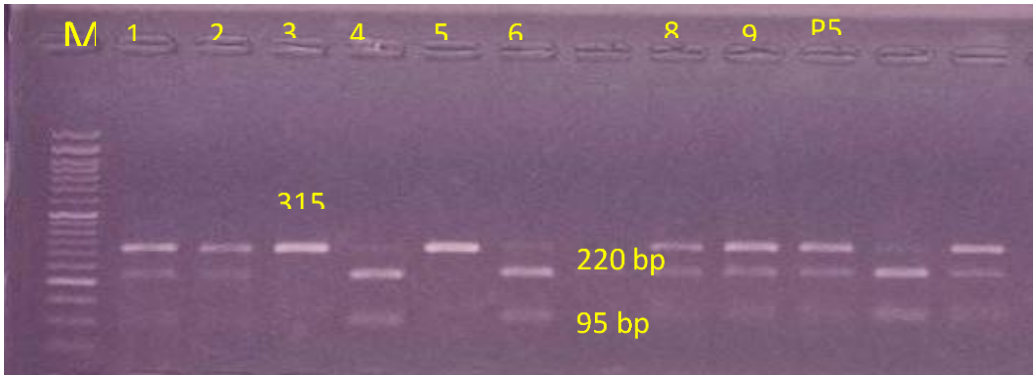

Figure 1: Shows agarose Gel Electrophoresis stained by Redsafe after Pcr-Rflp. Lanes 3, 6, and 9 show the AA, AT, And TT, respectively.

5. Discussion

Autism spectrum disorder (ASD) is a neuropsychiatric developmental condition that is highly heritable and multifactorial. It may rise due to a complex interplay between environmental and genetic risk factors. The genetics of autism spectrum disorder (ASD) are complicated and involve several genes, each of which plays a key role in the development of neural structures in children. Our research attempted to determine if there was an association between polymorphisms in the CNTNAP2 gene and autism. In Iraq children diagnosed with autism, the correlation between the GABRB3, MTR, and MTHFR gene polymorphisms and autism risk has been established (Jabbar & Jebor, 2018; Ma et al., 2005; Muftin et al., 2020). CNTNAP2, also known as NRXN4, is a protein found in the postsynaptic

membrane, which acts as a scaffolding component (Bourgeron, 2009). The CNT AP2 gene is crucial for proper cerebral development, and any disruption to its function significantly increases the likelihood of neurological impairment. Deficits in CNTNAP2 have been associated with ASD-related behaviors such as hyperactivity and epilepsy (Peñagarikano & Geschwind, 2012). An Amis family with autistic traits, cortical dysplasia, and focal epilepsy was found to have a mutation in the CNTNAP2 gene that made it less active (Strauss et al., 2006).

It was also demonstrated that uncommon variations in CNTNAP2 may play a role in the pathogenesis of ASD (Bakkaloglu et al., 2008). The rs7 94745 CNTNAP2 gene polymorphism was studied in a case-control study involving 50 autistic children and 40 controls. Our study's results suggest no significant association of rs7794745 A→T polymorphism to autism (p-value 0.9). like-wi e, no positive association was found between the CNTNAP2 polymorphism and ASD (Jonsson et al., 2014; Poot, 2014; Sampath et al., 2013). Previous research, however, has linked rs7794745 to autism in a variety of populations, including Brazilians and Iranians population (Nascimento et al., 2016). According to a recent study conducted in the Pakistani community, it was discovered that rs7794745 is strongly linked to ASD (Khalid et al., 2020). Additionally, al, research has also shown a direct association between rs7794745 and ASD in the Han Chinese (Lia et al., 2010). A few different factors could have caused these varying findings. Genetic diversity between populations initially had an impact on the results of the association studies. Second, a larger sample size would reduce the sampling error. Third, SD is a highly heterogeneous condition, and the vast majority of prior research has relied on the recruitment of patients with ASD. However, to limit variability, only families with typically developing autistic children were included in this study.

6. Conclusion

rs7794745 CNTNAP2 gene polymorphism was shown to have a non-significant connection with autism in the analyzed community. This is the conclusion that can be drawn from the findings. However, to arrive at a conclusive result, it was necessary to conduct the research with a more significant sample population and diverse ethnic groupings.

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