Letter to the Editor

The Association between Folate Pathway Genes and Cleft Lip With or Without Cleft Palate in a Chinese Population*

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NSCL/P is a common congenital defect and gene-environmental factors involve in this disorder. Periconceptional intake of folate may reduce the risk of NSCL/P. The present study investigated three SNPs (rs1801198, rs955516, and rs3733890) in three folate pathway genes, including *TCN2, MTR,* and *BHMT* among 481 patients and 558 healthy subjects. Rs955516 showed allelic association with NSCL/P. More patients carry rs955516 AA and rs3733890 AA genotypes. The gene-gene interaction test showed *trans*-phase combination effects for *MTR* and *BHMT* genes. Our study suggests that the interaction of *MTR* and *BHMT* genes play a vital role in the pathogenesis of NSCL/P in Chinese population.

Non-syndromic cleft lip with or without cleft palate (NSCL/P)(OMIM 119530) is one of the most common birth defects in humans and derived by the interaction between genetic and environmental factors[1]. In recent years, great efforts have been made to identify the genes involved in the susceptibility to NSCL/P and to disclose their relationship with specific environmental risk factors, to get information about the pathogenic mechanism leading to the malformation. Significant progress has been made recently due to advances in sequencing and genotyping technologies, primarily through the use of whole exome sequencing and genome-wide association studies[2-3]. Several loci including 8q contribute to NSCL/P etiology has begun to be identified but the majority remains unexplained.

Periconceptional vitamin supplementation with folic acid has been indicated as a preventative measure that reduces the risks of this oral facial cleft. Although the precise mechanisms have not been determined, the genetic variation in genes that alter cellular absorption, transport, and metabolism of folate/homocysteine may either confer or reduce susceptibility to the disease[4]. In this study, we focus on three genes belong to folate metabolism and methionine synthesis pathway including tanscobalamins II (TCN2), methionine synthase (MTR), and betaine-homocysteine methyltransferase (BHMT). Transcobalamin is a plasma protein which transports vitamin B12 to cells and therefore determines cellular availability. MTR, a vitamin B12 dependent enzyme, is essential for the remethylation of homocysteine to methionine. BHMT is a zinc dependent cytosolic enzyme that catalyses the conversion of betaine and homocysteine to dimethylglycine and methionine, respectively.

A cohort of 481 NSCL/P patients, including isolated cleft lip with or without palate, was identified from the Beijing Stomatological Hospital, Capital Medical University, Beijing and First People's Hospital of Jinzhong, Shanxi, China. Those patients whose mothers declared use of priconceptional drugs, or have other syndrome/malformation, were excluded from study. A total of 558 healthy samples were provided by the Cardiovascular Institute and Fuwai Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China. These control samples were obtained from workers on the Qingdao pier, Shandong province, and were absence of both other severe forms of physical diseases and a family history of any birth defects. All participants are of Han Chinese origin. Patients (300 men and 181 women) were aged between five months and 43 years, and control subjects (324 men and 234 women), between 18 and 49 years. This study was approved by the Ethics Committee of Beijing Stomatological Hospital and Fuwai Hospital. Written informed consent had been obtained from study participants or their guardians.

Genomic DNA was extracted from peripheral

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blood leukocytes using a genomic DNA extraction kit (Relaxgene Blood DNA kit, Tiangen, Beijing, China). Three single nucleotide polymorphisms (SNPs) (rs1801198, rs955516, and rs3733890) present in the *TCN2*, *MTR*, and *BHMT* genes respectively were selected based on their presence in the HapMap database for the Han Chinese in Beijing (CHB) population (www.hapmap.org) and their minor allele frequency (MAF). Rs1801198 and rs3733890 are functional SNPs, while rs955516 is intronic. The SNPs were genotyped by MALDI-TOF MS using the Mass ARRAY system (Sequenom, San Diego, CA, USA) and analyzed with MassARRAY Typer software by BGI Company.

The genotypic distributions of the three SNPs showed no deviation from Hardy-Weinberg Equilibrium by performance of Haploview program (Version 4.0). Rs955516 in *MTR* gene showed allelic association with NSCL/P (χ^2=11.200, *P*=0.0008) (Table 1). Rs955516 AA genotype and rs3733890 AA genotype increase the risk of NSCL/P with OR of 1.84 and 1.65 (Table 2). Due to the different chromosomes which three genes located in, genotype model was selected to test for *trans*-phase interaction between any two of three genes. The gene-gene interaction test showed evidence of *trans*-phase interaction for the *MTR* and *BHMT* gene combinations (χ^2=20.320, df=6, *P*=0.0024) (Table 3).

Table 1. Allelic Frequencies in Patients and Controls

Gene	SNPs	Allele	Controls (Percentage)	CL/P cases (Percentage)	χ^2	*P*
TCN2	rs1801198	G	541 (58.7)	491 (57.2)		
		C	381 (41.3)	367 (42.8)	0.383	0.5355
MTR	rs955516	T	663 (59.4)	485 (52.0)		
		A	453 (40.6)	447 (48.0)	11.200	0.0008*
BHMT	rs3733890	G	775 (70.0)	640 (66.5)		
		A	333 (30.0)	322 (33.5)	2.778	0.0956

Note. *P<0.001.

Table 2. Genotypic Association between Three SNPs and Risks for CL/P

Gene	SNPs	Allele	Controls (Percentage)	CL/P cases (Percentage)	Odds Ratio	95% CI[a]
TCN2	rs1801198	GG	155 (33.6)	138 (32.2)		
		GC	231 (50.1)	215 (50.1)	1.05	0.78-1.40
		CC	75 (16.3)	76 (17.7)	1.14	0.77-1.69
MTR	rs955516	TT	195 (35.0)	126 (27.0)		
		TA	273 (48.9)	233 (50.0)	1.32	0.99-1.76
		AA	90 (16.1)	107 (23.0)	1.84	1.29-2.63
BHMT	rs3733890	GG	265 (47.8)	219 (45.5)		
		GA	245 (44.2)	202 (42.0)	1.00	0.77-1.29
		AA	44 (8.0)	60 (12.5)	1.65	1.08-2.53

Note. [a]CI, confidence interval.

Table 3. Analysis of the Trans-phase Interactions between Any Two of Three Genes

Gene-Gene	χ^2	df	*P*
TCN2-MTR	3.270	6	0.7743
MTR-BHMT	20.320	6	0.0024*
TCN2-BHMT	5.770	6	0.4495

Note. *P<0.05.

Although Martinelli reported that more frequent C allele of rs1801198 in *TCN2* gene were significantly over transmitted to affected individuals with NSCL/P in Italian case-parent triad study[5], no association was found between this functional SNP and NSCL/P in our study. This inconsistent may result from either the unconformity of two population or the different features of SNP. According to 1000 genomes database, minor allele of rs1801198 is C in Han Chinese population and its frequency is 0.4227, however, minor allele of this SNP is G and its frequency is 0.3878 for Italian. It seems that the rs1801198 provides protective effect on Italian NSCL/P, but deleterious for Chinese population.

We selected rs955516 to investigate because it had been reported to be associated with two other congenital defects, conotruncal heart defects and spina bifida[6], though this SNP is intronic. Our findings showed the strong relationship between SNP rs955516 in *MTR* gene and NSCL/P based on either allelic or genotypic analysis. This evidence suggests that the potential variation of *MTR* gene, which linked with rs955516, function the pathogenesis of NSCL/P in Chinese population. Very recent study from Chinese scientists also provided consistent clues with ours and revealed that haplotypes of two other SNPs in *MTR* are more perilous for NSCL/P[7].

In our study, the proportion of rs3733890 AA genotype in *BHMT* gene is higher in patients with NSCL/P compared to healthy controls and it suggests that this exonic SNP may raise the risk of NSCL/P in China. It is also reported that homozygotes of rs3733890 have a low risk of NSCL/P where there has been maternal supplementation with >400 μg folate before or during conception[8]. Moreover, our previous study provide evidence that *BHMT* gene may confer genetic risk of NSCL/P in a recessive manner[9]. Then take into account the characteristic of NSCL/P being a complex disease, we tested interaction between any two of three genes for *trans*-phase under genotype model and found that *MTR* gene had a combined effect with *BHMT* gene on NSCL/P. Under normal metabolic circumstances, about 50% of the homocysteine formed is remethylated to methionine. Given that *MTR* and *BHMT* genes are important players in the homocysteine metabolism and methionine synthesis, dysfunction of their expression may lead to hyperhomocysteine. Maternal hyperhomocysteinemia has been implicated as a risk factor for some congenital anomalies such as NTDs, heart defects, and neruocristopathies. It estimates that there is a possible correlation between hyperhomocysteine and these congenital disorders through the involvement of abnormal DNA methylation, cellular proliferation, apoptosis, and migration during embryogenesis. The high plasma homocysteine levels have been observed in mothers after the birth of an infant with a clefting abnormality[10].

The limitation of our study is that only three variations in three genes present in folate pathway are studied. NSCL/P is complex disorder that is caused by the consequence of interactions of many genetic and environmental factors. Further studies should focus on screening of more specific genes related to folate metabolism and the precise mechanisms should be elucidated. That will shed light on the identification of at-risk mothers and the prevention of the disease based on a personalized approach.

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