MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

The association of ZIC5 gene rs965623242 polymorphism with neural tube defects

Ebru ONALAN[1], Yasemin ASKIN[1], Tugce KAYMAZ[1], Mehmet SARACOGLU[2], Ahmet KAZEZ[2], Tugba ONAL SUZEK[3], Vahit KONAR[4]

[1] Department of Medical Biology, Faculty of Medicine, Firat University, Elazig, Turkey
[2] Department of Pediatric Surgery, School of Medicine, Firat University, Elazig, Turkey
[3] Department of Bioinformatics, Graduate School of Applied and Natural Sciences, Mugla Sitki Kocman University, Mugla, Turkey, Faculty of Science
[4] Department of Biology, Faculty of Science, Amasya University, Amasya, Turkey

Corresponding Author: Tugce KAYMAZ
E-mail: tugcekaymaz.92@gmail.com

Submitted: 27.09.2021 **Accepted:** 26.02.2024

ABSTRACT

Objective: This study aims to evaluate the effects of the rs965623242 reference single nucleotide polymorphism (SNP) on the ZIC5 gene in patients with neural tube defect (NTD).

Patients and Methods: One hundred sixty-eight controls and one hundred sixty-eight NTD patients were included in the study. Deoxyribonucleic acid (DNA) isolation from peripheral blood samples was carried out for all participants. rs965623242 polymorphic region was amplified by polymerase chain reaction (PCR) and then sequenced.

Results: In the 5' untranslated region (UTR) of the first exon, guanine (G) to adenine (A) base change was detected in the 38th base of NM_033132.5. G to A base change was determined as *GG* genotype in 117 (69.6%), *AG* genotype in 30 (17.86%), and *AA* genotype in 21 (12.5%) patients. In the control group, *GG* genotype in 107 (63.7%), *AG* genotype in 23 (13.7%) and *AA* genotype in 38 (22.7%) were observed. The statistically significant difference was observed between the NTD and the control groups in ZIC5 genotypes or allele frequencies [p=0.044, odds ratio (OR)=0.49 (0.27-0.88) and p=0.021, OR=0.65 (0.46-0.93), respectively].

Conclusion: ZIC5 rs965623242 polymorphism may have a protective role in the NTD development in the Eastern Anatolian population, in Turkey. Although, these findings demonstrate that the rs965623242 polymorphism is associated with NTD, we do not clarify how its expression is affected during the embryonic period and ongoing processes. We will need advanced ongoing genetic and clinical studies to obtain more detail.

Keywords: Congenital anomalies, Neural tube defect, Spina bifida, ZIC5 gene, Polymorphism

1. INTRODUCTION

Spina bifida, also known as neural tube defects (NTDs), affects around 0.57–13.87% of the population and is the second most frequent birth condition globally [1]. In Turkey, the disease is seen in 1.5% to 6.3% of cases [2]. During the first three or four weeks of pregnancy, the neural tube begins to collapse. Delays in closure lead to NTD. In many parts of the world, there is a significant incidence of non-transferable diseases (NTDs), which varies according to the geographical region, season of pregnancy, gender of the diseased fetus, ethnicity, socioeconomic status of the family, and mother age [3]. The word "spina bifida" refers to a broad category of developmental defects. Spina bifida, meningocele, myelomeningocele, encephalocele, anencephaly, dermal sinus, stretched medulla spinalis, syringomyelia, diastemomyelia, and lipoma are the most prevalent of these abnormalities [4]. According to reports, genetic and environmental variables can have a role in the occurrence of NTD. The neural tube may not close properly as a result of maternal malnutrition during pregnancy, health issues such as high blood pressure, diabetes, obesity, and fever, as well as some medications and environmental contaminants [5].

Parallel to the new techniques used in molecular genetics, several possible gene polymorphisms and mutations that cause NTDs were identified. It is known that genetic research is being done to determine the cause of spina bifida. It has been proposed recently that the ZIC gene family may be involved in neural tube closure and abnormalities. NTD is commonly observed in humans with 13q deletion syndrome, which lends support to this observation. The 13q3.3 minimum deletion region is

How to cite this article: Etem Onalan E, Askin Y, Kaymaz T, et al. The association of RS965623242 polymorphism of ZIC5 gene with neural tube defects. Marmara Med J 2024: 37(2):248-255. doi: 10.5472/marumj.1493354

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1493354
Marmara Med J 2024;37(2): 248-255

where the ZIC2 and ZIC5 genes are located [6]. The human genome has five ZIC genes: ZIC1, ZIC2, ZIC3, ZIC4, and ZIC5. ZIC genes participate in neural crest induction, suppression of neurogenesis, and mediolateral segmentation during mouse brain development [7, 8].

Mice lacking ZIC2 showed signs of a broad range of NTDs, including spina bifida aperta, spina bifida oculus, anencephaly, and exencephaly [9–11]. In the Xenopus genus, neural crest development is known to be induced by the ZIC5 gene [12]. It has been demonstrated that ZIC5 deficiency in mice results in a variety of abnormalities, including NTDs and craniofacial abnormalities including hypoplasia. Despite evidence that the ZIC5 gene has a role in neural tube closure and neural crest development in experimental animals, ZIC5 gene mutation screening has not been carried out for human patients with neural tube defects [13]. The cerebellar chain-finger transcription factors encode the ZIC2 and ZIC5 genes. Every zinc finger motif has two histidines in the alpha (α) helix area, two tetrahedral structures in the beta (β)-sheet region, and a zinc atom in the center. The ZIC5 gene has three exons. In all five genes, the first intron-exon limit was maintained. In addition, the boundary of the second intron-exon is conserved in ZIC1, ZIC2 and ZIC3 but not in ZIC4 and ZIC5 [14-16].

Research indicates that the ZIC5 gene plays a role in both the etiopathogenesis and neurulation processes of NTD [12, 13]. There is no information in the literature about the relationship between the likelihood of acquiring neural tube abnormalities and mutations or polymorphisms in the ZIC5 gene. As a result, we looked into the ZIC5 gene's rs965623242 promoter polymorphism in patients with NTD and contrasted the findings with those of controls who resided in Turkey's Eastern Anatolian region.

2. PATIENTS and METHODS

After the approval of the Non-Interventional Research Ethics Committee of Firat University (Decision number: 158/9, dated 02.06.2011) was obtained, the study was carried out by obtaining verbal and written consent from the families. 168 patients who underwent surgery with the diagnosis of NTD at Fırat University Hospital Pediatric Surgery Clinic and 168 control individuals participated in the study between June 2011 and September 2016. Care was taken to collect the samples used in the study from similar geographical regions. For the genotype analyses, blood samples taken for the routine preoperative biochemistry tests from all participants, who were admitted to the outpatient clinic due to circumcision and trauma, were used. In the selection of control subjects, the criteria of absence of any congenital anomaly and genetic disease in the family, and similar age, gender, and region of residence were taken into consideration. Blood samples were collected into biochemistry tubes coated with ethylenediaminetetraacetic acid (EDTA) and for the genotyping analysis, they were conserved at – 20°C. An anamnesis form including each patient's demographic characteristics and clinical history was filled. The type of defect in the patients was recorded. The participants were asked about maternal age, whether there were similar cases in the family, and whether there was any consanguineous marriage. To reveal possible teratogenic causes (besides birth anamnesis); drug usage in the mothers' periconceptional period, whether or not the use of vitamin B12 and folic acid, and whether they had a feverish illness during the 3rd-4th weeks of pregnancy were asked. After a detailed examination of the patients, whether there was any other nervous system anomaly or other system anomalies accompanying spina bifidia was recorded.

DNA extraction

Genomic DNA extraction from blood samples was performed using Wizard® Genomic DNA Purification Kit (CAT # A1120, Promega, USA), designed for the DNA isolation from 300 µL of blood. After the samples were thawed and kept at room temperature, the isolation process followed the manufacturer's protocol and some modifications were made to the isolation protocol. 900 µL of cell lysis solution was added into the 1.5 mL tubes and then 300 µL blood was added into each tube after pipetting it 5-6 times. The tubes were kept at room temperature for 10 minutes and centrifuged at 15,000 x g speed for 20 seconds at 4°C temperature. After centrifugation, the supernatants were discarded, leaving approximately 10-20 µL of liquid at the bottom of the tube. The tubes were vortexed at high speed for 15-20 seconds, 350 µL of nuclei lysis solution was added into each tube and the vortexing process was performed again. After the tubes were centrifuged at 13.00x g speed for 1 min at 25°C temperature, the supernatants were discarded and the remaining liquid at the bottom of the tubes was pipetted 5-6 times with a Pasteur pipette to break down the white cells. To precipitate proteins in the blood, about 120 µL of precipitation solution was added to the nuclear lysate at room temperature. After vortexing the tubes vigorously for 10-20 seconds, small, different brown shades of protein clusters were seen. The samples were centrifuged at 15,000 x g for 3 minutes at 25°C temperature. 300 µL of isopropanol and the supernatants were added respectively into the fresh 1.5 mL centrifuge tubes. After the tubes were shaken thoroughly, DNAs were seen as white residue at the bottom of the tubes. The samples were centrifuged at 15,000 x g for 1 minute at 25°C temperature. 900 µL of 70% ethanol was added into each tube and the tubes were shaken gently. The centrifugation process was repeated at 15,000 x g for 1 minute at 25°C temperature. After the DNA washing process was completed, the tubes were placed on the clean paper as the tubes' mouths were faced upwards, so ethanol was exactly removed. Tubes were allowed to air dry for 5-10 minutes and 100 µL of DNA rehydration solution was added to each tube. The tubes were incubated at 37°C for 60 minutes to rehydrate the DNA and then stored at 2-8°C temperature. DNA concentration and purity were analyzed by a nanodrop device (Maestrogen, MaestroNanodrop, USA), and then DNA concentration was diluted to 1-10 ng.

http://doi.org/10.5472/marumj.1493354
Marmara Med J 2024;37(2): 248-255

Conventional polymerase chain reaction (PCR), DNA sequencing and genotyping

To evaluate the polymorphisms for the *ZIC5* gene, complete sequences of genes were obtained by using the Ensembl genome database [17]. Primers used to perform amplification of the *ZIC5* gene promoter region on human DNA, were designed with the help of a free primer designation tool [18] and purchased from the Sentebiolab (Ankara, Turkey). The nucleotide sequence for the forward (F) primer is 5′-CAGGCCAGGCTCAAACTTCTGCA-3′ and for the reverse (R) primer is 5′-GGGGCTCCATCAGAACTACACAATCA-3′. PCR conditions were set according to the protocol described by Zhang et al [19]. After the optimization process, the PCR protocol was adjusted to our laboratory conditions. The PCR reaction mix was prepared by using 8 µL of template DNA, 5µL of 10X PCR buffer, 5 µL of 25 mM $MgCl_2$, 0.5 µL of Taq DNA polymerase, 5 µL of 2.5 mM deoxyribonucleotide triphosphates (dNTPs), 0.5 µL of Taq DNA polymerase and 2µL each of 20 pmol of F and R primers. The total volume was completed with 50 µl of nuclease-free water. PCR denaturation step was carried out for 5 minutes at 95°C and 30 seconds at 95°C, the annealing step for 30 seconds at 58.4°C, the elongation step for 40 minutes at 72°C, and the extension step for 7 minutes at 72°C. The total PCR reaction consisted of 36 cycles and all steps were performed sequentially. To determine the specificity and quality of the DNA amplification process, PCR products were run on the 3% agarose gel. For the electrophoresis process, 5X Tris/Borate/EDTA (TBE) buffer was used. 6X loading buffer and PCR products were mixed and then loaded into the wells of the gel. 7 µL of 100 base pair (bp) DNA marker (Fermentas) was loaded into the first and last wells of the gel to determine the size of the bands. The gel was run at 60 V at room temperature for 1 hour and finally, it was visualized under ultraviolet (UV) light. A 431 bp product was obtained as a result of amplification of the *ZIC5* gene region containing the rs965623242 polymorphism (Figure 1).

A 401 bp product

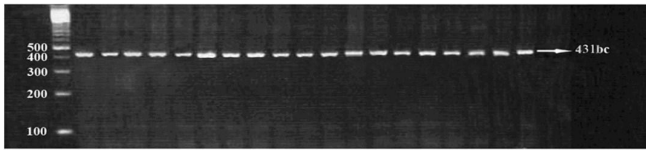


Figure 1. *Control of the PCR products by agarose gel electrophoresis. 100 bp of DNA marker and 431 bp PCR products belonging to exon 1 of the ZIC5 gene were obtained by 2% agarose gel electrophoresis with ethidium bromide (EtBr) staining. Forward (5'-CAGGCCAGGCTCAAACTTCTGCA-3') and reverse (5'-GGGGCTCCATCAGAACTACACAATCA-3') primers were used to amplify the promoter of ZIC5 gene..*

Polymerase chain reaction products were sent to the RefGen Gene Research and Biotechnology laboratory (Ankara, Turkey) for the sequencing process. The sequencing results were compared with a DNA baser program to determine whether polymorphism existed. For bioinformatics analysis of the ZIC5 gene variants, DIANA tools [20], miRDB [21], 1000 Genomes [22], The Exome Aggregation Consortium (ExAC) [23], the Single Nucleotide Polymorphism Database (dbSNP) of the [24] and Variation Viewer [25] were utilized.

Statistical Analysis

All descriptive and inferential statistical analyses were performed with IBM SPSS version 22.0 software (Chicago, IL, USA). Data are presented in counts, percentages, mean, and standard deviation (SD) values. The Kolmogorov-Smirnov test was conducted to determine whether the data were distributed normally. The deviation from Hardy-Weinberg equilibrium (HWE) was verified by using the Chi-square (χ^2) test which was also used to make a comparison between groups, for the analysis of the association between two qualitative variables and allele and genotype frequencies. The magnitude of the association between ZIC5 rs965623242 polymorphism and NTD was expressed with the help of odds ratio and 95% confidence interval (CI). Demographic, clinical, and laboratory data were compared using the χ^2 test, Student's t-test, and one-way ANOVA test. To decide the statistical significance or insignificance of the results, the p-value was accepted as < 0.05.

3. RESULTS

Demographic, clinical, and laboratory results of all subjects are indicated in Table I. 69.7% of the patients are from Elazig province and 31.3% from its surrounding provinces in the Eastern Anatolia Region of Turkey. Sex ratio of the children was 79 (47%) for females and 89 (53%) for males in the control group and 98 (58.3%) for females and 70 (41.7%) for males in the NTD group. The mean age of the children was 2.38±2.83 years [3.28 ± 4.21 for females (1 month-17 years old) and was 3.24 ± 4.15 years for males (1 month-14 years old)] for the control group and 2.22±3.13 years (2.24 ± 3.37 years for females and 2.38 ± 3.14 years for males) for the NTD group. There was no statistically significant difference in terms of the mean age of children and mothers between the control and NTD groups (p>0.05).

Table I. *Demographic, clinical, and laboratory findings for each group*

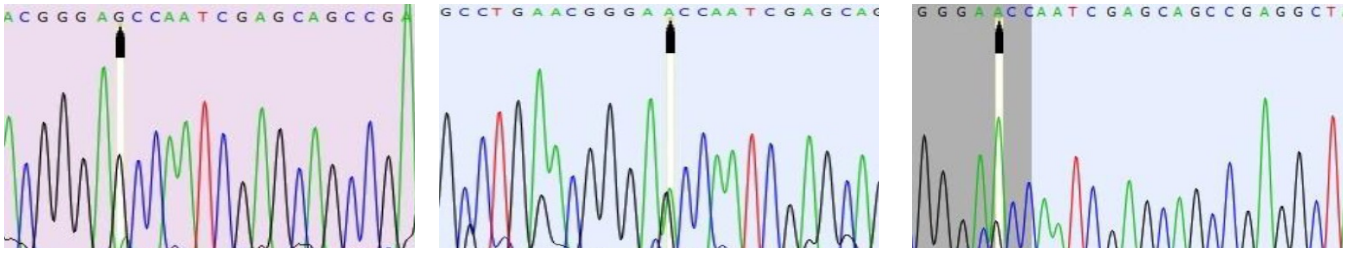
Parameters	NTD (n=168)	Control (n=168)	p-value
Age (years)	2.22 ± 3.13	2.38 ± 2.83	NS
Female/male	98/70	79/89	NS
Meningosel/ Meningomyelosel	10/158	——	——
History of the other baby with NTD in the family	21 (12.5%)	0 (0%)	0.0000
Consanguinity between parents	46 (27.4%)	16 (9.52%)	0.0001
Iron supplements used during pregnancy	25 (14.8%)	30 (17.9%)	NS
Mother's age during pregnancy (years)	27.35 ± 5.31	26.22±5.68	NS

NTD: Neural tube defect, NS: Non-significant. Results are expressed as mean ± standard deviation (SD). p < 0.05 was considered significant when compared with the control group.

http://doi.org/10.5472/marumj.1493354
Marmara Med J 2024;37(2): 248-255

Genotype and allele distributions

All samples were successfully sequenced and the sequencing results are indicated in Figure 2a. The specific DNA sequence obtained from the sequencing analysis is illustrated in the Figure 2b.



(A) GG genotype (B) GA genotype (C) AA genotype

Figure 2a. *Typical raw data for rs965623242 (G>A) polymorphism obtained by using Sanger sequencing instruments. Polymorphic nucleotide was represented with a black arrow. G nucleotide was the ancestral allele and A nucleotide was the polymorphic allele. (a) Single "G" peak shows a GG homozygous genotype. (b) The presence of both "G" and "A" peaks indicates a G/A heterozygous genotype. (c) Single "A" peak demonstrates a AA homozygous genotype. GenBank accession number of ZIC5 gene was NG_053065.1.*

3'-CGGCCGCAAGCACGGGGGCGAATCCCCGCTGGGTCGAGGGCCTGAACGGGAG/ACCAATCGAGCAGCCGAGGCTACTGCCA
ATCACGCGGCTCCCTCCAATCCCACCCGTGCCATTTCCAAAATCTCGGTCCCACTGTGCAGCTCAAATGTGGTGTTCACTCTGCCAATC
GCTGGAGGATAGAGTGGGAACAGGAATAAGCAGAGTTAAGAGGCCAGGACAAAAGAAGTTAAAGAGCGCCCAATACATAC-5'

Figure 2b. *5' untranslated region (UTR) sequence of ZIC5 gene. The genomic position of rs965623242 polymorphism was NC_000013.10:g.100624126C>T, which is located in the 5' UTR of the ZIC5 gene.*

The distribution of genotypes and allele frequencies of *ZIC5* gene variants are presented in Table II. All SNPs were analyzed in the HWE in NTD and control subjects (p>0.05). Between the patient and control groups, statistical significance was detected in genotype distributions and allele frequencies of the analyzed SNP (p=0.044, OR=0.49 (0.27-0.88) for patients; p=0.021, OR= 0.65 (0.46 – 0.93) for controls). Genotype distribution of rs965623242 *G>A* SNP was determined in 117 (69.6%) NTD patients for wild-type genotype, 30 (17.86%) for heterozygote genotype, and 21 (12.5%) for polymorphic genotype. Furthermore, for rs965623242 *G>A* SNP, 107 (63.7%) of control subjects were homozygous for wild type genotype, 23 (13.7%) were heterozygous, and only 38 (22.7%) were polymorphic homozygous. Allele frequencies in NTD patients were 0.79 for the wild *G* allele and 0.21 (12.5%) for the polymorphic A allele. The frequency of wild type allele was 0.71 and that of the polymorphic allele was 0.29, which represented 22.7% of the examined control subjects. The frequency of the AA genotype in NTD patients (12.5%) was significantly lower compared to control male subjects (22.7%).

Table II. *Genotypes and allele frequencies of ZIC5 gene variant i.e. rs965623242 in control and NTD groups.*

rs16835198	NTD (n=168)	Control (n=168)	p and OR (95% CI) values
Genotypes			
GG	117 (69.6%)	107 (63.7%)	**0.044**
GA	30 (17.86%)	23 (13.7%)	0.71 (0.39-1.26)
AA	21 (12.5%)	38 (22.7%)	
Alleles			
G	0.79	0.71	**0.02**
A	0.21	0.29	0.65 (0.45-0.92)

NTD: Neural tube defect, OR: Odds ratio, CI: Confidence interval, NS: Nonsignificant. χ2 and Fischer tests were performed. χ2 analysis of the genotypes and alleles for NTD subjects was performed versus control subjects. Results are expressed as percentages. p < 0.05 was considered significant when compared with the control group.

http://doi.org/10.5472/marumj.1493354
Marmara Med J 2024;37(2): 248-255

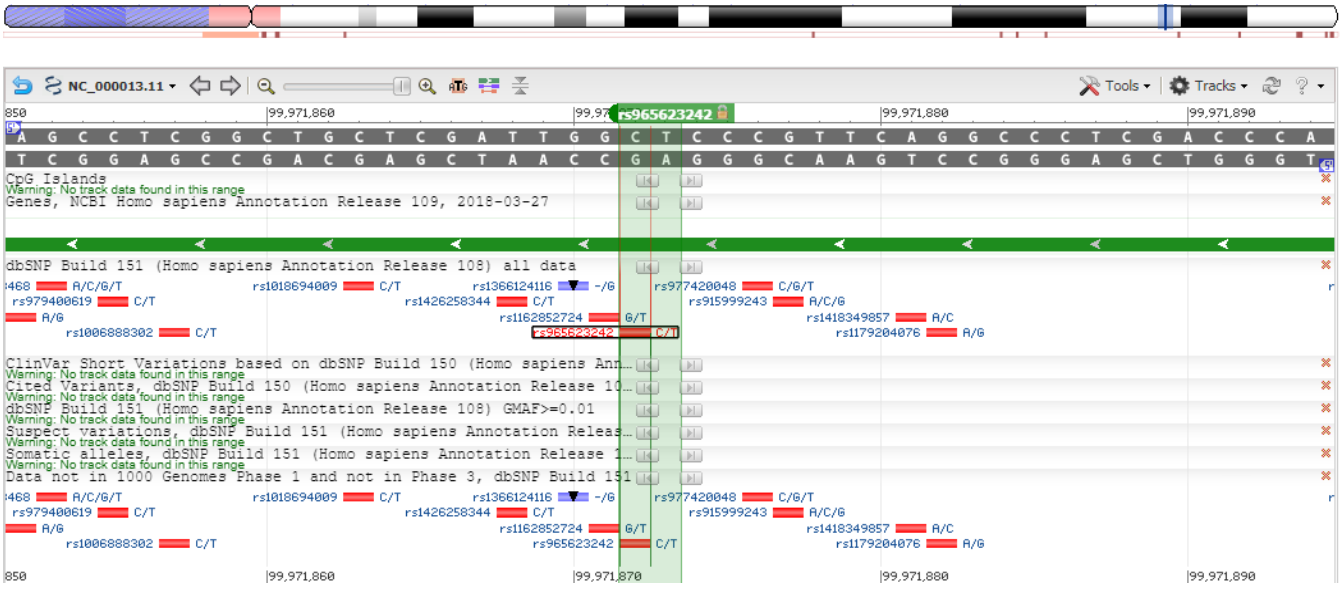


Figure 3. *Reference SNP (rs) reports [26].*

As of the submission date, no report of this variant's allele frequency has been found in the Turkish population or any other populations. Potential microribonucleic acid (miRNA) binding sites of the polymorphic region were investigated using DIANA tools and miRDB [20,21]. It was predicted that miRNA binding sites which were not determined, were found in the polymorphic region. Reference SNP reports are illustrated in Figure 3.

4. DISCUSSION

In the current study, we examined the rs965623242 polymorphism in the 5'-UTR region of the *ZIC5* gene in the formation of NTDs. The decreased frequency of the rs965623242 AA genotype may be a protective factor rather than a risk factor for NTD. This is the first study worldwide to examine this polymorphism in terms of such birth defects.

In our literature review, however, we could not find any study on the effects of this polymorphism on NTD and other neurological diseases. However, two studies are searching for the relationships between *ZIC2* gene polymorphisms and NTD. In the first of these, Brown et al. [27], screened for the polyhistidine tract polymorphism in the *ZIC2* gene in 192 NTD patients but they did not find any association between this polymorphism and NTDs. In other studies, Costa Lima et al. [28], similarly analyzed the same polymorphism in 138 NTD patients and their families, but they also did not find a significant relationship between the mentioned polymorphism and NTD. Recently, two important studies have been conducted that may be indicative of the pathogenic effects of the *ZIC5* gene, especially on fetal development. In the first study, a match between rare copy number variations (CNV) in the *ZIC5* gene and abnormal voluntary movement was identified in a mouse model of global developmental disorder [29]. In the second study, a Whole Exome Sequencing (WES) study was performed in couples with recurrent miscarriages, and missense, non-pathogenic changes were detected in the *ZIC2* and *ZIC5* genes in the patient group [30]. In the present study, the polymorphic variant appears to be protective for NTD. However, the mechanisms underlying the conservation of the rs965623242 polymorphism remain unknown. A small part of SNPs have significant effects on the phenotypes, while most of them have no or very limited effects [31]. SNPs can convert an amino acid to another one (nonsynonymous), be silent (synonymous) or be seen in a noncoding region. Although the promoter activity, messenger ribonucleic acid (mRNA) conformation and translational effects of the rs965623242 polymorphism are not yet known, our bioinformatics analysis showed that this region did not coincide with any miRNA or transcription factor binding sites [20, 21]. Although the transcriptional effects of the rs965623242 polymorphism in the *ZIC5* gene have not been revealed by luciferase reporter experiments, the phenotypic effects of the *ZIC5* AA genotype can be explained by the effects of the *ZIC5* gene on cell proliferation. The central nervous system (CNS) develops from a special region of the ectoderm layer called the neural plate, which is located in the dorsal midline of the embryo. Since the rate of cell division is faster along the edges of the neural plate than in the middle section, the neural plate thickens, expands and takes the shape of a groove during the development of the neural tube [32]. In this process, regulation of the cell division rate is critical. It has been demonstrated that high expression of the *ZIC5* gene accelerates the progression of lung, liver, melanoma and colorectal cancers, increasing cell proliferation through the activation of signaling pathways such as CCNB1/CDK1, Wnt/β catenin, FAK/STAT3 and

http://doi.org/10.5472/marumj.1493354
Marmara Med J 2024;37(2): 248-255

CDK1/CDC25c, while its suppression has antiproliferative and antineoplastic effects [33-38]. Recently, the *ZIC5* gene has been shown to play a role in induction along with Wnt downstream signals and Notch upstream signals [38]. Prevention of neuronal closure (NC) induction occurs through early embryonic exposure to ketamine, which inhibits notch-targeted gene expression, including the *ZIC5* gene [40]. Based on the effects of the *ZIC5* gene on cell proliferation, the AA genotype may cause low expression levels, and NC problems by producing a negative effect on the cell division along the edges of the neural plate.

In the present study, the *G* allele frequency was 0.71 and the *A* allele frequency was 0.29 in the control group. However, in the literature reviews, the frequency of the *T* allele was reported as 3/125568 in the TOPMED project [41] and 0/2188 in the Alfa Project [42]. In our study, unlike these two projects, the frequency of the *T* variant allele was found to be higher in the population of Elazig and its surrounding provinces. Different geographically separated populations may be exposed to different selective environmental conditions, but population-specific selection may result in an increase in population differentiation of the target locus. Nevertheless big differences in rare allele frequencies (RAF) caused by geographical region differences are more prone to be caused by *allelic surfing* which is defined as genetic drift during population expansion after a bottleneck, rather than natural selection. By looking at our findings in the genetic epidemiological scope, it is necessary to investigate the RAF differences that occur in NTD susceptibility variants and can be explained by natural selection or allele surfing [43-45].

The most interesting aspects of the current study are that the frequency of the rs965623242 polymorphism in our society is defined regionally, the frequency in our society is higher than in other societies and, the NTD and *ZIC5* gene relationship is revealed for the first time at the polymorphism level. The most important limitations of our study are that it was performed in a small cross-sectional patient group and only promoter polymorphisms were analyzed. Although it was scheduled to sequence the entire gene at the beginning of the current study, serious problems were experienced in duplicating the target region despite many primer combinations being tried. For this reason, we continued to work with the promoter region primers that worked best. In line with our experience, it is recommended that the gene region contains a high percentage of GC and that it be sequenced by amplifying it in very small regions.

Conclusion

In conclusion, since, multifactorial events including environmental and genetic factors are very important for the etiology of NTD, reporting the prevalence of this variant in different populations and revealing the disease relationships are very important. The effects of factors such as maternal age, health problems (e.g. obesity), consanguinity degree, drugs used by the mother during the pregnancy and maternal nutrition on the functioning of this allele are also unknown. To determine whether rs965623242 and the other polymorphisms in the *ZIC5* gene are susceptible to NTD disease, genetic studies including a higher number of patients in both the Turkish population and other populations are needed.

Compliance with Ethical Standards

Ethical approval: The study was approved by the Non-Interventional Research Ethics Committee of Firat University with approval number 58/9 and date 02.06.2011.

Conflict of interest: There are no conflicts of interest related to this article.

Financial disclosure: This study was supported by the Fırat University Scientific Research Projects Coordination Unit (FUBAP) under project number TF.11.58.

Author contributions: EO: Design, Project administration, statistical analysis Conceptualization, Writing-Reviewing and Editing, Investigation, YA: Methodology, Validation, Resources, Visualization, MS and AK: Patient selection and exclusion, Methodology, VK: Validation, Resources, Visualization, TK: Drafting and revision of the manuscript.

REFERENCES

[1] Nikkila A, Rydhström H, Kallen B. The incidence of spina bifida in Sweden 1973-2003: The effect of prenatal diagnosis. Eur J Public Health 2006; 16: 660-2. doi: 10.1093/eurpub/ckl053.

[2] Sanri A, Karayel M, Abur U, et al. Frequency and risk factors of neural tube defects in Samsun province. Croat Med J 2018; 40: 413-20. doi:https://doi.org/10.7197/223.vi.441516.

[3] Tinkle MB, Sterling BS. Neural tube defects: A primary prevention role for nurses. J Obstet Gynecol Neonatal Nurs 1997; 26: 503-23. doi: 10.1111/j.1552-6909.1997.tb02153.x

[4] Neyzi O, Ertugrul T. 3rd ed. Pediatri 2, Istanbul, Turkey: Nobel Tıp Kitabevleri, 2002:1338-41.

[5] Akan N. Nöral tüp defektli bebek doğurma riski azaltılabilir. Cumhuriyet Üniversitesi Hemşirelik Yüksekokulu Dergisi 2002; 6: 42-48.

[6] Brown S, Russo J, Chitayat D, Warburton D. The 13q-syndrome: the molecular definition of a critical deletion region in band 13q32. Am J Hum Genet 1995; 57: 859-66.

[7] Merzdorf CS. Emerging roles for zic genes in early development. Dev Dyn 2007; 36: 922-40. doi: 10.1002/dvdy.21098.

[8] Grinberg I, Millen KJ. The ZIC gene family in development and disease. Clin Genet 2005; 67: 290-6. doi: 10.1111/j.1399-0004.2005.00418

[9] Elms P, Siggers P, Napper D, Greenfield A, Arkell R. Zic2 is required for neural crest formation and hindbrain patterning during mouse development. Dev Biol 2003; 264: 391-406. doi: 10.1016/j.ydbio.2003.09.005.

[10] Klootwijk R, Groenen P, Schijvenaars M, et al. Genetic variants in ZIC1, ZIC2, and ZIC3 are not major risk factors for neural tube defects in humans. Am J Med Genet 2004; 124A: 40-7. doi: 10.1002/ajmg.a.20402.

[11] Nagai T, Aruga J, Minowa O, et al. Zic2 regulates the kinetics of neurulation. Proc Natl Acad Sci 2000; 97: 1618-23. doi: 10.1073/pnas.97.4.1618.

[12] Nakata K, Koyabu Y, Aruga J, Mikoshiba K. A novel member of the Xenopus Zic family, Zic5, mediates neural crest development. Mech Dev 2000; 99 : 83-91. doi: 10.1016/s0925-4773(00)00480-9.

[13] Inoue T, Hatayama M, Tohmonda T, Itohara S, Aruga J, Mikoshiba K. Mouse Zic5 deficiency results in neural tube defects and hypoplasia of cephalic neural crest derivatives. Dev Biol 2004; 270: 146-62. doi: 10.1016/j.ydbio.2004.02.017.

[14] Ali RG, Bellchambers HM, Arkell RM. Zinc fingers of the cerebellum (Zic): Transcription factors and co-factors. Int J Biochem Cell Biol 2012; 44: 2065-8. doi: 10.1016/j.biocel.2012.08.012.

[15] Sakai-Kato K, Umezawa Y, Mikoshiba K, Aruga J, Utsunomiya-Tate N. Stability of the folding structure of Zic zinc finger proteins. Biochem Biophys Res Commun 2009; 384: 362-5. doi: 10.1016/j.bbrc.2009.04.151.

[16] Mizugishi K, Aruga J, Nakata K, Mikoshiba K. Molecular properties of Zic proteins as transcriptional regulators and their relationship to GLI proteins. J Biol Chem 2001; 276: 2180-8. doi: 10.1074/jbc.M004430200.

[17] ZIC5 gene, https://www.ensembl.org/Homo_sapiens/Gene/Summary?g=ENSG000.001.39800;r=13:99962.964.99971767;t=ENST000.002.67294, 05.02.2023

[18] Primer design, https://eu.idtdna.com/pages, 02.03.2011

[19] Zhang Y, Liu T, Meyer CA, et al. Model-based analysis of ChIP-Seq (MACS). Genome Biol 2008; 9: R137. doi: 10.1186/gb-2008-9-9-r137.

[20] ZIC5 gene, rs965623242, http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=site/page&view=software),referans, 22.05.2020

[21] miRNA binding site, http://mirdb.org/, 22.05.2020

[22] ZIC5 gene, rs965623242, https://www.internationalgenome.org/, 21.10.2020

[23] ZIC5 gene, rs965623242, https://exac.broadinstitute.org/, 22.11.2020

[24] ZIC5 gene, rs965623242, https://www.ncbi.nlm.nih.gov/snp/, 25.11.2020

[25] ZIC5 gene, rs965623242, https://www.ncbi.nlm.nih.gov/variation/view/, 25.11.2020

[26] ZIC5 gene, rs965623242, https://www.ncbi.nlm.nih.gov/snp/rs965623242#history, 25.11.2020

[27] Brown LY, Hodge SE, Johnson WG, Guy SG, Nye JS, Brown S. Possible association of NTDs with a polyhistidine tract polymorphism in the ZIC2 gene. Am J Med Genet 2002; 108: 128-31. doi: 10.1002/ajmg.10221.

[28] Costa-Lima MA, Meneses HN, El-Jaick KB, Amorim MR, Castilla EE, Orioli IM. No association of the polyhistidine tract polymorphism of the ZIC2 gene with neural tube defects in a South American (ECLAMC) population. Mol Med Rep 2008; 1: 443-6.

[29] Shaikh TH, Haldeman-Englert C, Geiger EA, Ponting CP, Webber C. Genes and biological processes commonly disrupted in rare and heterogeneous developmental delay syndromes. Hum Mol Genet 2011; 20:880-93.

[30] Mou JT, Huang SX, Yu LL, Xu J, Deng QL, Xie YS, Deng K. Identification of genetic polymorphisms in unexplained recurrent spontaneous abortion based on whole exome sequencing. Ann Transl Med 2022; 10: 603. doi: 10.21037/atm-22-2179.

[31] Wang L, Shen H, Liu H, Guo G. Mixture SNPs effect on phenotype in genome-wide association studies. BMC Genom 2015; 16: 3. doi: 10.1186/1471-2164-16-3.

[32] Botto LD, Moore AC, Khory MJ, Erickson DJ. Neural-tube defects. N Eng J Med 1999; 1509-19.

[33] Dong C, Li X, Li K, Zheng C, Ying J. The expression pattern of ZIC5 and its prognostic value in lung cancer. Cancer Biother Radiopharm 2021; 36: 407-11.

[34] Zhao Z, Wang L, Bartom E, et al. Beta-Catenin/ Tcf7l2-dependent transcriptional regulation of GLUT1 gene expression by Zic family proteins in colon cancer. Sci Adv 2019; 5: eaax0698.

[35] Satow R, Inagaki S, Kato C, Shimozawa M, Fukami K. Identification of zinc finger protein of the cerebellum 5 as a survival factor of prostate and colorectal cancer cells. Cancer Sci 2017; 108: 2405-12.

[36] Sun Q, Shi R, Wang X, Li D, Wu H, Ren B. Overexpression of ZIC5 promotes proliferation in non-small cell lung cancer. Biochem Biophys Res Commun 2016; 479: 502-9.

[37] Liu L, Hu X, Sun D, Wu Y, Zhao Z. ZIC5 facilitates the growth of hepatocellular carcinoma through activating Wnt/beta-catenin pathway. Biochem Biophys Res Commun 2018; 503: 2173-9.

[38] Maimaiti A, Aizezi A, Anniwaer J, Ayitula, Ali B, Dilixiati M. Zinc finger of the cerebellum 5 promotes colorectal cancer cell proliferation and cell cycle progression through enhanced CDK1/CDC25c signaling. Arch Med Sci 2021; 17:449-61.

[39] Nyholm MK, Wu SF, Dorsky RI, Grinblat Y. The zebrafish zic2a-zic5 gene pair acts downstream of canonical Wnt signaling to control cell proliferation in the developing tectum. Development 2007; 134: 735-46. doi: 10.1242/dev.02756.

[40] Shi Y, Li J, Chen C, et al. Ketamine modulates Zic5 expression via the Notch signaling pathway in neural crest induction. Front Mol Neurosci 2018; 11: 9. doi: 10.3389/fnmol.2018.00009.

[41] https://topmed.nhlbi.nih.gov/topmed-whole-genome-sequencing-project-freeze-5b-phases-1-and-2 Accessed

[42] https://www.ncbi.nlm.nih.gov/snp/docs/gsr/alfa/

[43] Excoffier L, Ray N. Surfing during population expansions promotes genetic revolutions and structuration. Trends Ecol Evol 2008; 23: 347-51. doi: 10.1016/j.tree.2008.04.004.

[44] Hofer T, Ray N, Wegmann D, Excoffier L. Large allele frequency differences between human continental groups are more likely to have occurred by drift during range expansions than by selection. Ann Hum Genet 2009; 73: 95-108. doi: 10.1111/j.1469-1809.2008.00489.x.

[45] Novembre J, Di Rienzo A. Spatial patterns of variation due to natural selection in humans. Nature Reviews Genetics 2009; 10: 745-55. doi: 10.1038/nrg2632.