

Lack of association of polymorphic variants of genes encoding zinc transporters with the risk of orofacial cleft-affected pregnancies

Kamil K. Hozyasz¹, Adrianna Mostowska², Margarita Lianeri², Barbara Offert³, Pawel P. Jagodzinski²

¹Department of Paediatrics, Institute of Mother and Child, Warsaw, Poland

²Department of Biochemistry and Molecular Biology, University of Medical Sciences, Poznan, Poland

³Department of Paediatric Surgery, Institute of Mother and Child, Warsaw, Poland

Abstract: Maternal zinc deficiency seems to be a risk factor for orofacial clefts in offspring. This study was undertaken to investigate the involvement of polymorphic variants of genes for zinc transporters in the susceptibility of clefting. PCR-RFLP analysis was used to analyze single nucleotide polymorphisms of *SLC30A1* (rs7526700, rs2278651, rs611386), *SLC30A4* (rs2453531, rs8029246), *SLC30A5* (rs351444, rs164393, rs6886492), *SLC39A1* (rs10127484, rs11264736), and *SLC39A3* (rs759071, rs4806874, rs10415622) in mothers of children with non-syndromic cleft lip with or without cleft palate (CL/P) and control mothers. The allele, genotype, and haplotype distribution was found to be similar among case and control mothers. Also, the gene-by-gene interaction analysis conducted using the Multifactor Dimensionality Reduction approach revealed no significant interactive genetic effect on having a child with a cleft. In conclusion, our results demonstrated that the analyzed polymorphic variants of genes for zinc transporters are not implicated in abnormal palatogenesis in the investigated group of women from the Polish population.

Key words: CL/P, Zn, zinc transporters, SLC30A, SLC39A, polymorphism

Introduction

Zinc (Zn) is an essential dietary micronutrient that plays fundamental integrative roles in human physiology. The functions of Zn can be divided into three categories: structural, catalytic, and regulatory. It is estimated that 3-10% of human genes code for proteins with Zn binding domains [1]. Since Zn cannot passively diffuse across cell membranes its transport is carried out by Zn transporters. Two major unrelated families of mammalian Zn transporters have been identified: solute-linked carriers 30A (SLC30A, also called ZnTs) and SLC39A (ZIPs) [2]. Zinc uptake is mediated by the SLC39A protein family, while members of the SLC30A family transport zinc from the cytoplasm to the lumen of organelles or the extracellular space [3]. There is no storage form of Zn in the body that can be readily mobilized when dietary intake

is inadequate. However, it has been shown that Zn transporter expression can be regulated by both transcriptional and post-transcriptional mechanisms in response to Zn availability to ensure an appropriate plasma concentration of this nutrient [3]. Zn transporters have been strongly implicated in embryonic, fetal, and neonatal development [2-5]. SLC30A1 and SLC30A5 play a key role in regulating delivery of maternal Zn to the developing embryo [2,5]. Cadmium exposure, which is known to be risk factor for congenital anomalies, down-regulates *Slc30a1* expression, indicating that maternal cadmium exposure may alter Zn homeostasis in the conceptus [5]. Mutations in *ZnTs* and *ZIPs* reveal their physiological functions [3]. Mutations in *SLC30A4* have been associated with a loss or reduction of Zn secretory activity of some glandular epithelial cells, however the regulation of Zn transporter proteins in histiotrophic nutrition in early pregnancy has yet to be elucidated [3,6,7]. Mutations in human *SLC39A4* cause the rare disease acrodermatitis enteropathica [8]. Interestingly, it was been found that in the mouse, maternal

Corresponding: K.K. Hozyasz, 17a Kasprzaka Str, 01-211 Warsaw, Poland; tel./fax.: (+4822) 3277190, e-mail: khozyasz@verco.com.pl

Table 1. Conditions for the identification of investigated SNPs

Gene symbol	rs no.	Location ^a	SNP function ^b	Primers for PCR amplification (5' – 3')	Annealing temp. (°C)	PCR product length (bp)	Restriction enzyme	Alleles ^c
<i>SLC30A1</i>	rs7526700	chr1: 211739824	N/A	F: AGTACAGGATAAAAGGACCAAGGT R: TTGATTTGTGGTITTAAGGTAGG	57.4	352	<i>TaqI</i>	C/g
<i>SLC30A1</i>	rs2278651	chr1: 211751060	intron	F: GGTTTACACGCTCTGAATGC R: CAATACCAGCAACTCCAACG	59.7 with 10% DMSO	566	<i>MboI</i>	C/t
<i>SLC30A1</i>	rs611386	chr1: 211770318	N/A	F: GCCAAGGATGTTTTCTCCA R: TGGTTTGTGGTTGTGATTGC	67	679	<i>MboII</i>	a/G
<i>SLC30A4</i>	rs2453531	chr15: 45779810	cds-synon Asp305Asp	F: TCAGAATGTCCCCTGACTCC R: GTCCCATGAGGCAAGTAACG	67	374	<i>BspPI</i>	A/g
<i>SLC30A4</i>	rs8029246	chr15: 45821158	N/A	F: CGGCCTATCCAGTGTATGA R: GAAAGCAACCCCAAGTAAGAT	67	360	<i>TaqI</i>	A/g
<i>SLC30A5</i>	rs351444	chr5: 68377251	N/A	F: GGGACATACTTCCAGCTTGC R: TTAGCTTGGCACATGAAATCICC	67	536	<i>HpyF3I</i>	C/g
<i>SLC30A5</i>	rs164393	chr5: 68409094	intron	F: ACCCTTGA AAAAGGICATCCCTTG R: GTAAGAGAAGAGTTGGGGCTGA	67	587	<i>MnlI</i>	G/t
<i>SLC30A5</i>	rs6886492	chr5: 68425699	UTR-3	F: CCATGAAATACTGCAAAGATGG R: TTACAATGGAGATGCTTCCTGA	66.3	370	<i>TaqI</i>	A/g
<i>SLC39A1</i>	rs10127484	chr1: 153900980	N/A	F: GAAGGAGAGCAGGCGTATCA R: GCCCAGCCTAGATATCTTATGG	67	601	<i>Eam1104I</i>	A/g
<i>SLC39A1</i>	rs11264736	chr1: 153939130	intron	F: AGTGTGAAGAACATGGGCTGA R: GAAGATTTGAACGGGCTAAGG	66.3	461	<i>BccI</i>	C/t
<i>SLC39A3</i>	rs759071	chr19: 2728577	N/A	F: TGTGGGCTCAAGTAATCTGC R: GGGGACATCTGTGGTTGTCT	67	414	<i>MboI</i>	A/g
<i>SLC39A3</i>	rs4806874	chr19: 2738352	intron	F: GATGCAGAGAAGCAAGAAAGAA R: ACAAATTCGGCTCCTCAGIC	67	370	<i>NspI</i>	A/g
<i>SLC39A3</i>	rs10415622	chr19: 2739696	intron	F: ATCTCAGCTCCTCCCTGTC R: GACTCTACCCGACGTTAGC	66.3	532	<i>BseGI</i>	A/g

^aBased on UCSC Human Genome Browser, February 2009 (GRCh37) assembly; ^bAccording to the Single Nucleotide Polymorphism database (dbSNP); ^cUppercase denotes the more frequent allele in the control samples.

Slc39a4 haploinsufficiency results in increased sensitivity to the effects of Zn deficiency and the risk of abnormal embryonic development [8]. Recently, Kambe *et al.* [9] demonstrated that the *Slc39a1*, 2, and 3 genes evolved to decrease sensitivity to the stress of dietary Zn deficiency in the reproducing mouse. The high degree of evolutionary conservation of *ZIP1*, 2, and 3 suggests the possibility of these genes' involvement in protection against Zn deficiency in human pregnancies [9].

Cleft lip with or without cleft palate (CL/P) is one of the most common congenital malformations in humans. The aetiology of CL/P is highly complex and associated with both genetic and multiple environmental factors, including micronutrient deficiencies [10]. The correlation between abnormal palatogenesis and Zn dyshomeostasis has been clearly documented in laboratory animals [11]. In humans, poor maternal Zn status may be a risk factor for clefting when the micronutrient deficiency reaches a certain severity [12,13]. Observations in the rodent model indicate that the Zn transporter genes can be silent when dietary intake of Zn is normal in pregnancy, but

they might be involved in buffering the micronutrient deficiency and the success of reproduction can be dramatically compromised [14,15]. Such findings have prompted the search for cleft-associated variants of Zn transporters. Therefore, the present study was designed to test single nucleotide polymorphisms (SNPs) of the *SLC30A* and *SLC39A* genes as risk factors of having a child with CL/P in the Polish population.

Materials and methods

Participants. The study was approved by the local ethics committee. Index patients with non-syndromic cleft lip with or without cleft palate were chosen by clinicians using detailed diagnostic information from medical records in the Department of Paediatrics and Department of Paediatric Surgery at the Institute of Mother and Child in Warsaw. Peripheral blood samples from 149 healthy mothers of children with orofacial clefts were obtained. In addition, 100 healthy mothers of children without congenital anomalies were used as controls (control mothers). All participating omnivorous women, aged 18-45 years, were Caucasians of Polish origin recruited from the same geographic region. Written and oral consent was obtained from all subjects.

Genotyping. DNA was isolated from peripheral blood lymphocytes by salt extraction. All 13 analyzed polymorphic variants of *SLC30A1*,

Table 2. Genotype frequencies of the investigated polymorphisms in mothers of children with CL/P and control mothers

Gene	rs no.	Genotype	CL/P mothers (frequency)	Control mothers (frequency)	Odds ratio (95% CI)	p ^a
<i>SLC30A1</i>	rs7526700	CC	90 (0.63)	56 (0.57)	Referent	-
		CG	52 (0.36)	40 (0.40)	0.809 (0.476 - 1.375)	0.4328
		GG	2 (0.01)	3 (0.03)	0.415 (0.067 - 2.561)	0.3794 ^b
		CG + GG	54 (0.37)	43 (0.43)	0.781 (0.464 - 1.316)	0.3533
		MAF	0.19	0.23	-	-
<i>SLC30A1</i>	rs2278651	CC	33 (0.24)	24 (0.25)	Referent	-
		CT	76 (0.54)	49 (0.50)	1.128 (0.597 - 2.133)	0.7101
		TT	31 (0.22)	24 (0.25)	0.939 (0.444 - 1.986)	0.8700
		CT + TT	107 (0.76)	73 (0.75)	1.066 (0.582 - 1.951)	0.8357
		MAF	0.49	0.50	-	-
<i>SLC30A1</i>	rs611386	GG	86 (0.58)	56 (0.57)	Referent	-
		AG	59 (0.40)	39 (0.39)	0.985 (0.582 - 1.667)	0.9554
		AA	3 (0.02)	4 (0.04)	0.488 (0.105 - 2.266)	0.4400 ^b
		AG + AA	62 (0.42)	43 (0.43)	0.939 (0.561 - 1.571)	0.8101
		MAF	0.21	0.24	-	-
<i>SLC30A4</i>	rs2453531	AA	71 (0.48)	51 (0.53)	Referent	-
		AG	68 (0.46)	42 (0.44)	1.163 (0.687 - 1.970)	0.5741
		GG	9 (0.06)	3 (0.03)	2.155 (0.555 - 8.359)	0.3598 ^b
		AG + GG	77 (0.52)	45 (0.47)	1.229 (0.735 - 2.056)	0.4317
		MAF	0.29	0.25	-	-
<i>SLC30A4</i>	rs8029246	AA	72 (0.50)	55 (0.58)	Referent	-
		AG	58 (0.41)	35 (0.37)	1.266 (0.732 - 2.188)	0.3979
		GG	13 (0.09)	5 (0.05)	1.986 (0.668 - 5.906)	0.2106
		AG + GG	71 (0.50)	40 (0.42)	1.356 (0.805 - 2.287)	0.2532
		MAF	0.29	0.24	-	-
<i>SLC30A5</i>	rs351444	CC	55 (0.37)	30 (0.32)	Referent	-
		CG	73 (0.49)	44 (0.46)	0.905 (0.506 - 1.619)	0.7363
		GG	21 (0.14)	21 (0.22)	0.545 (0.257 - 1.156)	0.1117
		CG + GG	94 (0.63)	65 (0.68)	0.789 (0.457 - 1.362)	0.3938
		MAF	0.39	0.45	-	-
<i>SLC30A5</i>	rs164393	GG	63 (0.43)	45 (0.47)	Referent	-
		GT	73 (0.50)	41 (0.43)	1.272 (0.740 - 2.185)	0.3834
		TT	10 (0.07)	10 (0.10)	0.714 (0.274 - 1.859)	0.4892
		GT + TT	83 (0.57)	51 (0.53)	1.162 (0.693 - 1.951)	0.5686
		MAF	0.32	0.32	-	-
<i>SLC30A5</i>	rs6886492	AA	89 (0.64)	58 (0.60)	Referent	-
		AG	45 (0.32)	32 (0.33)	0.916 (0.523 - 1.606)	0.7605
		GG	6 (0.04)	7 (0.07)	0.559 (0.179 - 1.746)	0.3112
		AG + GG	51 (0.36)	39 (0.40)	0.852 (0.500 - 1.451)	0.5557
		MAF	0.20	0.24	-	-
<i>SLC39A1</i>	rs10127484	AA	37 (0.25)	21 (0.21)	Referent	-
		AG	74 (0.50)	58 (0.58)	0.724 (0.383 - 1.368)	0.3193
		GG	37 (0.25)	21 (0.21)	1.000 (0.469 - 2.133)	1.000
		AG + GG	111 (0.75)	79 (0.79)	0.797 (0.434 - 1.465)	0.4654
		MAF	0.50	0.50	-	-
<i>SLC39A1</i>	rs11264736	CC	42 (0.30)	22 (0.23)	Referent	-
		CT	69 (0.48)	56 (0.58)	0.645 (0.345 - 1.206)	0.1683
		TT	31 (0.22)	19 (0.19)	0.855 (0.396 - 1.845)	0.6890
		CT + TT	100 (0.70)	75 (0.77)	0.698 (0.385 - 1.268)	0.2370
		MAF	0.46	0.48	-	-
<i>SLC39A3</i>	rs759071	AA	81 (0.56)	51 (0.53)	Referent	-
		AG	47 (0.33)	38 (0.39)	0.779(0.448 - 1.354)	0.3749
		GG	16 (0.11)	8 (0.08)	1.259 (0.503 - 3.155)	0.6222
		AG + GG	63 (0.44)	46 (0.47)	0.862 (0.514 - 1.446)	0.5743
		MAF	0.27	0.28	-	-
<i>SLC39A3</i>	rs4806874	AA	68 (0.46)	44 (0.44)	Referent	-
		AG	63 (0.42)	48 (0.48)	0.849 (0.498 - 1.448)	0.5484
		GG	18 (0.12)	8 (0.08)	1.456 (0.583 - 3.636)	0.4195
		AG + GG	81 (0.54)	56 (0.56)	0.936 (0.562 - 1.558)	0.7990
		MAF	0.33	0.32	-	-
<i>SLC39A3</i>	rs10415622	AA	64 (0.45)	38 (0.39)	Referent	-
		AG	61 (0.43)	50 (0.51)	0.724 (0.418 - 1.254)	0.2487
		GG	16 (0.12)	10 (0.10)	0.950 (0.391 - 2.305)	0.9097
		AG + GG	77 (0.55)	60 (0.61)	0.762 (0.451 - 1.288)	0.3092
		MAF	0.33	0.36	-	-

^aChi-square analysis; ^bFisher exact test; MAF, minor allele frequency.

Table 3. Haplotypes and their association with maternal risk of having CL/P progeny

Haplotype	Frequency	Case, control ratios (freq.)	Chi-square	p value
<i>SLC30A1</i> (rs7526700, rs2278651, rs611386)				
CTG	0.296	0.298, 0.293	0.013	0.9106
CCG	0.279	0.301, 0.246	1.843	0.1746
GCG	0.198	0.182, 0.224	1.335	0.2479
CTA	0.192	0.184, 0.204	0.308	0.5789
CCA	0.024	0.024, 0.025	0.005	0.9420
GTA	0.010	0.011, 0.008	0.093	0.7607
<i>SLC30A4</i> (rs2453531, rs8029246)				
AA	0.697	0.675, 0.729	1.646	0.1995
GG	0.267	0.292, 0.229	2.370	0.1237
GA	0.034	0.032, 0.036	0.044	0.8341
<i>SLC30A5</i> (rs351444, rs164393, rs6886492)				
CGA	0.437	0.447, 0.421	0.307	0.5796
GTA	0.221	0.207, 0.244	0.914	0.3389
GCG	0.104	0.095, 0.119	0.754	0.3853
CGG	0.086	0.086, 0.086	0.000	0.9973
CTA	0.064	0.081, 0.037	3.821	0.0506
GGA	0.060	0.064, 0.055	0.155	0.6935
GTG	0.028	0.021, 0.038	1.280	0.2580
<i>SLC39A1</i> (rs10127484, rs11264736)				
AC	0.499	0.495, 0.505	0.047	0.8288
GT	0.466	0.464, 0.469	0.014	0.9057
GC	0.029	0.034, 0.020	0.781	0.3768
<i>SLC39A3</i> (rs759071, rs4806874, rs10415622)				
AAA	0.646	0.653, 0.636	0.147	0.7010
GGG	0.264	0.264, 0.264	0.000	0.9898
AGG	0.042	0.043, 0.040	0.014	0.9051
AGA	0.017	0.017, 0.017	0.002	0.9629
GAG	0.017	0.014, 0.021	0.296	0.5867
AAG	0.014	0.009, 0.022	1.357	0.2440

SLC30A4, *SLC30A5*, *SLC39A1*, and *SLC39A3* were identified using PCR followed by appropriate restriction enzyme digestion (PCR-RFLP). DNA fragments were separated using electrophoresis on 2% agarose gel and visualized using ethidium bromide staining. Primer sequences and conditions for PCR-RFLP analyses are presented in Table 1. The selection of SNPs was conducted through the use of the genome browser of the International HapMap Consortium (www.hapmap.org). SNPs were prioritized according to their validation status, functional relevance and importance, and minor allele frequency MAF>0.1 in the Caucasian population.

Statistical analysis. The differences in allele and genotype frequencies between case mothers and controls were determined using standard χ^2 and Fisher exact tests. The odds ratio (OR) and associated 95% confidence intervals (95% CI) for mothers of children with CL/P versus control mothers were also calculated. Haplotype analysis was performed using Haploview 4.0 (<http://www.broadinstitute.org/mpg/haploview>). Associations between the investigated polymorphisms and the risk of having a child with an orofacial cleft were tested using the nonparametric and genetic model-free Multifactor Dimensionality Reduction (MDR) approach (MDR version 2.0 beta 5). Statistical significance was evaluated using a 1,000-fold permutation test (MDR permutation testing module 0.4.9 alpha). A p-value of <0.05 was considered statistically significant.

Results

For all analysed polymorphisms of the *SLC30A1*, *SLC30A4*, *SLC30A5*, *SLC39A1*, and *SLC39A3* genes there was no evidence for both allelic and genotyping association with the risk of being a case mother in the Polish population. The genotyping results are presented in Table 2. No deviation from Hardy-Weinberg equilibrium in either CL/P mothers or control mothers at the 0.05 level was observed. The minor allele frequency for all SNPs was at least 0.19. Haplotype analysis did not detect differences in haplotype distribution between case and control mothers (Table 3). Moreover, the exhaustive MDR analysis revealed no significant interactive genetic effect on having a child with CL/P for all analysed SNPs (Table 4). All possible two-, three- and four-way SNP interactions were tested using 10-fold cross validation in an exhaustive search (considering all possible SNP combinations). All of the "best models" (showing highest testing balanced accuracy and cross validation consistency >5 out of 10) did not reach the statistical significance evaluated using a 1,000-fold permutation test.

Discussion

Genetic studies in humans have shown associations between expression profiles of genes for Zn transporters and chronic diseases (diabetes, asthma) as well as carcinogenesis [16,17]. Since craniofacial malformations arise from misregulation of normally coordinated tissue patterns during early embryogenesis [10], another interesting aspect of Zn transporter function is the possibility of these proteins' involvement in early embryonic nutrition. It has been demonstrated that the expression of the *Slc30a1* gene is highly active in the yolk sac of the developing mouse embryo, and this expression is partially dependent on maternal Zn intake [18]. Due to the key role of Zn in human physiology, the aim of this study was to verify possible contributions of Zn transporter gene SNPs to orofacial clefting. To our knowledge, the present report is the second association study examining the potential role of genes encoding Zn transporters in the aetiology of non-syndromic structural malformations in affected progeny. In a sample of Dutch triads of patients and their parents, no association was observed between the 1069C>T (rs2272662) variant of *SLC39A4* and spina bifida [19]. In our study the investigated SNPs were not found to be associated with the women's risk of having children with CL/P, analyzed either separately or in combination. We analyzed three polymorphisms of the gene for the SLC30A1 protein, which plays an essential function in transporting maternal Zn into the embryo, and two polymorphic variants of the gene for SLC39A1 protein, which has been detected in a wide variety of tissues and cell types [3].

Table 4. Results of gene-gene interactions analyzed by MDR method

Genes and rs numbers	Testing balanced accuracy ^a	Cross validation consistency ^b	p value ^c
<i>SLC39A1</i> rs10127484	0,5729	9 / 10	0,281
<i>SLC30A4</i> rs2453531, <i>SLC39A1</i> rs10127484	0,5862	6 / 10	0,186
<i>SLC30A1</i> rs7526700, <i>SLC30A1</i> rs2278651, <i>SLC30A4</i> rs8029246	0,4920	2 / 10	0,895
<i>SLC30A1</i> rs611386, <i>SLC30A1</i> rs2278651, <i>SLC39A1</i> rs10127484, <i>SLC39A1</i> rs11264736	0,4855	2 / 10	0,922

^aTesting balanced accuracy is the accuracy of classification of cases and controls in the testing dataset calculated as (Sensitivity+Specificity)/2;

^bCross validation consistency is the number of times the model was selected as the best model after 10-fold cross-validation runs;

^cSignificance of accuracy (empirical p value based on 1,000 permutations).

It has been shown that mRNA levels of both *SLC30A1* and *SLC39A1* may be potential Zn status indicators in the human lymphoblastoid cell line [20]. In women of childbearing age supplemented with Zn, the expression of *SLC39A1* in leucocytes has been reported to decrease significantly, whereas there were no changes in the mRNA levels of *SLC30A1* [20]. Therefore, *SLC39A1* mRNA expression is considered a potential biomarker of Zn status in humans. In contrast to these predictions, the allele and genotype distribution of *SLC30A1* and *SLC39A1* polymorphic variants was found to be similar among the investigated mothers of CL/P affected children and controls.

In the present study, the allele distribution of *SLC30A5* rs351444 showed the highest difference between case and control mothers. Moreover, the risk of having a CL/P affected child for the maternal rs351444 GG genotype compared with the wild type tended to be decreased (OR=0.55; 95%CI: 0.26-1.16; p=0.11). Interestingly, haplotype analysis of *SLC30A5* polymorphic variants (rs351444, rs164393, and rs6886492) showed borderline association between the CTA haplotype and increased risk of clefting (p=0.051).

Expression of *SLC30A4* appears to be independent of Zn status, which is contrary to *SLC30A1*, *SLC30A5*, *SLC39A1*, and *SLC39A3* [21]. It is noteworthy that there was no evidence for association of polymorphic variants of *SLC30A4* with CL/P risk. The analysis of epistasis is helpful in determining the true contribution of genetic factors to congenital anomaly susceptibility [22]. However, in our study the exhaustive MDR analysis revealed no significant interactive effect of polymorphic variants of the genes for Zn transporters on abnormal palatogenesis. There are no previously published clinical association studies of the SNPs analyzed in the present report, and so we are unable to compare our results with data from other studies.

However, our negative results do not exclude the potential role of polymorphisms of Zn transporter genes in the aetiology of CL/P. The results might be influenced by the sample size, which may not be large enough to detect a modest effect of the analyzed gene variants. We cannot exclude that SNPs in genes for Zn

transporters may influence palatogenesis via the embryonic, rather than the maternal, genotype. In humans, some of genes for Zn transporters may have a restricted expression to only fetal tissues [23]. Therefore, the exclusion of the investigated SNPs and other variants of genes encoding Zn transporters as risk factors of CL/P in the Polish population requires further analyses, which should be performed in larger groups of case and control mothers as well as in CL/P-affected children. The relatively high and fluctuating frequency of orofacial clefts is attributable to the sensitivity of facial development to environmental insult. Recent progress in our understanding of the molecular functions of Zn transporters and their clinical associations has revealed that additional analysis involving polymorphisms of *SLC30A8* and *SLC39A14* may throw a new light on the aetiology of CL/P. Maternal diabetes and infection with the common cold during early pregnancy are well known risk factors for abnormal palatogenesis [10,24]. *SLC39a14* plays a major role in hypozincemia induced by inflammation, which is among the classical changes observed during acute infections [3]. In rodents, dietary Zn supplementation decreases the frequency of malformations caused by bacterial lipopolysaccharides [25]. The association between some polymorphisms of *SLC30A8* and the onset of diabetes mellitus is well documented in humans [3].

In summary, the presented results did not support any association between maternal polymorphisms of *SLC30A* and *SLC39A* genes and the risk of having an infant with CL/P in the investigated Polish population. However, given the high heterogeneity of the association between the maternal Zn blood level and the risk of clefting [13], we suspect that genes acting in Zn homeostasis could be involved in CL/P onset and deserve further investigation.

Acknowledgments: The investigators thank the multitude of mothers who consented to participate in this study. We are also greatly thankful to all of the research nurses who were involved in the collection of blood samples. This work was supported by grant no. 502-01-01124182-07474 from the University of Medical Sciences in Poznan.

References

- [1] Andreini C, Banci L, Bertini I, Rosato A. Counting the zinc-proteins encoded in the human genome. *J Proteome Res.* 2006;5:196-201.
- [2] Ford D. Intestinal and placental zinc transport pathways. *Proc Nutr Soc.* 2004;63:21-29.
- [3] Lichten LA, Cousins RJ. Mammalian zinc transporters: nutritional and physiologic regulation. *Annu Rev Nutr.* 2009;29:153-176.
- [4] Yamashita S, Miyagi C, Fukada T, Kagara N, Che YS, Hirano T. Zinc transporter LIV1 controls epithelial-mesenchymal transition in zebrafish gastrula organizer. *Nature.* 2004;429:298-302.
- [5] Fernandez EL, Dencker L, Tallkvist J. Expression of ZnT-1 (Slc30a1) and MT-1 (mt1) in the conceptus of cadmium treated mice. *Reprod Toxicol.* 2007;24:353-358.
- [6] Huang L, Girchier J. A novel gene involved in zinc transport is deficient in the lethal milk mouse. *Nat Genet.* 1997;17:292-297.
- [7] Burton GJ, Watson AL., Hempstock J, Skepper JN, Jauniaux E. Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. *J Clin Endocrinol Metab.* 2002;87:2954-2959.
- [8] Andrews GK. Regulation and function of Zip4, the acrodermatitis enteropathica gene. *Biochem Soc Trans.* 2008;36:1242-1246.
- [9] Kambe T, Geiser J, Lahner B, Salt DE, Andrews GK. Slc39a1 to 3 (subfamily II) Zip genes in mice have unique cell-specific functions during adaptation to zinc deficiency. *Am J Physiol Regul Integr Comp Physiol.* 2008;294:R1474-1481.
- [10] Murray JC. Gene/environment causes of cleft lip and/or palate. *Clin Genet.* 2002;61:248-256.
- [11] Hurley LS, Gowan J, Swenerton H. Teratogenic effects of short-term and transitory Zinc deficiency in rats. *Teratology.* 1973;4:199-204.
- [12] Hozyasz KK, Kaczmarczyk M, Dudzik J, Bulska E, Dudkiewicz Z, Szymanski M. Relation between the concentration of zinc in maternal whole blood and risk of an infant being born with an orofacial cleft. *Br J Oral Maxillofac Surg.* 2009;47:466-469.
- [13] Munger RG, Tamura T, Johnston KE, Feldkamp ML, Pfister R, Carey JC. Plasma zinc concentrations of mothers and the risk of oral clefts in their children in Utah. *Birth Defects Res A.* 2009;85:151-155.
- [14] Dufner-Beattie J, Huang ZL, Geiser J, Xu W, Andrews GK. Mouse ZIP1 and ZIP3 genes together are essential for adaptation to dietary zinc deficiency during pregnancy. *Genesis.* 2006;44:239-251.
- [15] Helston RM, Phillips SR, McKay JA, Jackson KA, Mathers JC, Ford D. Zinc transporters in the mouse placenta show a coordinated regulatory response to changes in dietary zinc intake. *Placenta.* 2007;28:437-444.
- [16] Devirgiliis C, Zalewski PD, Perozzi G, Murgia C. Zinc fluxes and zinc transporter genes in chronic diseases. *Mut Res.* 2007;622:84-93.
- [17] Sun D, Zhang L, Wang Y *et al.* Regulation of zinc transporters by dietary zinc supplement in breast cancer. *Mol Biol Rep.* 2007;43:241-247.
- [18] Langmade SJ, Ravindra R, Daniels PJ, Andrews GK. The transcription factor MTF-1 mediates metal regulation of the mouse ZnT1 gene. *J Biol Chem.* 2000;44:34803-34809.
- [19] Groenen PM, Klootwijk R, Schijvenaars MM, Straatman H, Mariman EC, Franke B, Steegers-Theunissen RP. Spina bifida and genetic factors related to myo-inositol, glucose, and zinc. *Mol Genet Metabol.* 2004;82:154-161.
- [20] Andree KB, Kim J, Kirschke CP, *et al.* Investigation of lymphocyte gene expression for use as biomarkers for zinc status in humans. *J Nutr.* 2004;134:1716-1723.
- [21] Liuzzi JP, Blanchard RK, Cousins RJ. Differential regulation of zinc transporters 1,2, and 4 mRNA expression by dietary zinc. *J Nutr.* 2001;131:46-52.
- [22] Musani SK, Shriner D, Liu N, Feng R, Coffey CS, Yi N, Tiwari HK, Allison DB. Detection of gene x gene interactions in genome-wide association studies of human population data. *Hum Hered.* 2007;63:67-84.
- [23] Seve M, Chimienti F, Devergnas S, Favier A. In silico identification and expression of SLC30 family genes: an expression sequence tag data mining strategy for the characterization of zinc transporters' tissue expression. *BMC Genomics.* 2004;5:32.
- [24] Acs N, Banhidy F, Horvath-Puho E, Czeizel AE. Population-based case-control study of the common cold during pregnancy and congenital anomalies. *Eur J Epidemiol.* 2006;21:65-75.
- [25] Chua JS, Rofe AM, Coyle P. Dietary zinc supplementation ameliorates LPS-induced teratogenicity in mice. *Pediatr Res.* 2006;59:355-358.

Submitted: 13 January, 2010

Accepted after reviews: 15 June, 2010