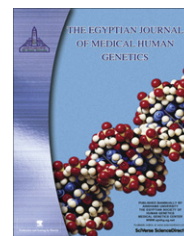




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ORIGINAL ARTICLE

## Role of ZIP14 (SLC39A14) gene histidine rich regions in neural tube defects

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### KEYWORDS

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**Abstract** Neural tube defects (NTDs) comprise a group of congenital malformations that includes spina bifida, anencephaly, meningomyelocele and encephalocele. Reports have implicated zinc deficiency as one of the causative factors of NTDs. Both environmental and genetic factors are involved in the etiology of NTDs. Inadequate folate intake and nutritional deficiency are important environmental risk factors. The aim of this study was to determine the relation of a zinc related gene ZRT and IRT like protein 14 (ZIP14) and neural tube defects in Turkish patients. The case control study included seventy Turkish mothers who gave birth to NTD infants. Two hundred and thirty-nine healthy controls were consecutively selected without any congenital defects or familial NTD history. Following DNA extraction, PCR, SSCP and DNA sequencing analysis of exons of the ZIP14 gene were performed. Our data revealed that no relation of neural tube defects and ZIP14 was detected in Turkish NTD patients. Zinc deficiency have been reported as a risk factor for Turkish population and other possible zinc related gene defects may have importance.

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### 1. Introduction

Zinc is an essential metal for the organism, and has a vital role for the function and structure of cells. It is required for the catalytic activity of several enzymes, protein synthesis, DNA synthesis, wound healing enhancement and cell division [1]. Zinc transporters control the level of intracellular zinc in cells. In mammals, zinc transporters are controlled with two gene families: the ZnT (solute linked carrier 30 (SLC30)) and the ZIP (ZRT/IRT like proteins (solute linked carrier 39(SLC39)). They appear to have opposite roles in cellular zinc homeostasis. ZnT transporters reduce intracellular cytoplasmic zinc by promoting zinc efflux from cells into intracellular vesicles, while ZIP transporters are responsible for the control of zinc transport into the cell cytosol [2].

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Neural tube defects (NTDs) are one of the commonest congenital malformations of the central nervous system and caused by misclosure of the neural tube resulting in serious complications. Various studies had shown the high incidence of neural tube defects (NTD) in Turkey. The estimated incidence is around three per 1000 live births [3,5].

Several factors influence the development of NTDs. Zinc deficiency is one of the proposed factor for the pathogenesis of NTD's. Either nutritional factors and/or genetic defects related to zinc may cause zinc deficiency among women which then, they may predispose to NTD babies [4,6,10].

Acrodermatitis enteropathica (AE, OMIM #201100, located on 8q24.3) is an autosomal recessive disorder affecting the uptake of zinc, with severe intestinal mucosal atrophy that can be reversed by effective oral zinc supplementation [7]. In seven pregnancies in patients with AE, there was one spontaneous abortion and two major congenital malformations including NTD's. Conversely, pregnancy outcome was good when a patient with acrodermatitis enteropathica was given supplemental zinc throughout her pregnancy [8]. Our group previously found nutritional defective zinc absorption in Turkish women with NTD babies and following Zn treatment, they delivered a normal full term child [9]. Also a case of a nutritionally zinc deficient young Turkish woman was reported who had previously delivered two anencephalic stillborn infants. After zinc supplementation she delivered a normal full term child [10,11].

Recently, we have reported a polymorphism at exon 5 of the ZnT4 gene 915 T-C alteration which may play a role in neural tube defects causing a 2.6 risk [12].

The mouse and human genomes contain 14 highly conserved SLC39 genes. ZIP14 is a member of the SLC39A zinc transporter family, which is involved in zinc uptake by cells. Member of ZIP family consist of 220-430 amino acid residues, where their aminic and carboxylic ends are directed toward the center of the cell. ZIP14 was localized on plasma membrane and have eight transmembrane domains. Transmembrane domains III and IV also contain a high amount of histidine, and mutations within those segments lead to the loss of the transporting function of the protein [13].

The histidine-rich repeats which is important for zinc transport considered to be a metal binding domain.

The SLC39A14 gene (ZIP14, OMIM #608736) is located on 8q21.3 and has 13 exons [13]. Various experiments have demonstrated that ZIP14 expression is up regulated through IL-6, and that this zinc transporter plays a major role in the mechanism responsible for hypozincemia that accompanies the acute phase response for inflammation and infection [14].

So, we aimed to study the ZIP14 gene defects which may have a possible role in the pathogenesis of NTD's. We choose promoter region and exon 7 which is histidine rich motif of ZIP14. Because promoter region can affect gene expression and HHH motif is have major role of zinc into the cell.

## 2. Subjects and methods

### 2.1. Subjects

Sample population consisted of 70 newborns with NTDs, 70 mothers who gave birth to NTD babies. 239 controls

contained healthy (mothers and newborns) were consecutively selected without any congenital defects or familial NTD history. An informed consent for genetic analysis was obtained from the parents and/or from the individual controls.

### 2.2. Methods

#### 2.2.1. DNA isolation and PCR amplification

DNA was extracted by conventional methods. Following DNA extraction, promoter region and exon 7 (histidine-rich domain-HHH motif) of the ZIP 14 (SLC39A14) gene was amplified by polymerase chain reaction (PCR). PCR reaction was carried out in a reaction volume of 20  $\mu$ l containing 100 ng of genomic DNA, 10 pmoles of each primer, 0.5 U Taq polymerase, 200  $\mu$ M of each dNTP and 2.5 mM MgCl<sub>2</sub> in a total reaction volume 50  $\mu$ l. The PCR reaction started after 5 min at 95 °C, followed by 34 cycles of 50 s of denaturation at 94 °C, 50 s of annealing at 55 °C and 1 min extension at 72 °C. Two different primer sets (F: 5'TCACCCCAAATTAACATTTCT3'; R: 5'GCTAGGCGGTGAGAGCTTC3') were used for amplifying the promoter region using a Whatman Biometra Thermal Cycler (Germany). PCR revealed a 333 bp-amplified product. For amplification of promoter region II; two primer sets used (F: 5'CCAGGGAGCAGGTCTTCAC3'; R:5'TGACGCGCCGGGTATATAG3') and PCR revealed 261 bp amplified product.

Two primers (F: 5'CCTCCCTGTCACCCTTCC3'; R: 5'TGCTTGGTGGAGCCTCTAAG3') used for the amplification of exon 7. PCR revealed a 246 bp amplified product.

Single strand conformation was performed as follows: Aliquots of 10  $\mu$ l of the PCR products were mixed with denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 95 °C and chilled on ice. Denatured DNA was subjected to acrylamide bisacrylamide gel electrophoresis in Tris-Boric acid EDTA buffer at constant voltage 5–8 V/cm for 14–15 h.

The gel was stained with 0.1% silver nitrate. Seventy newborn samples were sequenced, using a DNA sequencer (Beckman Coulter DNA Sequencer, USA) (Figs. 1 and 2).

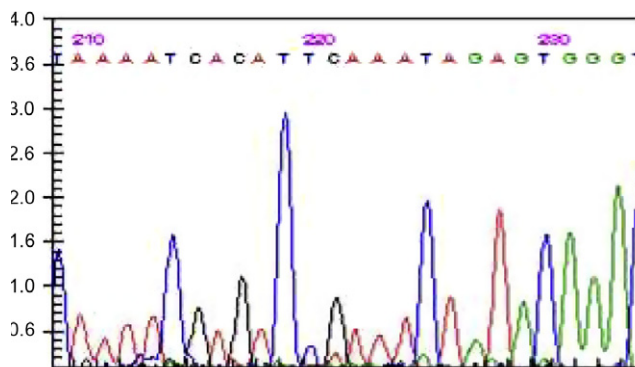


Figure 1 HHH motif sequence in exon 7.

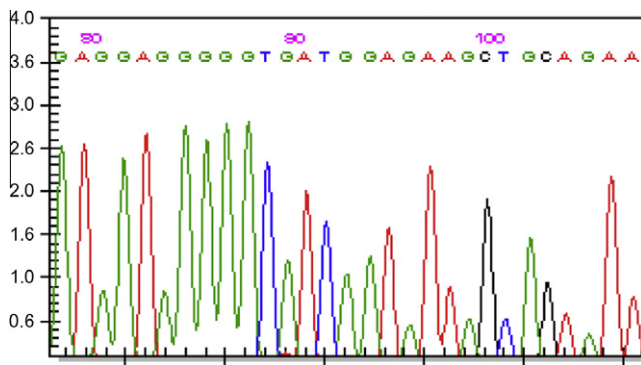


Figure 2 Promotor region sequence.

### 3. Results and discussion

None of the patients had a gene alteration at HHH motif or promotor region. Our data revealed that no relation of neural tube defects and ZIP14 was detected in Turkish NTD patients.

NTD's are congenital malformations caused by misclosure of the neural tube that result in serious complications. Various studies have shown the high incidence of neural tube defects (NTD) in Turkey (2,4). Several factors influence the pathogenesis of neural tube defects. Zinc deficiency is one of the possible factor for the etiopathogenesis of NTD's [3].

Previous data in human studies have shown a possible role of zinc metabolism in at least some of the mothers with NTD babies. Either nutritional factors and/or genetic defects related to zinc may cause zinc deficiency among women. Recent studies showed that concerning maternal plasma zinc levels in pregnancies associated with NTD revealed that some of the affected women have defective zinc absorption due to chronic zinc deficiency, which returned to normal after zinc supplementation [1,6].

Solute carrier family (SLC) group of membrane transport proteins include over 300 members, organized into 47 families. ZNT4 gene (also known as SLC30A4), belongs to the ZNT family of zinc transporters. ZNTs are involved in transporting zinc out of the cytoplasm and have similar structures, consisting of 6 transmembrane domains and a histidine-rich cytoplasmic loop [13].

In a recent study SLC30A4 seems to be a good candidate gene for spina bifida especially in cases where low zinc concentrations are observed. Furthermore, ZnT4 915T-C reported to have a functional role in zinc absorption and 915CC homozygosity was higher in NTD mothers than control group; bringing a two fold risk [12].

In our study we analyzed the two regions of the gene in a group of NTD mothers and their babies compared to healthy controls. We studied the possible gene defects in the promotor and histidine rich region(exon7) of the SLC39A14(ZIP14). Because promotor region can affect gene expression and HHH motif have a major role on zinc.

Although we were not able to show a link between NTD and SLC39A14; other possible genes related to zinc metabolism should be studied.

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