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Original Article

Association between MTHFR gene polymorphism and NTDs in Chinese Han population

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Abstract: Objective: This study aims to investigate the single nucleotide polymorphisms (SNPs) of 5,10-methylenetetrahydrofolate reductase (MTHFR) gene and neural tube defects (NTDs) in Chinese population. Method: A total of 271 NTDs cases and 192 healthy controls were used in this study. Fifty-two selected single nucleotide polymorphism (SNP) sites in the MTHFR gene were analyzed with next-generation sequencing method. A series of statistical methods were carried out to investigate the correlation between the SNPs and the patient susceptibility to NTDs. Results: Statistical analysis showed a significant correlation between the SNP sites rs1801133 in MTHFR gene and NTDs. The GG genotype, G allele of rs1801133 in MTHFR significantly decreased the incidence of NTDs (OR = 0.449, 95% CI: 0.255-0.789 with genotype, and OR = 0.669, 95% CI: 0.508-0.881 with allele). Conclusions: The gene polymorphism loci rs1801133 in MTHFR gene maybe potential risk factors for NTD in Chinese population.

Keywords: Neural tube defects (NTDs), single nucleotide polymorphisms (SNPs), 5,10-methylenetetrahydrofolate reductase (MTHFR)

Introduction

Birth defects are the leading cause of infant mortality in developed countries and a major cause of health problems in surviving children. Neural tube defects (NTDs) are a common group of central nervous system anomalies affecting 0.5-2 per 1000 pregnancies worldwide [1, 2]. NTDs arise when the neural tube, the embryonic precursor of the brain and spinal cord, fails to close during neurulation. The high prevalence and traumatic consequences affected children and their families, however, the causes of NTD are poorly understood. It is found that the majority of NTDs appear to result from a combination of genetic and environmental factors [3, 4]. Maternal nutritional status is a key determinant of pregnancy outcome. Studies have been focused on folic acid, a water-soluble B vitamin which acting as a cofactor in one-carbon transfer reactions and plays a key role in DNA methylation, synthesis, and repair [5-7]. The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) plays an important role in the folate metabolism pathway and regulates the intracellular folate pool for synthesis and methylation of DNA folate metabolism [8, 9]. The MTHFR gene locates at chromosome 1p36.3 and is 2.2 kb in length with a total of 11 exons [10]. MTHFR mutations are commonly related with hyperhomocysteinemia. MTHFR also have been implicated as risk factors for NTDs and unexplained recurrent embryo losses in early pregnancy through defects in homocysteine metabolism and several single nucleotide polymorphisms in the MTHFR gene have been found to be associated with NTDs [11-13]. In this study, we explored the association between the SNPs of MTHFR genes and the risk of NTDs in Chinese Han population.

Materials and methods

Subjects

Stillborn neural tube defects subjects were collected from a region in Northern China with a neural tube defect prevalence of 199.38/10,000 based on the local epidemiologic surveillance data collected during January 2002 and Dece-

Table 1. Genotype and alleles for rs1801133 variants of MTHFR in control and NTD

	case	control					
	n = 269	n = 189					
MTHFR: rs1801133	n (%)	n (%)	p*	OR	95% CI	OR (logistic)	95% CI
AA	104 (38.7%)	52 (27.5%)	0.0156	1.6606	1.11-2.484	1	
AG	130 (48.3%)	98 (51.9%)		0.8684	0.599-1.26	0.663	0.434-1.013
GG	35 (13%)	39 (20.6%)		0.5753	0.349-0.949	0.449	0.255-0.789
A	338 (62.8%)	202 (53.4%)	0.0045	1.4725	1.127-1.924	1	
G	200 (37.2%)	176 (46.6%)		0.6791	0.52-0.888	0.669	0.508-0.881

mber 2004 [14]. The enrolled pregnant women were diagnosed by trained local clinicians using ultrasonography and then registered in a database. Fetuses aborted for nonmedical reasons were also obtained from the same areas for use as control participants. The surgical details were as previously described [15]. This study was approved by the Committee of Medical Ethic in the Capital Institute of Pediatrics (Beijing, China). Written informed consents were obtained from the parents on behalf of fetus.

Determination of folate

Folate was determined by using a competitive receptor binding immunoassay (Chemiluminescent Immunoenzyme Assay Access Immunoassay system II; Beckman Coulter, Krefeld, Germany). It was performed according to the manual of Access II kits. High folate ≥ 0.11 and low folate < 0.11.

DNA extraction and next generation sequencing

Genomic DNA was isolated from the individual human samples using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). It was performed following the manual of kits. DNA purity is tested using Invitrogen Qbit Spectrophotometer. Genomic DNA was shearing by CovarisTM system. Then sample preparation by following the manufacturer's standard procedure using Truseq DNA Sample preparation Kit (Illumina, Inc, San Diego, CA). We order the Agilent Custom enrichment array (Probe Code: BI426526171) and construct the library with Agilent Custom SureSelect Enrichment Kit. Custom capture oligos were designed using SureDesign website of Agilent technologies. The hybridization reactions were carried out on AB 2720 Thermal Cycler (Life Technologies Corporation, USA) with the following hybridization conditions: Incubate the hybridization mixture for 16 or 24 hours at 65°C with a heated lid at 105°C. Then capture and wash the hybridization mixture with magnetic beads (Invitrogen, USA) and SureSelect Target Enrichment Kit (Agilent technologies, Inc, USA) after the hybridization reactions.

The capture production was enriched with following cycling conditions: The cycling program was 98°C for 30 s; 10 cycles of 98°C for 10 s; 60ºC for 30 s; 72°C for 30 s; 72°C for 5 min. Twelve libraries were pooled, and then bridge amplification on cBot (Illumina, Inc, San Diego, CA) following the manufacturer's standard cluster generation protocols. After hybridization of sequencing primer, base incorporation was carried out on Genomic analyzer II Sequencer (Illumina, Inc, San Diego, CA) following the manufacturer's standard sequencing protocols, for 101 cycles of sequencing per read to generate paired-end reads including 100 bps at each end and 6 bps of the index tag.

Sequence analysis, variant calling and genotyping

For each individual, the sequences' alignment was performed using BWA software in hg19 database. They were sorted and PCR repeat was removed after Comparison. Realignment and recalibrate (GATK, Samtools pileup (MAPQ 15) and Varscan SNP and indel calling with loose standard (min-coverage = 1, min alternative allele reads = 1, min-var-freq > 0.03)) and then SNV calling (Samtools pileup (MAPQ 30) and Varscan SNP and indel calling with strict standard (min-coverage = 2 min alternative allele reads = 2, min-var-freq > 0.1), data for SNV library building). Data for following genotype calling were noted using ANNOVAR software. The sequence alignment against the reference genomic sequence in hg19 and the sin-

Table 2. Comparison of genotype and alleles for rs1801133 variants in control and NTD between high and low folate

	high folate (≥ 0.11)					low folate (< 0.11)				
	case	control				case	control			
MTHFR: rs1801133	n = 43 (%)	n = 103 (%)	P	OR	OR (logistic)	n = 69 (%)	n = 18 (%)	P	OR	OR (logistic)
AA	17 (39.5%)	27 (26.2%)	0.1846	1.8405 (0.867-3.9068)	1	23 (33.3%)	6 (33.3%)	0.7789	1 (0.3327-3.0054)	1
AG	17 (39.5%)	57 (55.3%)		0.5277 (0.2557-1.0887)	0.474 (0.21-1.068)	34 (49.3%)	5 (27.8%)		2.5257 (0.8124-7.8522)	1.774 (0.484-6.505)
GG	9 (20.9%)	19 (18.4%)		1.1703 (0.4818-2.8428)	0.752 (0.277-2.042)	12 (17.4%)	7 (38.9%)		0.3308 (0.1065-1.0281)	0.447 (0.123-1.632)
A	51 (59.3%)	111 (53.9%)	0.4715	1.2471 (0.7489-2.0769)	1	80 (58%)	17 (47.2%)	0.3331	1.5416 (0.7381-3.2197)	1
G	35 (40.7%)	95 (46.1%)		0.8019 (0.4815-1.3354)	0.797 (0.474-1.338)	58 (42%)	19 (52.8%)		0.6487 (0.3106-1.3548)	0.671 (0.33-1.367)

Table 3. Comparison of genotype and alleles for rs1801133 variants of MTHFR between males and females in case and control group

	Male					Female				
	case	control				case	control			
MTHFR: rs1801133	n = 111 (%)	n = 56 (%)	P	OR	OR (logistic)	n = 147 (%)	n = 71 (%)	P	OR	OR (logistic)
AA	50 (45%)	19 (33.9%)	0.1178	1.5962 (0.8187-3.1122)	1	50 (34%)	18 (25.4%)	0.3077	1.5178 (0.8049-2.862)	1
AG	48 (43.2%)	24 (42.9%)		1.0159 (0.5309-1.9439)	0.76 (0.37-1.562)	77 (52.4%)	39 (54.9%)		0.9026 (0.5111-1.5938)	0.711 (0.367-1.378)
GG	13 (11.7%)	13 (23.2%)		0.4388 (0.1879-1.0248)	0.38 (0.15-0.966)	20 (13.6%)	14 (19.7%)		0.6412 (0.3025-1.3588)	0.514 (0.215-1.227)
A	148 (66.7%)	62 (55.4%)	0.0575	1.6129 (1.0126-2.569)	1	177 (79.7%)	75 (67%)	0.1738	1.3515 (0.9024-2.0239)	1
G	74 (33.3%)	50 (44.6%)		0.62 (0.3893-0.9875)	0.639 (0.405-1.007)	117 (52.7%)	67 (59.8%)		0.7399 (0.4941-1.1081)	0.716 (0.467-1.099)

gle nucleotide variation was annotated in HGVs. Poor confidence 'variants' were excluded by visual inspection of sequence alignment and read coverage data.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Science (SPSS, version 16.0). All Statistical tests were 2-sided, and $p < 0.05$ was considered to be significant. Hardy-Weinberg equilibrium was assessed by Chi-square test, meanwhile Chi-square and Fisher's exact tested were executed to compare frequencies of pregnant week, gender and genotype/allele. Adjusted odds ratios with 95% confidence interval (CI) were performed by multiple logistic regression to estimate the risk of NTDs related to the polymorphism.

Results

52 selected single nucleotide polymorphism (SNP) sites in the MTHFR gene were analyzed in this study. The two groups contained both male and female. There did not appear to be any significant difference with respect to sex between the two study groups.

Associations were discovered between the rs1801133 polymorphism in the MTHFR gene and NTD (**Table 1**). For genotypes and alleles, The GG genotype, G allele of rs1801133 in MTHFR significantly decreased the incidence of NTDs (OR = 0.449, 95% CI: 0.255-0.789 with genotype, and OR = 0.669, 95% CI: 0.508-0.881 with allele).

When the data were analyzed on the basis of folate content, it showed that there was no significant difference between the case and control group (**Table 2**).

When the data were analyzed on the basis of gender, it showed that there was no significant difference between the case and control group in male and female. The results were shown in **Table 3**.

Discussion

The vertebrate neural tube serves as the precursor to the central nervous system (CNS): the brain and spinal cord. NTDs arise as a defect in embryonic development. The CNS normally develops as a flat sheet of cells that subsequently rolls up and fuses shut to form the hollow neural tube in embryogenesis. NTDs arise when this process of neural tube closure (NTC) is disrupted. A cluster of neurodevelopmental conditions associated with failure of neural tube closure during embryonic development are a multifactorial disorder which arising from a complex interaction of genetic and environmental factors [3]. Several studies have evaluated specific genetic NTDs risk factors and identified more than 100 genes. However, the mechanism of NTDs is still not fully understood [1, 3, 16]. We analyzed the association between MTHFR polymorphisms and risk of NTDs in this study. Statistical analysis showed that there was a significant correlation between the SNP sites rs1801133 in MTHFR gene and NTDs. The GG genotype, G allele of rs1801133 in MTHFR significantly decreased the incidence of NTDs.

The folate metabolism pathway plays an important role in DNA methylation, DNA synthesis, cell division, and tissue growth, especially in the rapidly developing cells [17]. Thus, a defective folate metabolism could result in an impaired DNA synthesis or DNA methylation involved in the neurulation process. Folate metabolism is complex and involves several regulatory mechanisms. Genetic variations affecting protein function at any step may alter the balance of metabolites and gene-gene interactions [18]. MTHFR is an important enzyme in the folate metabolism pathway. The combination of MTHFR and cystathione-b-synthase (CBS) mutations was reported to have a fivefold increase in the risk for spina bifida compared with each variant alone [19], indicating the presence of gene-gene interactions. Other previous studies had found that the MTHFR 677 TT genotype in infants and mothers has been associated with increased risk for NTDs [20], studies in Irish populations found significantly more individuals with NTDs with the TT and CT genotypes and more parents of affected individuals with the TT genotype [21, 22]. The MTHFR 1298A > C variant also results in reduced enzyme activity although its role in NTD risk is less clear [20, 23].

It was reported that the combination of MTHFR mutations and low folate concentrations could lead to a hypomethylation of homocysteine to methionine, enhancing the impairment of folate

metabolism and increasing the risk for NTDs [24], suggesting a strong genetic-nutritional interaction. However, in this study when the data were analyzed on the basis of folate content, it showed that there was no significant difference between the case and control group. There were no statistically significant differences of the distribution of genotype and allele between the case and control groups when they were stratified by gender in this study. This may be due to small sample size.

In conclusion, the gene polymorphism loci rs1801133 in MTHFR gene maybe potential risk factors for NTDs in Chinese population.

Disclosure of conflict of interest

None.

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References

[1] Greene ND, Stanier P and Copp AJ. Genetics of human neural tube defects. Hum Mol Genet 2009; 18: 113-129.

[2] Yaliwal LV and Desai RM. Methylenetetrahydrofolate reductase mutations, a genetic cause for familial recurrent neural tube defects. Indian J Hum Genet 2012; 18: 122-124.

[3] Wallingford JB, Niswander LA, Shaw GM and Finnell RH. The continuing challenge of understanding, preventing, and treating neural tube defects. Science 2013; 339: 1222002.

[4] Detrait ER, George TM, Etchevers HC, Gilbert JR, Vekemans M and Speer MC. Human neural tube defects: developmental biology, epidemiology, and genetics. Neurotoxicol Teratol 2005; 27: 515-524.

[5] Hosseini M, Houshmand M and Ebrahimi A. MTHFR polymorphisms and breast cancer risk. Arch Med Sci 2011; 7: 134-137.

[6] Zeisel SH. Importance of methyl donors during reproduction. Am J Clin Nutr 2009; 89: 673S-677S.

[7] Yan L, Zhao L, Long Y, Zou P, Ji G, Gu A and Zhao P. Association of the maternal MTHFR C677T polymorphism with susceptibility to neural tube defects in offsprings: evidence from 25 case-control studies. PLoS One 2012; 7: e41689.

[8] Das PM and Singal R. DNA methylation and cancer. J Clin Oncol 2004; 22: 4632-4642.

[9] Ueland PM, Hustad S, Schneede J, Refsum H and Vollset SE. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci 2001; 22: 195-201.

[10] Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG and Rozen R. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat Genet 1994; 7: 195-200.

[11] Sameer AS, Shah ZA, Nissar S, Mudassar S and Siddiqi MA. Risk of colorectal cancer associated with the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in the Kashmiri population. Genet Mol Res 2011; 10: 1200-1210.

[12] Cadenas-Benitez NM, Yanes-Sosa F, Gonzalez-Meneses A, Cerrillos L, Acosta D, Praena-Fernandez JM, Neth O, Gomez de Terreros I and Ybot-González P. Association of neural tube defects in children of mothers with MTHFR 677TT genotype and abnormal carbohydrate metabolism risk: a case-control study. Genet Mol Res 2014; 13: 2200-2207.

[13] Liu J, Qi J, Yu X, Zhu J, Zhang L, Ning Q and Luo X. Investigations of single nucleotide polymorphisms in folate pathway genes in Chinese families with neural tube defects. J Neurol Sci 2014; 337: 61-66.

[14] Gu X, Lin L, Zheng X, Zhang T, Song X, Wang J, Li X, Li P, Chen G, Wu J, Wu L and Liu J. High prevalence of NTDs in Shanxi Province: a combined epidemiological approach. Birth Defects Res A Clin Mol Teratol 2007; 79: 702-707.

[15] Zhang Q, Xue P, Li H, Bao Y, Wu L, Chang S, Niu B, Yang F and Zhang T. Histone modification mapping in human brain reveals aberrant expression of histone H3 lysine 79 dimethylation in neural tube defects. Neurobiol Dis 2013; 54: 404-413.

[16] Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, Mulinare J, Zhao P, Wong LY, Gindler J, Hong SX and Correa A. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med 1999; 341: 1485-1490.

[17] Morrison K, Papapetrou C, Hol FA, Mariman EC, Lynch SA, Burn J and Edwards YH. Susceptibility to spina bifida; an association study of five candidate genes. Ann Hum Genet 1998; 62: 379-396.

[18] Robien K and Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. Am J Epidemiol 2003; 157: 571-582.

[19] Botto LD and Mastroiacovo P. Exploring gene-gene interactions in the etiology of neural tube defects. Clin Genet 1998; 53: 456-459.

[20] Botto LD and Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol 2000; 151: 862-877.
[21] Carter TC, Pangilinan F, Troendle JF, Molloy AM, VanderMeer J, Mitchell A, Kirke PN, Conley MR, Shane B, Scott JM, Brody LC and Mills JL. Evaluation of 64 candidate single nucleotide polymorphisms as risk factors for neural tube defects in a large Irish study population. Am J Med Genet A 2011; 155A: 14-21.
[22] Kirke PN, Mills JL, Molloy AM, Brody LC, O'Leary VB, Daly L, Murray S, Conley M, Mayne PD, Smith O and Scott JM. Impact of the MTHFR C677T polymorphism on risk of neural tube defects: case-control study. BMJ 2004; 328: 1535-1536.
[23] Wang XW, Luo YL, Wang W, Zhang Y, Chen Q and Cheng YL. Association between MTHFR A1298C polymorphism and neural tube defect susceptibility: a metaanalysis. Am J Obstet Gynecol 2012; 206: 251.e1-7.
[24] van der Put NM, van Straaten HW, Trijbels FJ and Blom HJ. Folate, homocysteine and neural tube defects: an overview. Exp Biol Med (Maywood) 2001; 226: 243-270.