Short Communication

Evaluating the role of maternal folic acid supplementation in modifying the effects of methylenetetrahydrofolate reductase (C677T and A1298C) gene polymorphisms in oral cleft children

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Background: We studied the role of maternal folic acid supplementation in modifying the effects of methylenetetrahydrofolate reductase (*MTHFR* C677T and A1298C) gene polymorphisms in Iranian children with oral clefts. **Materials and Methods:** Forty-seven newborn infants with orofacial cleft and their mothers were selected randomly. Mothers were matched regarding dietary folate intake. The genotyping on venous blood was carried out. Consistency between maternal and child genotypes was analyzed. **Results:** Genotype consistency was not statistically significant in both C677T and A1298C gene variants ($P > 0.05$). **Conclusion:** Maternal folic acid consumption may not have any significant effect on modifying C677T and A1298C polymorphisms in children.

Key words: Folic acid supplementation, methylenetetrahydrofolate reductase gene, orofacial clefts, polymorphism

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INTRODUCTION

Orofacial clefts are common malformations involving the maxillofacial structure. In general, Asian or Amerindian populations have the highest birth prevalence, often as high as 1/500.[1]

Multiple genes are associated with nonsyndromic orofacial clefts.[2] The role of folate deficiency as a risk factor for orofacial clefts is important. Genes involved in the folic acid metabolism could also be associated with the risk of development of oral clefts. Since embryonic tissues require a high amount of DNA production and folic acid is needed for DNA synthesis, any event which reduces the supply of folic acid can theoretically result in orofacial clefts.[3]

Methylenetetrahydrofolate reductase (*MTHFR*) located on the short arm of chromosome 1 is one of these genes. Two of the *MTHFR* polymorphic variants C677T (locus; rs1801133) and A1298C (locus; rs1801131) are the most important ones in patients with cleft lip and palate.[4]

Low folate concentrations in association with a reduced MTHFR enzyme activity result in an increase in the homocysteine levels and a decrease in plasma methionine which could be related to DNA-methyltransferase inhibition and DNA hypomethylation.[5]

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Folic acid supplements during early pregnancy may reduce the risk of oral clefts by about 33%[6] though controversy exists.

A study in Brazil suggested that high-dose (4 mg) folic acid supplements taken by pregnant women could decrease their chance of having the second affected child with orofacial clefts significantly.[7]

The aim of this study was to evaluate the presence of genotype consistency of MTHFR (C677T and A1298C) between children with oral cleft and their mothers and the role of folic acid supplementation on modifying the gene expression.

MATERIALS AND METHODS

In this case–control study, we included 47 patients with nonsyndromic cleft palate and their mother from Mofid Hospital in Tehran, Iran, in 2012–2013. Ethical approval and informed consent were obtained.

Mothers were asked to complete a questionnaire about history of folate intake during periconceptional period (from 3 months prior to 1 month after conception). A food frequency questionnaire was used to select mothers who were matched regarding dietary folate. Questions consisted of dosage, duration, and frequency of supplementary folate intake.[8] Peripheral venous blood was taken for DNA extraction.

Genotyping for C677T and A1298C gene mutations was performed by enzymatic restriction digestion of polymerase chain reaction (PCR) products with HinfI and MboII (New England Biolabs Inc., CA, USA), respectively.

For screening 677C-T and 1298A-C variants in the *MTHFR* gene, exon 4 and 7 of the gene were amplified by PCR with the use of modified primers (4F: 5′-TCTTCATCCCTCGCCTTGAAC-3′; 4R: 5′-AGGACGGTGCGGTGAGAGTG-3′) and (7F: 5′ CTTCTACCTGAAGAGCAAGTC 3′ 7R: 5′-CATGTCCACAGCATGGAG-3′), respectively. DNA fragments were separated and visualized by electrophoresis using 8% polyacrylamide gels. The wild product of MboII enzyme for A1298C genotype included 176 bp while PCR products for C677T genotype were 198 bp.

Statistical analysis

Data were analyzed by SPSS 11.5 (SPSS Inc., Chicago, USA). Fisher's exact test and Chi-square test were used for analysis. P <0.05 was assumed as statistically significant.

RESULTS

Forty-four (23 females and 21 males) and 47 (25 females and 22 males) newborn samples were available for C677T and A1298C analysis, respectively.

In C677T and A1298C groups, 29 and 26 mothers reported the history of using folic acid supplementation during pregnancy, respectively. Allelic and genotypic frequency in children and their mothers are shown in Table 1.

As it is demonstrated in Table 2, genotype consistency between mothers and children was not statistically significant for the C677T and A1298C variants in both

Table 1: Allelic and genotypic frequency of C677T and A1298C among cleft children and their mothers

Genotype/allele	Children (%)	Mothers (%)
CC	16 (34.0)	22 (46.8)
TT	17 (36.1)	14 (29.7)
CT	14 (29.7)	11 (23.4)
C	46	55
T	48	39
AA	15 (34.0)	18 (38.2)
CC	5 (10.6)	5 (10.6)
AC	24 (54.5)	24 (51.0)
A	54	60
C	34	34

Table 2: Genotype consistency of A1298C and C677T between orofacial patients and their mothers according to the history of acid folic supplementation

Genotype (mother)	Genotype (child)	Folic acid consumption, *n* (%)		*P*
		Yes	No	
AA	AA	5 (71.4)	8 (61.5)	>0.99
	AC	2 (28.6)	4 (30.8)	
	CC	0 (0)	1 (7.7)	
AC	AA	4 (36.4)	2 (16.7)	0.49
	AC	7 (63.6)	9 (75)	
	CC	0 (0)	1 (8.3)	
CC (A1298C)	AC	2 (66.7)	0 (0)	>0.99
	CC	1 (33.3)	1 (100)	
CC (C677T)	CC	4 (66.7)	7 (58.3)	0.62
	CT	2 (33.3)	2 (16.7)	
	TT	0 (0)	3 (25)	
CT	CC	2 (33.3)	1 (12.5)	0.80
	CT	2 (33.3)	4 (50)	
	TT	2 (33.3)	3 (37.5)	
TT	CC	1 (33.3)	1 (11.1)	0.71
	CT	1 (33.3)	2 (22.2)	
	TT	1 (33.3)	6 (66.7)	

HWE *P* value for A1298C=0.347. This genotype is consistent with HWE. HWE *P* value for C677T=0.007. This genotype is not consistent with HWE. HWE = Hardy-Weinberg equilibrium

negative and positive history groups ($P > 0.05$). Thus, maternal history of folic acid consumption may not have any significant effect on modifying C677T and A1298C polymorphisms in children.

DISCUSSION

Orofacial cleft is a major public health concern in many countries. In the present study, we concluded that maternal folic acid supplementation did not have a major role in modifying the genotype of *MTHFR* gene.

MTHFR single nucleotide polymorphism (SNP) C677T was the first common *MTHFR* variant found and was shown to reduce enzyme activity in both heterozygous and homozygous mutated forms.[9] Another common variant A1298C reduces enzyme activity only in homozygous mutant CC form. The 677 heterozygous computed tomography/homozygous TT significantly reduces enzyme activity since SNP is in the catalytic domain of the enzyme. In addition, it has shown to cause hyperhomocysteinemia, especially if folate deficiency is also present. A1298C only reduces the enzyme activity less than C677T since SNP is located in the presumed regulatory domain of *MTHFR* and not associated with hyperhomocysteinemia unless concomitant *MTHFR* C677T variant is also present. There is a significant role of *MTHFR* SNP C677T in the causation of several major congenital malformations including orofacial clefts.[10]

Mills *et al*. evaluated the role of polymorphisms of different genes related to folate metabolism in cleft lip and palate. Results showed that no significant relationship exists between *MTHFR* C677T polymorphism and cleft lip and palate. However, the authors recommended additional investigations to prove the validity of their results.[11]

Another study has shown that maternal C677T is a protective genotype for orofacial clefts, and this protective effect was higher when mothers did not take folic acid supplements. Hence, there is a possibility that folate has no protective effect against orofacial clefts. However, the authors mentioned that more studies with improved power and sample sizes were needed.[12]

Semiç-Jusufagiç *et al*. reported that low dietary folate intake during the first trimester of pregnancy along with maternal TT genotype could have a damaging impact on the embryonic organogenesis.[13]

A recent meta-analysis performed by Zhao *et al*. revealed that 677T variant of MTHFR population. However, no significant relationship between MTHFR A1298C polymorphism and nonsyndromic cleft lip with or without cleft palate was found in this meta-analysis.[14]

According to the difficulty of collecting sufficient blood sample from newborn infants, we try to increase the sample size in the next phase.

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Conflicts of interest

There are no conflicts of interest.

AUTHORS' CONTRIBUTION

- AE coordinated the study and carried out the design and prepared the manuscript.
- NA prepared the manuscript and provided assistance in sample collection.
- HRKK coordinated all the experiments,
- KK coordinated the statistical analysis and participated in the manuscript preparation.
- MSZ participated in most of the experiments and manuscript preparation.

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