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Association of Vitamin D Receptor Gene Polymorphisms (rs731236 and rs7975232) among Iraqi Children with Autism Spectrum Disorder

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Abstract

Background: Vitamin D receptor gene (VDR) is implicated in several aspects of human psychiatric disorders, one of them is autism spectrum disorder. Autism spectrum disorder is neurodevelopment disorder characterized by some degree of difficulty with social interaction and communication. The etiology of this disease has been linked to both hereditary and environmental factors. The aim of this study is to determine the association of single-nucleotide polymorphisms(rs731236and rs7975232) in VDR gene with susceptibility of ASD childhood .

Method: A total of 44 children with ASD and 44 controls from Iraqi children were chose, with age of 2 – 11 years old. This study took place between May 2022 and March 2023.Single-Nucleotide Polymorphism SNP genotyping was carried out by Sanger sequencing using (genomic DNA extracted) from blood cells. The number of samples were too low due to the difficulty of drawing blood from a vein due to the child's hyperactivity.

Result: Among two examined SNPs, the AG category of rs731236 demonstrated a significant effect, B = 1.54, OR = 4.68, p = 0.002. This finding suggests that the presence of the AG category of rs731236 increases the odds of observing the Patient category within the Group by approximately 368.24%, in comparison to the AA category of rs731236. In contrast, the GG category of rs731236 did not exhibit a significant effect, B = 0.97, OR = 2.63, p = 0.515, indicating no considerable impact on the likelihood of observing the Patient category within the Group. Furthermore, the AA category of rs7975232 revealed no significant effect, B = -0.37, OR = 0.69, p = 0.489, suggesting that it did not notably influence the odds of observing the patient category within the Group. Similarly, the CC category of rs7975232 had no significant, B = 0.71, OR = 2.03, p = 0.294.The findings of this study, support the hypothesis that rs731236 implicated in the pathophysiology of autism.

Conclusion: The combination of AG genotype of rs731236 is associated with a higher risk of ASD childhood and it is considered a promising target in the diagnosis of this disease.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition marked by repetitive behaviors, limited interests, and difficulties with social communication[1]. Both genetic and environmental factors have been identified as significant risk factors for ASD, even if the exact cause of the disorder is still unknown. Finding these factors could help determine the disease's etiology and lead to the development of innovative disease prevention and treatment strategies [2]. Vitamin D is a neurosteroid hormone that is essential for embryogenesis and neurodevelopment because it influences immunological regulation, neuronal differentiation, neurotrophic and neuroprotective effects, and all of these processes. 25-hydroxyvitamin D3, the active form of vitamin D, interacts to the vitamin D receptor (VDR) and regulates the synthesis of several proteins[3, 4]. The malfunctions of the vitamin D pathway may thus be attributable to the development of ASD. Vitamin D demonstrates its efficacy by binding to VDR in various tissues including brain and peripheral nerves [5]. According to recent studies, Vitamin D**3** has several biological properties, including the ability to down regulate inflammation, and it may also have a role in the pathogenesis of ASD[6] .The VDR gene, which has nine exons and eight introns, is found on chromosome 12q13 [7]. This gene has many SNPs that have been linked to autism spectrum disorder [5].

Methods

Participants

A Case –Control study of (44) children with autism spectrum disorder and (44) healthy controls were enrolled in this study from the Iraqi population, with age of 2 – 11 years old. The study was conducted between May 2022 and March 2023.Ref ID of VDR (Gene ID: 7421), this study was conducted after reviewing numerous studies proving the implication of VDR gene in ASD Iraqi children.

Ethical approval

Ethical approval was obtained with (the approval of the Research Ethical Committee) from the ministry of higher education and scientific research / Al-Nahrain University / College of Medicine / Baghdad (according to the decision for research ethics approval with No:2022144 (IRB/80) in 12/27/2023 with the approval of the families of healthy and ASD children.

Blood sample Collection and Preservation and Preparation

Disposable syringes and vein punctures were used to collect (2) ml of blood sample. A venous whole blood sample collected in a tube contains EDTA and maintained kept at (20-25 C˚) until used for SNPs genotype investigations of VDR gene.

SNPs genotyping was carried out by Sanger sequencing using genomic DNA extracted from blood cells. Forty micro liters of PCR product were sending to Macrogene/ Korea for Sanger sequencing. After trimming of each sequence, the result was blasted in NCBI to check the similarities and differences with the database. Finch TV version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; (http://www.geospiza.com) was employed to check VDR gene polymorphism.

Exclusion criteria

Children with aged > 11 years, Liver diseases, renal diseases, attention deficit/hyper-activity disorder ADHAD, mental retardation, case of diabetes were excluded from this study.

Statistical analysis

The chi-square analysis was determined in both controls and cases to find the frequencies of genotype and alleles differences. 95% confidence intervals (CIs) and Odds ratios (ORs) were calculated. P-values less than 0.05 were regarded as significant.

Results

The logistic regression results with predicting group and genotype and Allele frequencies of rs731236 and rs7975232 polymorphisms in ASD and Control groups

The AG category of rs731236 demonstrated a significant effect, B = 1.54, OR = 4.68, p = 0.002. This finding suggests that the presence of the AG category of rs731236 increases the odds of observing the Patient category within the Group by approximately 368.24%, in comparison to the AA category of rs731236. In contrast, the GG category of rs731236 did not exhibit a significant effect, B = 0.97, OR = 2.63, p = 0.515, indicating no considerable impact on the likelihood of observing the Patient category within the Group. Furthermore, the AA category of rs7975232 revealed no significant effect, B = -0.37, OR = 0.69, p = 0.489, suggesting that it did not notably influence the odds of observing the Patient category within the Group. Similarly, the CC category of rs7975232, B = 0.71, OR = 2.03, p = 0.294 also had no significant .

Variable	*B*	*SE*	χ^2	*p*	*OR*	95.00% CI
(Intercept)	-0.60	0.41	2.15	.142	-	-
rs731236AG	1.54	0.50	9.41	.002	4.68	[1.75, 12.56]
rs731236GG	0.97	1.48	0.42	.515	2.63	[0.14, 48.18]
rs7975232AA	-0.37	0.53	0.48	.489	0.69	[0.25, 1.96]
rs7975232CC	0.71	0.67	1.10	.294	2.03	[0.54, 7.61]
Note. $\chi^2(4) = 12.14$, $p = .016$, McFadden $R^2 = 0.10$.						

Table 1: The logistic regression results with rs731236 and rs7975232 predicting group.

rs731236	Control	Patient
AA	33 [26.00]	19 [26.00]
AG	10 [17.00]	24 [17.00]
GG	1 [1.00]	1 [1.00]
p-value		.004

Table 2: Observed and expected frequencies for rs731236 polymorphism in case and control groups.

rs7975232	Control	Patient	χ2	df	p-value
CA	20 [22.00]	24 [22.00]	1.85	2	0.397
AA	18 [15.00]	12 [15.00]			
CC	6 [7.00]	8 [7.00]			

Table 3: Observed and expected frequencies for rs7975232 polymorphism in case and control groups.

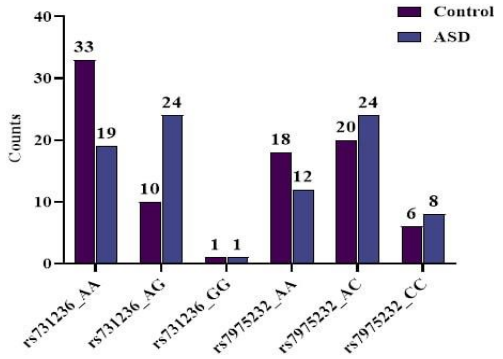

Figure 1: Genotypic frequencies of rs731236 and rs7975232 in ASD Cases and Controls.

Genotypic frequencies of rs731236 in ASD Cases and Controls

The frequency of A in control group was 50% (43 out of 86) and G was 30.56% (11 out of 36). while for ASD, the frequency of A was 50% (43 out of 86) and the frequency of G was 69.44% (25 out of 36). The p-value was 0.0714, which showed there is no significant association between the genetic Alleles (A or G) and ASD.

Data analyzed	control	Freq.	ASD	Freq.	Total	P-value	OR	95% CI
A	43	50%	43	50%	86	0.0714NS	2.273	0.9908 to 4.965
G	11	30.56%	25	69.44%	36			
Total	54		68		122			

Table 4: Allelic frequencies of rs731236 in ASD Cases and Control.

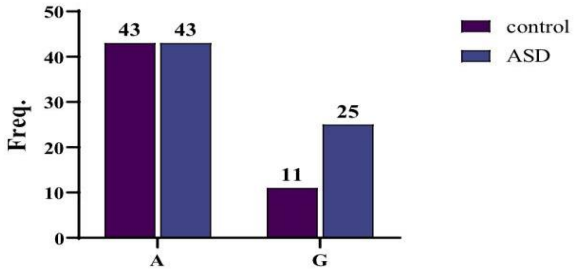

Figure 2: Allelic frequencies of rs731236 in ASD Cases and Controls.

Data analyzed	control	Freq.	ASD	Freq.	Total	P value	Odds ratio	95% CI
A	38	51.35%	36	48.65%	74	0.4868 NS	1.299	0.6604 - 2.596
C	26	44.83%	32	55.17%	58			
Total	64		68		132			

Table 5: Allelic frequencies of rs7975232 in ASD Cases and Controls.

Genotypic frequencies of rs7975232 in ASD Cases and Controls

In the control group, the frequency of allele A was 51.35% (38 out of 74) and the frequency of allele C was 44.83% (26 out of 58). In the ASD group, the frequency of allele A was 48.65% (36 out of 74) and the frequency of allele C was 55.17% (32 out of 58). The p-value was 0.4868, which meant no significant association between rs7975232 alleles (A or C) and ASD.

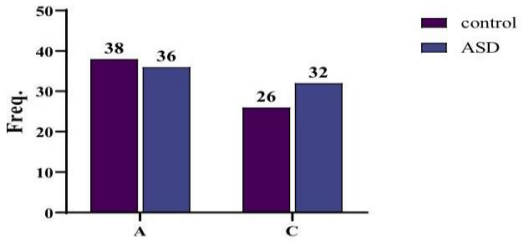

Figure 3: Allelic frequencies of rs7975232 in ASD Cases and Controls.

Regarding the drug safety, only one patient in the G-CSF group developed a mild fever with the first dose of G-CSF, which was treated with a paracetamol tablet (500 mg/8 hours) orally for three days, and no fever developed with subsequent doses of G-CSF in the same patient.

Discussion

Many researchers agree that there are multiple variables that contribute to autism, including both environmental and genetic influences [8]. As a result, epigenetics plays a significant role in the etiology of ASD and combines environmental and genetic factors to deregulate neurodevelopment processes. SNP allelic association approaches are effective tools for identifying genetic variables that predispose to the majority of prevalent diseases, as demonstrated by Nowotny *et al.*, [9]. Therefore, by identifying risk factors for autism through their presence as genetic markers, single nucleotide polymorphism research can identify this disease's contributors. Some studies have reported that vitamin D receptor (VDR) signaling has been shown to affect neurodevelopment, which may contribute to the risk of autism spectrum disorder (ASD). Previous research linking autism with vitamin D deficiency during fetal development is not entirely consistent, but it does suggest a potential effect of sunlight exposure at the time of birth [10-12]. In this work, researchers employed single nucleotide polymorphism (SNP) analysis to determine the differences in the distribution of VDR gene (rs731236 and rs7975232) genotypes between children with ASD and non-ASD control children (Table 1, 2, 3, 4, and 5).

In this study the AG category of rs731236 demonstrated a significant effect . This finding

suggests that the presence of the AG category of rs731236 increases the odds of observing the patient category within the Group by approximately 368.24%. in comparison to the AA category of rs731236 and GG category of rs731236 did not exhibit a significant effect. Furthermore, the AA category of rs7975232 (p = 0.489) and CC category of rs7975232 (p = 0.294), revealed no significant effect which indicating no considerable effect on the likelihood of observing the patient category within the Group.

Even if these data need to be confirmed in larger groups ,this finding indicate that the AG allele combination is over 2.5 times more frequent in children with ASD than children without ASD and that mean a strong association between (AG) rs731236 and ASD .So far, this study was similar with data presented by Coskun *et al.*, [13] and Zhang *et al.*, [14] showed correlation between Taq-I (rs 731236) genotype and ASD. The VDR gene polymorphism was also linked to epileptic patients , and skin diseases [12,15, 16].

These finding indicate that there was also no statistical correlation between alleles at the polymorphic site of rs7975232 and susceptibility to ASD which agree with Coşkun S. *et al.*, study [13]. In contrast to other study showed correlation between Apa-I (rs 7975232) of VDR gene and decreased ASD incidence [8].

Author Contributions

The research was interpreted and reviewed with R.S.B. and A.A.A., who also checked the results and confirmed the veracity of the research's finding. Implementing the research, writing this manuscript, and analyzing the findings were all done by R.A.K. The authors discussed the results and contributed to the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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