VITAMIN D RECEPTOR GENE RS 731276 (TAQ1) POLYMORPHISM AND AUTISM SUSCEPTIBILITY IN CHILDREN: A SINGLE CENTER STUDY

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ABSTRACT

BACKGROUND: The exact molecular and biochemical cause of autism is unknown. Some experts believe that various genetic disorders are linked to autism. This study aimed to evaluate the association between gene polymorphism of vitamin D receptor (VDR) Taq1 (rs 731276) and autism susceptibility and severity in children.

METHODS: We enrolled 40 autistic children, and 40 age-gender matched healthy controls. Complete clinical examinations were performed, with a focus on neurological examination, and clinical data from each autistic patient's medical records were reviewed. Polymerase chain reaction-restriction fragment length polymorphism was used to detect Taq1 (rs731276) gene polymorphism in all participants.

RESULTS: Males represented 70% of the autistic group and 75% of the control group. The mean age was 5.5±0.5 and 5.6±0.5 years in the autistic, the control groups, respectively. There were significant differences between the autistic and control groups regarding gestational age (p  <0.001), family history of psychiatric disorders (p <0.001), and paternal age (p= 0.004). The dominant genotype in the autistic group was the AG (heterozygous) genotype (70%), however, in the control group, the dominant genotype was the AA (wild) genotype (87.5%). There was a significant association between VDR Taq1 (rs731276) genotypes and autism susceptibility (p<0.001). Furthermore, there was no significant association between Taq1 (rs731276) gene polymorphism and the severity of autism (p= 0.138).

CONCLUSIONS: Our results reveal a significant association between vitamin D receptor gene polymorphism Taq1 (rs 731276) and autism susceptibility in children, but there is no significant association between this gene polymorphism and the severity of autism.

Keywords: Autism; vitamin D receptor gene; rs 731276; Taq1; Autism severity.

I. INTRODUCTION

The pattern of autism exhibited in children include an impairment existing in social and communication skills, abnormal language development, and stereotypic behavior (1).

Since autism is heterogeneous, it is difficult to accurately detect the underlying genetics, so various approaches are necessary to examine the loci that are responsible for autism. The risk factors for neurodevelopmental disorders result from health behaviors are related to socioeconomic factors, parental mental health disorders, and genetic influences (2).

Increased vulnerability to oxidative stress could impair vitamin D metabolism and Vitamin D deficiency and the vitamin D receptor gene mutations have been related to autism (3, 4).

Vitamin D conducts signaling via attaching to its receptor (VDR). The gene of VDR is located on chromosome 12q13 and consisting of 8 introns and 9 exons (5).
The role of VDR gene mutations in the development of autism has not been well investigated, and the findings are still ambiguous (6, 7). This study aimed to assess the association between vitamin D receptor gene rs 731276 (Taq1) polymorphism and autism susceptibility and severity in children.

II. SUBJECTS AND METHODS

Study design
This case-control study was conducted in the Children Hospital of Zagazig University, in Egypt. The study included 40 children who fulfilled the inclusion criteria of the study as the autistic group, and 40 age-gender matched healthy participants as the control group. The total duration of the study was one year, from February 14, 2019, to February 13, 2020.

Inclusion and Exclusion Criteria
We included children diagnosed with autism using the Childhood Autism Rating Scale (CARS) (8) and the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) (9). Children with autistic characteristics caused by definitive causes such as chromosomal abnormalities, neurological syndromes, and brain injuries were excluded.

Data Collection
All participants were subjected to detailed history taking including three-generation pedigrees construction, with detailed peri-natal history. Similarly affected cases and other family findings. Pre, peri, and postnatal history were also included in the study. Complete clinical examinations were performed, with a focus on neurological examination, and clinical data from each autistic patient's medical records were reviewed. Polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) was used to detect Taq1 (rs731276) gene polymorphism in all participants.

Genetic analysis
To recognize the distribution of allele and genotype frequencies in the VDR gene, we used restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). The Genomic blood DNA extraction Mini Kit acquired from INTRONBIO (Catalog No: 17351) was used to extract genomic DNA from whole blood. All the tubes, tips pipettes used for DNA extraction were DNAse, RNase free tubes to avoid contamination purchased from Gentra (Minneapolis. the USA). DNA was eluted and stored at –20°C for the PCR procedure.

PCR amplification was conducted using the thermal cycler. An initial denaturation at 94°C for 3 min and proper denaturation at 94°C for 30 s were performed, then we attached the starters at 61°C for all genes for 30 s, then the synthesis at 72°C for the 30s and the final synthesis at 72°C for 5 min was performed, with the number of cycles were 40 and cooling at 4°C. The following primers: F: 5-GGATCCTAAATGCACGGAGA-3 and R: 5-AGGAAAGGGGTTAGGTTGGA-3 were used. The mixture consisted of Dream Taq™ Green Master Mix, specific primers, the DNA matrix, and ultrapure water. This Mix is a fitting mixture of the component required for PCR for SNP genotyping. The digestion was performed using restriction endonuclease Taq I. The digestion was performed in a total volume of 25µl. The amplification products were separated by electrophoresis through 3% agarose gel stained with ethidium bromide with a positive band at 345 bp. We got 15 µl of the PCR products for the VDR Taq I and mixed it with 1 µl (1 unit) of Fast Digest Taq1 restriction enzyme (provided by Fermentas) with 6.5 µl nuclease-free water and 2.5 µl of 10× Fast Digest Buffer. The mixture was well mixed and incubated at 65°C for 30 min then 10 µl of the products were loaded into 3% agarose gel containing ethidium bromide for electrophoresis.

Ethical Approval
The study was approved by the Institutional Review Board. Written informed consent was taken from the parents of enrolled patients before being included in the study.

Statistical Analysis
All data were collected, tabulated, and statistically analyzed using SPSS Statistics for Windows, Version 20.0. Categorical variables were expressed as frequencies and percentages, and continuous quantitative variables were
expressed as the mean ± SD. Categorized data were compared using the Chi-square (χ²) test. Independent Student t-test was used to compare two groups of normally distributed data. P ≤ 0.05 was considered statistically significant.

III. RESULTS

Males represent 70% of the autistic group and 75% of the control group. The mean age was 5.5±0.5 and 5.6±0.5 years in the autistic, the control groups, respectively. There were significant differences between the autistic and control groups regarding gestational age (p <0.001), family history of psychiatric disorders (p <0.001), and paternal age (p= 0.004), but there were no significant differences between the two groups regarding age or gender of the patients, maternal age, and maternal diseases (Table 1).

The dominant genotype in the autistic group was the AG (heterozygous) genotype (70%), however, in the control group, the dominant genotype was the AA (wild) genotype (87.5%). Regarding Taq1 (rs731276) gene polymorphism, there was a statistically significant difference between the autistic and control groups denoting a significant association between VDR Taq1 (rs731276) genotypes and autism susceptibility (p<0.001). (Table 2).

According to the autism severity, 15 patients had mild autism, 15 patients had moderate autism and 10 patients had severe autism. In addition, there was no significant association between the severity of autism and Taq1 (rs731276) gene polymorphism (p= 0.138) (Table 3).

IV. DISCUSSION

Vitamin D is a steroid hormone that is essential for calcium hemostasis and musculoskeletal health. It is also involved in neuronal differentiation, immunological function, antioxidation, and neurotrophic and neuroprotective functions. All of these are necessary for embryogenesis and neuropsychiatric health (10).

Disorders associated with vitamin D level abnormalities are related to the development of ASD, several studies indicated that there is an association between vitamin D insufficiency and incidence of autism (11).

In this case-control study, we determined the relationship between Taq1 (rs731276) gene polymorphism in VDR and childhood autism and its severity. There was a significant difference between the autistic and control groups regarding the family history of psychiatric disorders. Similar findings were reported by many studies (12, 13, 14).

According to El-Baz et al. (15), a family history of autism was found in 16 percent of autistic individuals compared to 1% in the control group. This result was explained by a common genetic tendency as well as the same environment and stressors. In comparison to the general population, if a family already has an autistic child, the chances of having another child with autism rise 25 times, and identical twins have a 60–90% probability of having autism, whereas non-identical twins have a 0–24 % lower risk (16).

Regarding the paternal age, in this study, there was a significant difference between autistic and control groups with no difference as regards maternal age and diseases. Advanced parental age (both paternal and maternal) has been linked to an increased likelihood of having a child with ASD, according to Sandin et al. (17).

Balta et al. (18) and Schmidt et al. (6) found a relation between paternal homozygous VDR Taq 1 mutation and ASD. VDR and vitamin D metabolizing enzymes are found in the male reproductive tract and the mature neck of spermatozoa during the late stages of spermatogenesis. Aneuploid sperm and sperm with chromosomal abnormalities are caused by a reduction in sperm motility, which can harm growing embryos (19, 20).

De novo genetic mutation had a significant role in the development of ASD and these de novo point mutations in protein-coding regions are mostly of paternal origin, and VDR has a more significant role in sperm production and motility (21, 22, 23).

The present study revealed that there was a significant difference between the autistic and control groups regarding gestational age (p <0.001), and this finding was consistent El-Baz et al (15) study that showed a significantly higher incidence of prematurity among autistic children than controls. LUU et al (24) stated that preterm children are at risk for poor neurodevelopment outcomes due to an immature nervous system.

Vitamin D regulates extracellular calcium, inflammation-mediated cytokines, and glucocorticoids, all of which have an impact on the developing brain. Vitamin D expresses its significant role in neurogenesis,
neurodifferentiation, and neuroprotection since the beginning of life by regulating these critical factors in the fetal brain (25).

Vitamin D insufficiency during pregnancy and after birth has been linked to later-life neuropsychiatric illnesses such as schizophrenia, autism, and multiple sclerosis (26, 27, 28).

As regarding Taq1 gene polymorphism, there was a statistically significant difference between the autistic and control groups (p<0.001) and the dominant genotype in the autistic group was the AG genotype (70%), however, in the control group, the dominant genotype was the AA genotype (87.5%). According to the autism severity, there was no significant association between the severity of autism and Taq1 (rs731276) gene polymorphism (p= 0.138). This finding is consistent with that of Coşkun et al. (7), who found that the frequency of Taq 1 genotypes differed significantly between children with ASD and controls (p=0.016) and that genotypes of these polymorphisms were significantly higher in patients compared to controls (p=0.005), implying that VDR polymorphisms play a role in ASD risk.

Schmidt et al. (6) examined common functional polymorphisms of vitamin D-relevant genes in maternal, paternal, and child samples to identify risk for ASD in children, and found that paternal and child variants in VDR, specifically Taq 1, may be associated with a risk for ASD, which is consistent with our findings.

Anna et al. (29) found similar results as regard VDR gene mutation in autistic patients with different results regarding the severity and their study revealed that there was a link between having the T allele at position Taq-I and having the A allele at position Apa-I of the VDR gene and having a lower risk of ASD, but there was no statistically significant link between VDR single nucleotide polymorphisms and vitamin D3 concentration in ASD children's serum. The genetic variability in two single nucleotide polymorphisms in VDR may be associated with the development of ASD symptoms via affecting the functioning of vitamin D3 metabolism, even though vitamin D3 levels were not considerably different between ASD and non-ASD children.

Uitterlinden et al. (30) and Yan et al. (31) earlier examined occurrences of VDR polymorphisms in bipolar disorder and schizophrenia and ASD. Although they did not find any suggestion of an association, their study included only 24 children with ASD, making it unsatisfactory for this type of detection.

Chugani (32) and Eyles et al. (33) stated that vitamin D receptor has been shown to be expressed more prominently in dopaminergic areas of the brain, which may be consistent with evidence that altered dopamine neurotransmission existed in autistic patients.

Patrick et al. (34) proposed that vitamin D modulates tryptophan metabolism and facilitates serotonin synthesis, both of which are disrupted in children with ASD, which could explain our findings regarding the VDR gene mutation and autism.

In a review of the literature, Cannell JJ, Grant WB. (35) hypothesized that vitamin D has numerous roles in ASD, including repairing DNA damage, decreasing inflammation, avoiding autoimmunity, and maintaining mitochondrial function. Cui X, et al. (36) suggested that low vitamin D levels in autistic children are negatively correlated to scores on the childhood autism rating scale (CARS).

Patients who had mutations in their VDR receptor gene had a unique stimulation threshold than those who had the receptor's wild type (37). Because the VDR is involved in all of 1,25(OH)2D3's molecular activities, polymorphisms in the VDR gene have been reported to control bone mineral density and induce inactivation of vitamin D receptor effects on skeletal and calcium homeostasis (38). Because SNPs can affect gene expression, genetic variation in the VDR receptor gene could be linked to altered VDR expression and function in the presence of functional 1,25(OH)2D3 rather than vitamin D3 or its precursors in the blood (39). Wong et al. suggested that ASD might be due to interaction of genetic with prenatal/perinatal factors (40).

Many conflicting studies were published on the effect of vitamin D deficiency on the development of diseases, including autism, and therefore, the attention should be focused on genes that control vitamin D metabolism and efficient functioning in the body. The outcome is a combination of an environmental factor (vitamin D) and genetic background (mutations of VDR gene) and is apparent in many tissues as the VDR is widely expressed (29).
The study had many strengths, including being a case-control study with a comparative assessment of this gene polymorphism in healthy control and autistic patients. The consistency of our findings, with other studies, about the genetic variations of the VDR gene and the detection of genetic polymorphisms in the VDR gene driving disease susceptibility, can be a useful instrument in preventive medicine. The primary limitation of this study was a relatively small sample size. Additional work is needed on a larger, multicenter prospective study that examines the association between this gene polymorphism and the risk of autism.

V. CONCLUSIONS

Our results reveal a significant association between the rs 731276 (Taq1) polymorphism in the vitamin D receptor gene and autism susceptibility in children, but there is no significant association between this gene polymorphism and the severity of autism.

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Authors’ contributions: THH and AAA designed the study, collected, and interpreted data, and wrote the manuscript. MAA and NR collected, evaluated the patient’s interpreted data, and wrote the manuscript. TAA, MA, and MMG collected, evaluated the patient’s interpreted data and wrote the manuscript. All the authors critically contributed to the discussion and data interpretation, reviewed, and approved the final manuscript.

REFERENCES


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Table 1: Sociodemographic and clinical characteristics of the study groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Autistic group</th>
<th>Test value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N= 40</td>
<td>N=40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30 (75%)</td>
<td>28 (70%)</td>
<td>0.251 X²</td>
<td>0.617 (NS)</td>
</tr>
<tr>
<td>Female</td>
<td>10 (25%)</td>
<td>12 (30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>5.6± 0.5</td>
<td>5.5 ± 0.5</td>
<td>1.574 t</td>
<td>0.119 (NS)</td>
</tr>
<tr>
<td>Mean± SD</td>
<td>5.6± 0.5</td>
<td>5.5 ± 0.5</td>
<td>1.574 t</td>
<td>0.119 (NS)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>37.4 ± 1.2</td>
<td>35.1 ± 1.8</td>
<td>6.852 t</td>
<td>&lt;0.001 (HS**)</td>
</tr>
<tr>
<td>Paternal age</td>
<td>32 ± 3.4</td>
<td>34.7 ± 4.5</td>
<td>3.007 X²</td>
<td>0.004 (S*)</td>
</tr>
<tr>
<td>Maternal age</td>
<td>29.5 ± 0.5</td>
<td>29.3 ± 0.4</td>
<td>-0.294 t</td>
<td>0.770 (NS)</td>
</tr>
<tr>
<td>Maternal medical illness</td>
<td>12 (30%)</td>
<td>18 (45%)</td>
<td>1.920 X²</td>
<td>0.166 (NS)</td>
</tr>
<tr>
<td>Family history of psychiatric disorders</td>
<td>4 (10%)</td>
<td>17 (42.5%)</td>
<td>10.912 X²</td>
<td>0.001 (S*)</td>
</tr>
</tbody>
</table>

* P ≤ 0.05 is statistically significant, ** p<0.001 is statistically highly significant.

1. Student’s t-test, χ²; 2. Chi-square test, HS: Highly Significant, NS: Non-Significant, S: Significant
Table (2): Taq 1 genotype in studied groups

<table>
<thead>
<tr>
<th>Taq 1 Gene</th>
<th>Case group n=40</th>
<th>Control group N=40</th>
<th>Test value</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA***</td>
<td>12 (30%)</td>
<td>35 (87.5%)</td>
<td>27.29 x²</td>
<td>&lt;0.001</td>
<td>1</td>
</tr>
<tr>
<td>AG</td>
<td>28 (70%)</td>
<td>5 (12.5%)</td>
<td></td>
<td>3.32</td>
<td>3.32 (2 – 5.53)</td>
</tr>
<tr>
<td>GG</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

χ²; Chi-square test, 
HS: Highly Significant 
** p<0.001 is statistically highly significant. 
***AA is the wild genotype, AG is the heterozygous genotype, and GG is the mutant genotype.

Table (3): Subgroup analysis of autistic group according to the severity

<table>
<thead>
<tr>
<th>Taq 1 Gene</th>
<th>Mild N=15</th>
<th>Moderate N=15</th>
<th>Severe N=10</th>
<th>Test value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA***</td>
<td>7 (46.7%)</td>
<td>2 (13.3%)</td>
<td>3 (30%)</td>
<td>3.968 x²</td>
<td>0.138 (NS)</td>
</tr>
<tr>
<td>AG</td>
<td>8 (53.3%)</td>
<td>13 (86.7%)</td>
<td>7 (70%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

χ²; Chi-square test, 
NS: Non-Significant, 
***AA is the wild genotype, AG is the heterozygous genotype, and GG is the mutant genotype.