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Association between *MTHFD1* polymorphisms and neural tube defect susceptibility

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ABSTRACT

Objectives: Neural tube defect (NTD) is a common disease among neonates with multiplex symptom and complex origins, and the exact mechanism of NTD has not been definitely elucidated. Nevertheless, it is hypothesized that NTD risk can be prevented by periconceptional folic acid in folate metabolism. The methylenetetrahydrofolate dehydrogenase (*MTHFD1*) gene has been proved to play an important role in folate metabolism, which was strongly associated with the high risk for NTD. We focused on three folate metabolism-related single-nucleotide polymorphisms (SNPs) on the *MTHFD1* gene to evaluate the associations between *MTHFD1* polymorphisms and NTD susceptibility.

Methods: We genotyped blood samples from 222 specimens (including 122 NTD-affected infants and 100 healthy controls) in a case–control study. We investigated the association between NTD and three selected tag-SNPs on *MTHFD1* gene: 401A>G (rs1950902), 2305C>T (rs17857382) and 1958G>A (rs2236225) by the SNapShot method. These SNPs were identified by Haploview 4.2 software with HapMap databases, and then these associations were evaluated by the Mann–Whitney test, one-way analysis of variance (ANOVA) and chi-square test. Furthermore, a meta-analysis of the association between *MTHFD1* 1958G>A and NTD risk was also performed.

Results: In our study, an increased risk of NTD was observed for 1958G>A of *MTHFD1* (AA vs. GG: OR = 2.63, 95% CI = 2.61–5.70; AA vs. GG + GA: OR = 2.10, 95% CI = 1.07–4.14; A vs. G: OR = 1.62, 95% CI = 1.11–2.36). However, the other two SNPs (401A>G and 2305C>T) displayed no statistically significant association with NTD risk. The overall result of the meta-analysis indicated that the 1958G>A variant might not be a genetic susceptible factor for the Caucasian population.

Conclusions: Our analysis implicated that *MTHFD1* 1958G>A was significantly associated with the susceptibility of NTD in a Chinese population. In addition, the AA homozygote carriers were more likely to suffer NTD, compared with the others with GA or GG genotypes. Validation of the risk effect and functional impact of this polymorphism is needed in future investigations.

1. Introduction

Neural tube defect (NTD) is among the most common and deadly human congenital malformations at birth and manifested as a wide range of phenotypes, primarily including anencephaly, spina bifida and encephalocele which result from failure of the neural tube to close properly in the developing brain or lower spine [1,2]. The incidence rate of NTD is about 0.2% worldwide, but this rate varies in different races and sections with a conspicuous diversity which may be a result of genetic diversity [3]. Some previous studies demonstrated that the etiology of NTD is implicated with several factors, like gene–environment interactions and complex genetic factors [4–7]. These unclear factors would lie in the increased recurrence risk in siblings of individuals with NTD, the increased risk for NTD among other relatives, and the increased risk for birth defects overall among both close and distant relatives [8]. Recent studies also revealed that the NTD incidence and overall birth defect risk are higher in maternal relatives than in paternal relatives [9,10]. Though the exact mechanism of NTD has not been completely elucidated, it is hypothesized that genes coding folate-dependent enzymes might have an impact on NTD risk. Epidemiological studies have revealed that periconceptional vitamin supplementation with folic acid substantially lowers the percentage of women with NTD-affected pregnancies [11]. Thus, elucidation of the mechanisms by which folate reduces the risk of NTD, currently focused on candidate genes that are involved in folate-related pathways could be informative for developing improved prevention strategies and reducing the global burden of NTD [12].

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Methylenetetrahydrofolate dehydrogenase (*MTHFD1*) is a nicotinamide adenine dinucleotide phosphate (NADP)-dependent trifunctional cytoplasmic enzyme (often referred to as "C1-THF synthase"), acting as 10-formyl, 5,10-methenyl, and 5,10-methylene derivatives [13]. This folate-dependent enzyme has been proved to play an important role in folate metabolism, which is an attractive candidate gene associated with the high risk of NTD, but these associations remain controversial since several studies suggest no associations. Hence, in our study, some potential SNPs in the *MTHFD1* gene were identified from public databases, and through analysis we finally choose 401A>G, 2305C>T and 1958G>A as representative SNPs to investigate their associations with NTD risk. Considering the limitation of an individual study with a small sample size, a further meta-analysis with public data for the purpose of getting an exhaustive conclusion of empirical evidences to prove the association between *MTHFD1* 1958G>A and NTD risk was also performed. Elucidation of the interaction might contribute to ameliorate the diagnosis and prognosis of NTD.

2. Material and methods

2.1. Study population

Our study was conducted in strict accordance with the protocol approved by the Jining No. 1 People's Hospital, and a written informed consent must be provided by each participant (or their guardian). We recruited 222 specimens in total from Jining No. 1 People's Hospital. Among them, 122 subjects were confirmed as NTD patients; the other 100 subjects were healthy infants used as normal controls in this study. The neonates of NTD cases within our samples consisted of 62 females and 60 males, while the control group consisted of 47 females and 53 males as outlined in Table 2. We collected 5 ml venous blood sample from each participant after their guardians provided a written informed consent. All the samples and complete follow-up data were available for each specimen (more clinical characteristics are presented in Table 2).

Table 2
Comparison of neural tube defect patients and controls by selective characteristics.

Clinical characteristics	NTD patients (N = 122)	Normal controls (N = 100)	*P*-value
Sex			
Male	60 (49.2%)	53 (53.0%)	0.571
Female	62 (50.8%)	47 (47.0%)	
Maternal age			
≤20	22 (18.0%)	23 (23.0%)	0.469
20–30	92 (75.4%)	68 (68.0%)	
≥30	8 (6.6%)	9 (9.0%)	
Gestational weeks			
≤20	76 (62.3%)	59 (59.0%)	0.725
20–25	31 (25.4%)	25 (25.0%)	
≥25	15 (12.3%)	16 (16.0%)	
Use of folic acid supplements			
Yes	66 (54.1%)	61 (61.0%)	0.301
No	56 (45.9%)	39 (39.0%)	
Daily dietary intake of folates			
Q1	30 (24.6%)	26 (26.0%)	0.810
Q2–Q4	92 (75.4%)	74 (74.0%)	
Overall intake of folates			
Low[a]	16 (13.1%)	12 (12.0%)	0.803
High[b]	106 (86.9%)	88 (88.0%)	

NTD, neural tube defect.
[a] Women in the lowest quartile of maternal folate intake (Q1 ≤ 309.49 μg/100 g/day) who did not take supplemental folic acid in the periconceptional period.
[b] All other combinations of folate intake (Q2, Q3 or Q4) and folic acid supplementation (yes/no).

2.2. DNA methods

The whole genomic DNA was extracted from blood samples of each specimen using Clotspin Baskets per manufacturer's protocol (Qiagen, Valencia, CA). Quantification was conducted by spectrophotometry (260 nm) and all samples were normalized to 10 ng/μl. For PCR reactions, 2 μl extracted genomic DNA (40 μl) was used [8]. In this study, SNapShot assay was used to genotype DNA samples for *MTHFD1* 401A>G, 2305C>T and 1958G>A according to the manufacturer's instructions (Applied Biosystems). The primers to amplify different fragments containing each SNP as shown in Table 2 were designed using PSQ Assay Design Software Version 1.0.6. Briefly, the amplification program was performed using the 9800 Fast PCR System according to manufacturer's suggested protocol (all the assays were performed in a 20 μl reaction mix containing 0.5 μM each of forward and reverse primers and Applied Biosystems GeneAmp® Fast PCR Master Mix), at the start of 95 μC for 2 min, followed by 33 cycles of 94 μC for 20 s, 56 μC for 30 s and then 72 μC for 40 s [14]. After amplification, the pyrosequencing primer was used to extend the PCR product by adding nucleotides in a specific sequential order based on the known sequence [8] as described previously. Appropriate controls were included in all assays and at least 10% of samples were repeatedly genotyped with >99% agreement. We use the signal peaks' resulting pyrogram to determine the genotype. The primers and conditions of all assays are available upon request.

2.3. Statistical analysis

We processed the data with Microsoft Excel in the first place, and then all statistical analyses were performed using SPSS 20.0 software. To verify the data quality, we implement a chi-square goodness-of-fit test to find out whether the variants among subjects have any significant variation from the Hardy–Weinberg equilibrium (HWE). The associations were evaluated by the Mann–Whitney test, one-way analysis of variance (ANOVA) and chi-square test. Genotypes were analyzed using an additive model and dichotomized using a recessive model; both were in accordance with previous studies [15]. A meta-analysis of the association between the *MTHFD1* 1958G>A polymorphism and NTD risk was conducted using the pooled odds ratios (ORs) with their corresponding 95% confidence intervals (CIs) under five genetic models. A two-tailed *P* value less than 0.05 was considered to be statistically significant for all analyses.

Table 1
Primers of *MTHFD1* gene polymorphisms for PCR amplification.

SNP	Chr pos	Gen pos	Alias name	Primers for PCR amplification	Restriction enzyme
rs1950902	64415662	Exon 5	401A>G	F: 5′-CAATCCTCCCACCTCTGC-3′ R: 3′-GAATGGGTAGGTGTAGGA-5′	AluI
rs17857382	64449470	Exon 20	2305C>T	F: 5′-TTTATAGGACGGATACAGAGT-3′ R: 3′-TGTTCGTGGGTCGTCGAA-5′	HinfI
rs2236225	64442127	Exon 19	1958G>A	F: 5′-TAATCACGAGGATAAGAGCA-3′ R: 3′-CTTTGGTCCTGTGGCACT-5′	EcoRII

SNP, single-nucleotide polymorphism; F, forward; and R, reverse.