Original Article
The relationship between cleft lip and palate children with their trace elements in serum

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Abstract: Objective: To study the relationship between Cleft lip and palate children with their trace elements in serum. Methods: All patients were divided into four groups. The first group of unilateral cleft lip was constituted with 72 patients. Accordance with the ratio 1:2 random survey, it selected the health children for control group. It detected the trace elements in serum by collecting 2-3 mL Fasting venous blood. Made determination of vitamin content in high performance liquid chromatography and detection of respiratory infection history by indirect immunoinfluscent assays for nine kinds of antibody for different pathogens. Then it analyzed the data by SPSS; Results: The incidence of disease children concentrated on the Genetic Factors and the Mining Area children (P<0.05). As for the zinc content, it was at lower level in every disease group compared with the control group. Selenium content was at considerable level between unilateral cleft lip groups with control group. However, other disease groups were at lower level. Used Chi-square test, it indicated that the changes of the three key trace elements could be Synergistic effect with Adenoviruses and respiratory syncytial virus infection; Conclusion: The differences of crucial trace elements (including: zinc, selenium, copper and vitamin A) in children's serum could have close relationship with inborn cleft lip and palate disease.

Keywords: Cleft lip, cleft palate, trace elements, respiratory pathogens

Introduction

CDC recently estimated that, each year in the United States, about 2,650 babies are born with a cleft palate and 4,440 babies are born with a cleft lip with or without a cleft palate. Isolated orofacial clefts, or clefts that occur with no other major birth defects, are one of the most common types of birth defects in the United States. Depending on the cleft type, the rate of isolated orofacial clefts can vary from 50% to 80% [1-3].

Cleft lip and cleft palate are birth defects that occur when a baby's lip or mouth do not form properly during pregnancy. Together, these birth defects commonly are called "orofacial clefts". These birth defects happen early during pregnancy. A baby can have a cleft lip, a cleft palate, or both a cleft lip and cleft palate [4, 5].

As for facial development, a baby's head has formed early during pregnancy. To make the face, body tissue and special cells from each side of the head grow toward the center of the face and join together. This joining of tissue forms the facial features, like the lips and mouth [6, 7].

The lip forms between the fourth and seventh weeks of pregnancy. A cleft lip happens if the tissue that makes up the lip does not join completely before birth. This results in an opening in the upper lip. The opening in the lip can be a small slit or it can be a large opening that goes through the lip into the nose. A cleft lip can be on one or both sides of the lip or in the middle of the lip, which occurs very rarely. Children with a cleft lip also can have a cleft palate [7, 8].

Cleft Palate: The roof of the mouth (palate) is formed between the sixth and ninth weeks of pregnancy. A cleft palate happens if the tissue that makes up the roof of the mouth does not join together completely during pregnancy. For some babies, both the front and back parts of

the palate are open. For other babies, only part of the palate is open [7-9].

Like the many families of children with birth defects, CDC wants to find out what causes them. Understanding the factors that can increase the chance of having a baby with a birth defect will help us learn more about the causes. CDC coordinates, funds, and collaborates on one of the largest studies in the United States-the National Birth Defects Prevention Study-to understand the causes of and risks for birth defects, including orofacial clefts [8-10].

The causes of orofacial clefts among most infants are unknown. Some children have a cleft lip or cleft palate because of changes in their genes. Cleft lip and cleft palate are thought to be caused by a combination of genes and other factors, such as things the mother comes in contact with in her environment, or what the mother eats or drinks, or certain medications she uses during pregnancy [11, 12].

Use of certain medicines-Women who used certain medicines to treat epilepsy, such as topiramate or valproic acid, during the first trimester (the first 3 months) of pregnancy have an increased risk of having a baby with cleft lip with or without cleft palate, compared to women who didn't take these medicines [13, 14].

Environment: Environmental influences may also cause, or interact with genetics to produce, orofacial clefting. An example for how environmental factors might be linked to genetics comes from research on mutations in the gene PHF8 that cause cleft lip/palate (see above). It was found that PHF8 encodes for a histone lysine demethylase, and is involved in epigenetic regulation. The catalytic activity of PHF8 depends on molecular oxygen, a fact considered important with respect to reports on increased incidence of cleft lip/palate in mice that have been exposed to hypoxia early during pregnancy, In humans, fetal cleft lip and other congenital abnormalities have also been linked to maternal hypoxia, as caused by e.g. maternal smoking, maternal alcohol abuse or some forms of maternal hypertension treatment [13-17].

Other environmental factors that have been studied include: seasonal causes (such as pes-ticide exposure); maternal diet and vitamin intake; retinoids- which are members of the vitamin A family; anticonvulsant drugs; alcohol; cigarette use; nitrate compounds; organic solvents; parental exposure to lead; and illegal drugs (cocaine, crack cocaine, heroin, etc.).

Materials and methods

The case survey

We selected the inborn cleft lip and palate children reported by the First Affiliated Hospital of Guangxi Medical University for the subject, from 2013 to 2015.

The definition of clinical, according to Classification criteria made by the Professional Committee of Oral and Maxillofacial Surgery, of Chinese Stomatological Association, was implemented.

All patients were divided into four groups by that standard. The first group of unilateral cleft lip was constituted with 72 patients. There were three different degree of cleft lip in that group: degree I with only Vermilion section split opening; degree II with Upper lip section spliced but complete nasal floor; degree III with whole upper lip and nasal floor spliced.

The second group of bilateral cleft lip was constituted with 77 patients.

The third group of cleft soft palate was constituted with 63 patients, who only had cracks in soft palate or vertical palatal whether left or right.

The fourth group of complete cleft palate was with 56 patients, who had cracks from vertical palatal to Incisors hole, whether left or right.

The case-control

We selected the common health cases reported by the First Affiliated Hospital of Guangxi Medical University for the case-control, from 2013 to 2015, in accordance with the ratio 1:2 random survey.

All patients surveyed in this study, were at age from 1 month to 18 months, with median age of 5.3 months.

There was no significant difference between the case control group with other four survey

groups in the age and gender comparison (P>0.05).

All patients surveyed in this study, were with body check, including liver and kidney function testing, CT examination and Electrocardiography (ECG). That was to exclude infectious diseases and other congenital diseases.

By Related epidemiological investigation, all patients were not exposure with metal, Glucocorticoids or Sedative.

All study participants provided written informed consent. The study was according to the declaration of Helsinki and world health organization guidelines to implement. It was under the supervision of the Ethics Committee of the Guangxi Medical University.

Detection of trace elements

It collected 2-3 mL Fasting venous blood, then separated the serum by centrifugal for each experimental group and the healthy control group. Saved that in -20°C for further detection.

The Standard Operating Procedures (SOP) for trace elements were according to Chinese National identification standard of trace elements. The substances of Standard concentrations were purchased from National standard test center. The blank control substance were used the Secondary deionized and Distilled water.

Emission spectrometer was inductively coupled plasma atomic, by which Equipment Type was (ICP-AES) Integra XL.

For the blank control testing, it measured for five times, and then calculated the average value. As for each four standard concentration, it was used the same measured and calculated method.

It could draw the standard curve line in Coordinate diagram by the each average value (in vertical axis) and each standard concentration (in Horizontal axis).

It measured every serum specimen using the same equipment, to get the average value for every patient by five times. Then it could calculate the trace element concentration in Coordinate diagram for each serum specimen.

Determination of vitamin content

It Vortex mixed the 200 µl serum with 200 µl Absolute ethanol, at radio as 1:1. Then it added 400 µl Hexane for Vortex mixing 3 min. The supernatant were separated after Centrifugation with 3000 rpm for 5 min. After Filtering by 0.45 µm membrane filter, it had already read for testing in High performance liquid chromatography (HPLC) (Cat.No. was HP1100).

The Analytical column was also purchased from Hewlett-Packard Company (USA), which Cat.No. Was Kromasil10025C18, with volume as 150×4.6 mm, 5 µm.

In The detection process, using Detection wavelength as 325 nm, it made Mobile phase for 98% methanol, with flow Velocity as 1.2 ml/min. The injection volume was 20 µl.

Desktops refrigerated centrifuge were purchased from Chinese Military Medical Sciences Experimental Instrument Factory (Cat.No. was TLL2C). The Standard Substance of Vitamin A was purchased from Sigma, USA. The Secondary Distilled water was used for the testing progress.

Detection of respiratory infection history by Immunological test IgM

Indirect immunoinfluscent assays (IFA) were used in the test. There were nine kinds of antibody for different pathogens, as: Legionella pneumophila, Mycoplasma pneumonia, Q fever Rickettsia, Chlamydia pneumonia, adenovirus, respiratory syncytial virus, influenza A virus, influenza B virus and The parainfluenza virus of Series from one to three. And the assays of the nine kinds of pathogens were integrated into one kit named nine Respiratory assay, which were purchased from AntuecoWise Biological Engineering Co., Ltd (Cat.No. S204887, Zhenzhou, China).

It operated the tests strictly were according to kit instructions.

Statistical analysis of data

All data are represented as means ± SD (x±s) of three or more independent experiments. The data are changed into normal distribution with logarithm if the original data are positive skewness distribution. Comparision among the experimental groups, and the correlations

Trace elements influenceed cleft clip and palate

Table 1. The results of the risk factors by univariate analysis between each group (n)

Risk factors	Compared items	Health control group	Unilateral cleft lip group	Bilateral cleft lip group	Cleft soft palate group	Complete cleft palate group	χ^2	p
Total people		38	72	77	63	56		
Family genetic	yes/no	5/33	38/34	59/18	56/7[a,b]	54/2[a,b]	25.088	<0.001
Living in the mining area	yes/no	8/30	40/32	51/26	53/10	52/4	3.087	0.543
Occupational distribution	Farmers/herders/Workers/Others	14/8/15/1	26/19/25/2	27/20/28/2	24/15/22/2	22/12/22/0	9.632	0.655
Detection of Adenovirus antibody	yes/no	6/29	15/57	16/61	14/49[a,b]	12/44[a,b]	33.652	<0.001
Detection of Respiratory syncytial virus antibody	yes/no	4/31	12/60	15/62	16/47	11/45	32.673	<0.001

Note: by Paired comparison; a: by comparing with control group, P<0.05; b: by comparing with Cleft palate group, P<0.05.

Table 2. Analyzed the difference about trace elements in each children serum ($\overline{X} \pm S$)

Trace elements	Health control group	Unilateral cleft lip group	Bilateral cleft lip group	Cleft soft palate group	Complete cleft palate group	F	p
Zinc (umol/L)	132.9±1.1	130.0±1.2	96.2±4.1	43.3±2.0	17.4±1.2	>1000	<0.001
Selenium (ug/L)	131.1±1.2	102.6±3.6	45.3±4.2	35.1±4.0	12.2±1.7	>1000	<0.001
Copper (umol/L)	9.3±4.0	18.9±4.2	18.2±4.5	18.8±3.6	52.9±44	573.467	<0.001
Cadmium (umol/L)	0.0038±0.0002	0.0056±0.0003	0.0056±0.0004	0.0044±0.0002	0.1832±0.0005	>1000	<0.001
Nickel (ug/L)	5.45±0.31	6.31±0.21	11.06±0.12	16.13±0.43	136.30±0.12	>1000	<0.001
Arsenic (ug/L)	1.10±0.45	5.18±0.12	5.80±0.38	5.11±0.25	16.38±0.17	>1000	<0.001
Cobalt (ug/L)	0.66±0.03	0.73±0.04	0.81±0.05	0.68±0.03	0.62±0.01	324.736	<0.001
Iron (mmol/L)	5.34±0.43	5.11±0.40	5.32±0.32	5.12±0.27	5.09±0.09	43.651	<0.001
Manganese (umol/L)	1.011±0.014	0.939±0.014	0.914±0.011	0.908±0.012	0.922±0.010	1.596	0.188
Vitamin A (µg/L)	478±32	863±45	869±39	1009±43	1023±30	>1000	<0.001

Note: Due to many groups to compared, values less than 0.01 were considered to be statistically significant, to reduce the I statistical risk.

Table 3. Studied correlation analysis of trace elements with adenovirus and respiratory syncytial virus infection (n)

Trace elements	Threshold value	Health control group	Antibody positive group for Adenovirus	Antibody positive group for Respiratory syncytial virus	χ^2	p
Zinc (umol/L)	≥70	38	7	2	167.448	<0.001
	<70	0	56	56		
Selenium (ug/L)	≥50	36	4	3	160.867	<0.001
	<50	2	59	55		
Copper (umol/L)	≥36	4	63	51	190.221	<0.001
	<36	34	3	7		
Vitamin A (µg/L)	≥500	8	53	49	181.335	<0.001
	<500	30	10	9		

between levels of each trace elements were analyzed. If the Data are homogenous, Analysis of variance, Student-Newman-Keulsa and Pearson's correlation will be used. If the data are not homogenous, Kruskal-Wallis, Games-Howell test, as well as spearman's correlation analysis will be used. All the analyses were carried out using the SPSS17.0 software (SPSS Inc, Chicago, IL, USA). Values less than 0.05 were considered to be statistically significant.

Results

Population distribution

After age matching for survey between four inborn cleft lip and palate groups with health control group, it studied the risk factors by univariate analysis. The results were shown in **Table 1**. It could be found that: The incidence of disease children concentrated on the Genetic Factors and the Mining Area children (P<0.05).

Furthermore, by Monitoring of the Respiratory pathogens about the inborn cleft lip and palate cases, the Adenoviruses and respiratory syncytial virus infection was an important risk factor (P<0.05).

However, respected the occupational distribution and advanced maternal age, there was no significant between them (P>0.05).

By pairwise comparison with health control group, in four disease groups, there was also conclusion made to.

After multivariate analysis, there were Statistical significance exist in genetic factors, residence factors, and history factors of respiratory infections for the inborn cleft lip and palate children.

The disease incidences were at high level in the population of farmers, herders and works.

After regression analysis, there was a significant correlation between the Adenoviruses and respiratory syncytial virus infection with severe degree of cleft lip and palate.

It only could find one kinds of virus infection in unilateral cleft lip group and bilateral cleft lip group. However, it could find two kinds of virus infection in cleft soft palate group and complete cleft palate group. And the trend was clearly and significant.

Analysis of trace elements concentration in serum

Each disease group and control group had been tested the trace elements concentration in serum. After calculating the average, Analysis of variance had been used to test the significant differences with average value. The changes of trace elements concentration in serum had significant differences (P<0.05).

The results of multiple comparisons had been shown in **Table 2**.

The copper content in serum had significantly increased in cleft soft palate group and complete cleft palate group, compared with control group (P<α). To further, the cadmium, nickel and arsenic content had be at significantly higher level in the complete cleft palate group (P<α).

As for the zinc content, it was at lower level in every disease group compared with the control group.

Selenium content was at considerable level between unilateral cleft lip groups with control group. However, other disease groups were at lower level.

More severe with Cleft lip and palate, more lower level of selenium in serum.

There was no statistically significant difference between groups in Iron, Cobalt and Manganese content, in the normal range level.

Analyzed the trace elements correlations with respiratory viruses infection

After IFA, it analyzed the positive rate of antibody of IgM for Respiratory viruses. It focused on three key trace elements which were zinc, Selenium and copper. Used Chi-square test, it indicated that the changes of the three key trace elements could be Synergistic effect with Adenoviruses and respiratory syncytial virus infection. That was shown in **Table 3**.

Discussion

Current research continues to investigate the extent to which folic acid can reduce the incidence of clefting. In analytical chemistry, a trace element is an element in a sample that has an average concentration of less than 100

parts per million measured in atomic count or less than 100 micrograms per gram. In biochemistry, a trace element is a dietary element that is needed in very minute quantities for the proper growth, development, and physiology of the organism. It had been Studied the Sources and Physiological Functions about trace elements [1, 3, 18].

Iron functions as a component of proteins and enzymes. Almost two-thirds of the iron in the body (approximately 2.5 grams of iron) is found in hemoglobin, the protein in red blood cells that carries oxygen to tissues, and about 15% is in the myoglobin of muscle tissue [21-23]. The average American diet provides 10-15 milligrams (mg) of iron daily in the form of heme and nonheme iron. Each day the body absorbs approximately 1-2 mg of iron to compensate for the 1 to 2 mg of iron that the body loses [10, 19, 20].

The currently recommended estimated average requirement (EAR) for selenium is 17 and 23 µg daily, respectively. These values are extrapolated from adult values by The National Academy of Sciences [24, 25].

In geochemistry, a trace element is a chemical element whose concentration is less than 1000 ppm or 0.1% of a rock's composition. The term is used mainly in igneous petrology. Trace elements will either prefer liquid or solid phase. If compatible with a mineral, it will prefer a solid phase (e.g., Ni compatible with Olivine). If it is incompatible with an element it will prefer a liquid phase. The measurement of this ratio is known as the partition coefficient. Trace elements can be substituted for network-forming captions in mineral structures. Minerals do not have to contain trace elements, i.e., they do not have to appear in the mineral's chemical formula. When practicing biodynamic farming it is important to utilize the trace elements of the soil, in order to give strength to the roots. Hydroponic practices however are decreasing the seed germination rate, causing an increase in pollution and waste [26-28].

Recently, the USA CDC reported on important findings from research studies about some factors that increase the chance of having a baby with cleft: Smoking and Diabetes [29, 30].

Iron deficiency with and without anemia; however, has been linked to negative effects on cognitive development among infants and adolescents. Iron overload is the accumulation of excess iron in body tissues, and it usually occurs as a result of a genetic predisposition to absorb iron in excess of normal. However, it can also be caused by excessive ingestion of iron supplements or multiple blood transfusions [31, 32].

By itself, selenium deficiency does not usually cause illness. Rather, it can make the body more susceptible to illnesses caused by other nutritional, biochemical, or infectious stresses. Three specific diseases have been associated with selenium deficiency. Cleft lip and palate disease occurs only in selenium-deficient children and is associated with heart disease. Lastly, myxedematous endemic cretinism, a condition that results in mental retardation, occurs in infants born to mothers deficient in both selenium and iodine [33].

Folic acid clearly reduces the risk of neural tube defects. Whereas early work showed that folic acid could reduce the risk of facial clefts in mouse models, epidemiologic studies of facial cleft shave provided inconsistent results. A recent meta-analysis of 17 prospective and case-control studies supported a protective effect of folic acid supplementation on cleft lip with or without cleft palate and perhaps cleft palate only. That had studied of the relationship between Cleft lip and palate children with their trace elements in serum [34, 35].

Disclosure of conflict of interest

None.

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References

[1] Loenarz C, Ge W, Coleman ML, Rose NR, Cooper CD, Klose RJ, Ratcliffe PJ, Schofield CJ. PHF8, a gene associated with cleft lip/palate and mental retardation, encodes for an N{varepsilon}-dimethyl lysine demethylase. Hum Mol Genet 2009; 19: 217-22.

[2] Margulis AV, Mitchell AA, Gilboa SM, Werler MM, Glynn RJ, Hernandez-Diaz S; National Birth Defects Prevention Study. Use of topira-

mate in pregnancy and risk of oral clefts. Am J Obstet Gynecol 2012; 207: 405, e1-7.

[3] Makhkamov EU, Murtazayev SM, Yuldashev AU. Early rehabilitation of children with cleft palate. Acta Chir Plast 1990; 32: 35-9.

[4] Sloan GM. Posterior pharyngeal flap and sphincter pharyngoplasty: the state of the art. Cleft Palate Craniofac 2000; 37: 112-22.

[5] Watkins SE, Meyer RE, Strauss RP, Aylsworth AS. Classification, epidemiology and genetics of orofacial clefts. Clin Plast Surg 2014; 41: 149-63.

[6] Eppley BL, van Aalst JA, Robey A, Havlik RJ, Sadove AM. The spectrum of orofacial clefting. Plast Reconstr Surg 2005; 115: 101e-114e.

[7] Pope AW, Snyder HT. Psychosocial adjustment in children and adolescents with a craniofacial anomaly: age and sex patterns. Cleft Palate Craniofac 2005; 42: 349-54.

[8] Cox TC. Taking it to the max: The genetic and developmental mechanisms coordinating midfacial morphogenesis and dysmorphology. Clin Genet 2004; 65: 163-176.

[9] Boyles AL, Wilcox AJ, Taylor JA, Shi M, Weinberg CR, Meyer K, Fredriksen A, Ueland PM, Johansen AM, Drevon CA, Jugessur A, Trung TN, Gjessing HK, Vollset SE, Murray JC, Christensen K, Lie RT. Oral facial clefts and gene polymorphisms in metabolism of folate/one-carbon and vitamin A: a pathway-wide association study. Genet Epidemiol 2009; 33: 247-55.

[10] Lawrence CW, Philips BJ. A telefluoroscopic study of lingual contacts made by persons with palatal defects. Cleft Palate J 1975; 12: 85-94.

[11] Alamoudi NM, Sabbagh HJ, Innes NP, El Derwi D, Hanno AZ, Al-Aama JY, Habiballah AH, Mossey PA. Prevalence and characteristics of non-syndromic orofacial clefts and the influence of consanguinity. J Clin Pediatr Dent 2014; 38: 241-6.

[12] Hou YX , Ren ZP, Li JF, Li XL, Ma SW, Cao HQ. [Presurgicalnasoalveolar molding in infants with cleft lip and palate: analysis of 29 cases]. Shanghai Kou Qiang Yi Xue 2011; 20: 641-4.

[13] Ettinger, Stephen J, Feldman, Edward C. Textbook of Veterinary Internal Medicine. 4th edition. W.B. Saunders Company; 2005.

[14] Parker SE, Mai CT, Canfield MA, Rickard R, Wang Y, Meyer RE, Anderson P, Mason CA, Collins JS, Kirby RS, Correa A; For the National Birth Defects Prevention Network. Updated national birth prevalence estimates for selected birth defects in the United States, 2004-2006. Birth Defects Res A Clin Mol Teratol 2010; 88: 1008-16.

[15] Little J, Cardy A, Munger RG. Tobacco smoking and oral clefts: a meta-analysis. Bull World Health Organ 2004; 82: 213-18.

[16] Cheng N, Zhang K, Song R. [Applied anatomy of arterial supply for the soft palate]. Zhonghua Zheng Xing Wai Ke ZaZhi 2000; 16: 208-11.

[17] Honein MA, Rasmussen SA, Reefhuis J, Romitti P, Lammer EJ, Sun L, Correa A. Maternal smoking, environmental tobacco smoke, and the risk of oral clefts. Epidemiology 2007; 18: 226-33.

[18] Yazdy MM, Autry AR, Honein MA, Frias JL. Use of special education services by children with orofacial clefts. Birth Defects Res A Clin Mol Teratol 2008; 82: 147-54.

[19] Parker SE, Mai CT, Canfield MA, Rickard R, Wang Y, Meyer RE, Anderson P, Mason CA, Collins JS, Kirby RS, Correa A; National Birth Defects Prevention Network. Updated National Birth Prevalence estimates for selected birth defects in the United States, 2004-2006. Birth Defects Res A Clin Mol Teratol 2010; 88: 1008-16.

[20] Correa A, Gilboa SM, Besser LM, Botto LD, Moore CA, Hobbs CA, Cleves MA, Riehle-Colarusso TJ, Waller DK, Reece EA. Diabetes mellitus and birth defects. Am J Obstet Gynecol 2008; 199: 237, e1-9.

[21] Werler MM, Ahrens KA, Bosco JL, Michell AA, Anderka MT, Gilboa SM, Holmes LB; National Birth Defects Prevention Study. Use of antiepileptic medications in pregnancy in relation to risks of birth defects. Ann Epidemiol 2011; 21: 842-50.

[22] Canfield MA, Collins JS, Botto LD, Williams LJ, Mai CT, Kirby RS, Pearson K, Devine O, Mulinare J; National Birth Defects Preventin Network. Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States: findings from a multi-state population-based study. Birth Defects Res A Clin Mol Teratol 2005; 73: 679-89.

[23] Boyles AL, Wilcox AJ, Taylor JA, Meyer K, Fredriksen A, Ueland PM, Drevon CA, Vollset SE, Lie RT. Folate and one-carbon metabolism gene polymorphisms and theirassociations with oral facial clefts. Am J Med Genet A 2008; 146A: 440-9.

[24] American Cleft Palate-Craniofacial Association. Parameters for evaluation and treatment of patients with cleft lip/palate or other craniofacial anomalies. Revised edition, Nov 2009. Chapel Hill, NC. P. 1-34.

[25] Boyles AL, Wilcox AJ, Taylor JA, Meyer K, Fredriksen A, Ueland PM, Drevon CA, Vollset SE, Lie RT. Folate and one-carbon metabolism gene polymorphisms andtheir associations with oral facial clefts. Am J Med Genet A 2008; 146: 440-449.

[26] Werler MM, Hayes C, Louik C, Shapiro S, Mitchell AA. Multivitamin supplementation and

risk of birth defects. Am J Epidemiol 1999; 150: 675-682.

[27] Wong WY, Eskes TK, Kuijpers-Jagtman AM, Spauwen PH, Steegers EA, Thomas CM, Hamel BC, BlomHJ, Steegers-Theunissen RP. Nonsyn0 dromic orofacial clefts: Association with maternal hyperhomocysteinemia. Teratology 1999; 60: 253-257.

[28] Weinberg CR, Wilcox AJ, Lie RT. A log-linear approach to case-parent-triad data: Assessing effects of disease genes that act either directly or through maternal effects and that may be subject to parental imprinting. Am J Hum Genet 1998; 62: 969-978.

[29] Van Rooij IA, Vermeij-Keers C, Kluijtmans LA, Ocke MC, Zielhuis GA, Goorhuis-Brouwer SM, van der Biezen JJ, Kuijpers-Jagtman AM, Steegers-Theunissen RP. Does the interaction between maternal folate intake and the methylenetetrahydrofolate reductase polymorphisms affect the risk of cleft lip with or without cleft palate? Am J Epidemiol 2003; 157: 583-591.

[30] Shaw GM, Carmichael SL, Laurent C, Rasmussen SA. Maternal nutrient intakes and risk of orofacial clefts. Epidemiology 2006; 17: 285-291.

[31] Shaw GM, Rozen R, Finnell RH, Todoroff K, Lammer EJ. Infant C677T mutation in MTHFR, maternal periconceptional vitamin use, and cleft lip. Am J Med Genet 1998; 80: 196-198.

[32] Relton CL, Wilding CS, Jonas PA, Lynch SA, Tawn EJ, Burn J. Genetic susceptibility to neural tube defect pregnancy varies with offspring phenotype. Clin Genet 2003; 64: 424-428.

[33] Pezzetti F, Martinelli M, Scapoli L, Carinci F, Palmieri A, Marchesini J, Carinci P, Caramelli E, Rullo R, Gombos F, Tognon M. Maternal MTHFR variant forms increase the risk in offspring of isolated nonsyndromic cleft lip with or without cleft palate. Hum Mutat 2004; 24: 104-105.

[34] Munger, RG. Maternal nutrition and oral clefts. In: Wyszynski, DF., editor. Cleft lip and palate: From origin to treatment. Oxford: Oxford University Press; 2002. pp. 170-192.

[35] Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. Lancet 1991; 338: 131-137.