Correlation analysis of MTHFD1 gene polymorphisms and neural tube defects in Han population of Northern China

Yulian Fang
Tianjin Children's Hospital  https://orcid.org/0000-0003-0136-0078

Yan Liu
Tianjin Children's Hospital

Jian-Bo Shu
Tianjin Children's Hospital

Lin-Sheng Zhao
Tianjin Children's Hospital

Lu Wang
Tianjin Children's Hospital

Xue-Tao Wang
Tianjin Children's Hospital

Xiu-Fang Zhi
Tianjin Medical University

Jie Zheng
Tianjin Medical University

Ou-Yan Shi
Tianjin Medical University

Chun-Quan Cai (✉ cqcns6@126.com)
Children's Hospital of Tianjin University  https://orcid.org/0000-0002-8812-4096

Research

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Abstract

Background

Neural tube defects (NTDs) is a common birth defects worldwide. The methylenetetrahydrofolate dehydrogenase1 (MTHFD1) gene has been proved to play an important role in folate metabolism, which was strongly associated with the increased NTDs risk. The study is aimed to explore the correlation of single nucleotide polymorphisms (SNPs) in MTHFD1 gene with NTDs susceptibility.

Methods

A case-control study was conducted on children who included 152 NTDs patients and 169 healthy controls. Tag-SNPs were identified in HapMap database. Then, we investigated the association between NTDs and four selected tag-SNPs in MTHFD1 gene: rs1950902, rs2236225, rs2236224, rs11849530. We also performed a meta-analysis based on previous published studies to further evaluate the association.

Results

The results indicated that rs2236225 polymorphism displayed a significant association with NTDs risk (AA vs. GG: OR = 2.862, 95%CI = 1.022–8.015; GA + AA vs. GG: OR = 1.619, 95%CI = 1.040–2.520; A vs. G: OR = 1.500, 95%CI = 1.061–2.120). In addition, rs2236224 polymorphism was correlated with increased NTDs risk (TT vs. CC: OR = 2.559, 95%CI = 1.128–5.804; CT + TT vs. CC; OR = 1.631, 95%CI = 1.041–2.556; T vs C: OR = 1.489, 95%CI = 1.072–2.068). Further analysis showed the harmful effect of rs2236225 polymorphism was further supported by the result of meta-analysis. Meanwhile, haplotype analysis results showed that A-A and T-A haplotypes were correlated with increased NTDs risk, but C-A haplotype might decrease NTDs risk.

Conclusions

The results indicated that rs2236225 and rs2236224 polymorphisms of MTHFD1 gene were significantly associated with NTDs susceptibility in Han population of Northern China.

Background

Neural tube defects (NTDs) are congenital malformations of the brain and spinal cord that usually occur in early pregnancy (21–28 days)[1], mainly including spina bifida, anencephaly and encephalocele. As reported, the prevalence is roughly 0.5–2 per 1000 births worldwide, which is higher in China with about 2.74 per 1000 births [2–3]. Related studies had revealed that pathogenesis of NTDs was believed to mainly involve genetic and environmental factors [4–5]. Furthermore, epidemiological studies testified that maternal folic acid supplement can reduce the incidence by 50–70%, suggesting that variants in genes involved in folate metabolism pathway may contribute to NTDs risk [6]. Methylenetetrahydrofolate dehydrogenase1 (MTHFD1) plays an important role in folate metabolism by catalyzing the conversion of tetrahydrofolate to the
corresponding 10-formyl, 5,10-methenyl and 5,10-methylen derivatives [7]. The MTHFD1 gene is located at chromosome 14q24 and its cDNA contains an open reading frame of 2805 bp that encodes a protein of 935 amino acids [8]. Related studies had indicated that MTHFD1 polymorphisms might be associated with increased NTDs risk, but the association remains controversial since several studies suggest no association. So, the tag-SNPs of MTHFD1 gene were retrieved in our study, including rs1950902 (401A > G), rs2236225 (1958G > A), rs2236224 (2136 + 31G > A), rs11849530 (2458-2060A > G) and rs35020344 (41 + 239A > G) from the HapMap database based on related criteria. Then, we conducted a case-control study to assess the association between MTHFD1 polymorphisms and the risk of NTDs. Considering the reason for small sample size of an individual study, a further meta-analysis was performed so as to comprehensively evaluate the correlation.

Methods

Subjects

All subjects were collected from the Department of Neurosurgery of Tianjin Children's Hospital in China from November 2010 to May 2014. Among them, 152 subjects were diagnosed with NTDs based on clinical manifestations and images. The control group was composed of the other 169 subjects who had the same ethnic background with cases. All participants were from Chinese Han population in the North, Northwest, and Northeast of China. The study protocols were by the Ethical Committee of Tianjin Children's Hospital in China, and the guidelines of the 2000 Declaration of Helsinki and the Declaration of Istanbul 2008 were followed. Written informed consent was obtained from their guardians.

SNPs selection

In this study, the potential tag-SNPs of MTHFD1 were confirmed by Haploview4.2 software in HapMap database according to the following criteria: Han Population of Northern China; HW_pval (P value of Hardy-Weinberg equilibrium test) > 0.05; MAF (Minor Allele Frequency) ≥ 0.05. The tag-SNPs selected by Haploview could represent all the genetic variants of MTHFD1 gene.

DNA extraction and genotyping

Peripheral blood samples were collected from all participants in our study after obtaining their consent. The tubes used to collect peripheral blood contained EDTA as anticoagulation. Extraction of genomic DNA was carried out from blood samples using the Qiagen DNA Blood Mini Kit (Qiagen, UK). The collected DNA samples were stored at -80℃ before use. Five tag-SNPs of MTHFD1 gene were genotyped via the Sequenom-based Mass ARRAY assay. Then, the data of genotype and allele distributions were incorporated and analyzed by the Filemaker Pro Database, which is a cross-platform relational database application from FileMaker Inc [9].

Statistical analysis

All statistical analysis were performed by SPSS19.0 software. Statistical significance was accepted at $P \leq 0.05$. The t-test and Chi-square test were used to estimate the difference in clinical characteristics, genotype and allele distributions between cases and controls. Hardy-Weinberg equilibrium was assessed by Chi-square
in the control group in order to detect group representation. Then, meta-analysis of all relevant studies on the correlation between \textit{MTHFD1} G1958A polymorphism and NTDs risk was conducted by Stata 12.0 software. The pooled OR were calculated under five genetic model to comprehensively assess the correlation. In meta-analysis, heterogeneity among studies was analyzed by Chi-square test-based Q-statistic. The pooled OR was calculated by fixed effect model when \( P \) value was more than 0.1; otherwise, the random effects models was applied. Moreover, the online SHEsis software (\((\text{http://analysis2.bio-x.cn/myAnalysis.php})\) was used to analyzed the linkage disequilibrium (LD) and haplotype construction. \( D' \) and \( r^2 \) were calculated for LD analysis.

\textbf{Results}

\textbf{Study characteristic}

In our research, 321 subjects were recruited, including 152 NTDs patients (87 males and 65 females) and 169 healthy controls (90 males and 79 females). As shown in Table 1, the average age in NTDs cases and healthy controls were 2.36 ± 1.21 and 2.41 ± 1.30, respectively, but the difference did not reveal any statistical significance (\( P = 0.722 \)). Importantly, compared with healthy controls, NTDs cases appeared to have higher concentrations of homocysteine and SAH (\( P = 0.010 \) and \( P = 0.012 \)). Moreover, the level of SAM in NTDs cases was lower than healthy controls (\( P = 0.021 \)). In addition, we found a lower level of folate in NTDs cases compared with healthy controls, but the difference showed no statistical significance (\( P = 0.063 \)).

| \( \text{NTDs(N = 152)} \) | \( \text{Control(N = 169)} \) | \( P \) Value |
|--------------------------|--------------------------|----------------|
| Age(years)               | 2.36 ± 1.21              | 2.41 ± 1.30    | 0.722          |
| sex                      |                          |                |                |
| male                     | 87(57%)                  | 90(53%)        | 0.474          |
| female                   | 65(43%)                  | 79(47%)        |                |
| Folate(nmol/L)           | 8.8 ± 1.5                | 9.4 ± 1.8      | 0.063          |
| Homocysteine(\( \mu \)mol/L) | 9.5 ± 1.6               | 8.8 ± 1.6    | \textbf{0.010} |
| SAM(nmol/L)              | 51.1 ± 2.6               | 52.2 ± 2.7     | \textbf{0.021} |
| SAH(nmol/L)              | 10.5 ± 1.4               | 9.8 ± 1.6      | \textbf{0.012} |
| SAM/SAH                  | 4.9 ± 0.6                | 5.5 ± 0.9      | \textless 0.001 |

\textbf{Note}: SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

\textbf{Selection of tag-SNPs}

Five tag-SNPs were identified from HapMap database according to above criteria. The LD plot of five tag-SNPs was presented in Fig. 1. Because rs35020344 site did not conformed to Hardy-Weinberg equilibrium...
test, four tag-SNPs (rs1950902, rs2236225, rs2236224 and rs11849530) were selected by Haploview 4.0 to represent all SNPs in \textit{MTHFD1} gene. The SNPs of rs1950902 and rs2236225 were located in the exons, whereas the SNPs of rs2236224 and rs11849530 were situated in introns.

\begin{center}
\textbf{Association between haplotypes of \textit{MTHFD1} gene and the risk of NTDs}
\end{center}

The haplotype was constructed to analyze the effect of the LD on NTDs in Han population of Northern China. Our results indicated that there was a statistical significance in haplotype A-A, C-A and T-A between the two groups ($P<0.05$). The OR with 95\%CI of A-A and C-A haplotypes were 1.951(1.057-3.600) and 1.529(1.041–2.246), suggesting that they were harmful factors for NTDs. However, the OR with 95\%CI of T-A haplotype was 0.682(0.497–0.93), indicating it was a protective factor for NTDs (Table 2).

\begin{center}
\textbf{Table 2}
\end{center}

\begin{center}
\textbf{Haplotype analysis results}
\end{center}

| Haplotype | Cases (%) | Controls (%) | $t$ | $\chi^2$ value | $P$ values | OR values | 95\%CI |
|-----------|-----------|--------------|-----|---------------|------------|-----------|--------|
| block 1   |           |              |     |               |            |           |        |
| A-A*      | 15(9.7)   | 9(5.2)       | 4.710 | 0.0300       | 1.951      | 1.057-3.600 |        |
| A-G       | 23(15.1)  | 31(18.2)     | 1.042 | 0.3075       | 0.804      | 0.529-1.223 |        |
| G-A       | 34(22.8)  | 32(19.0)     | 1.334 | 0.2481       | 1.252      | 0.855-1.835 |        |
| G-G       | 79(52.4)  | 97(57.6)     | 1.726 | 0.1890       | 0.811      | 0.594-1.109 |        |
| block 2   |           |              |     |               |            |           |        |
| C-A*      | 57(37.5)  | 79(46.8)     | 5.682 | 0.0172       | 0.682      | 0.497-0.935 |        |
| C-G       | 36(24.0)  | 40(23.6)     | 0.015 | 0.9027       | 1.023      | 0.711-1.472 |        |
| T-A*      | 37(24.3)  | 29(17.4)     | 4.722 | 0.0298       | 1.529      | 1.041-2.246 |        |
| T-G       | 22(14.2)  | 21(12.2)     | 0.532 | 0.4659       | 1.186      | 0.750-1.874 |        |

\begin{center}
\textbf{Association between \textit{MTHFD1} polymorphisms and the risk of NTDs}
\end{center}

Table 3 showed the distribution of genotype and allele frequencies. We found a significant association between \textit{MTHFD1} rs2236225 and the risk of NTDs. In homozygous comparision (AA vs. GG), the frequency of AA genotype was higher in the case group than that of controls, and the difference was significant (OR = 2.862, 95\%CI: 1.022 ~ 8.015). Under the dominant model (GA + AA vs. GG), children carrying GA and AA genotypes had greater risk of NTDs compared with individuals with GG genotype (OR = 1.619, 95\%CI: 1.040 ~ 2.520). Under allele model (A vs. G), comparision of A allele frequency revealed a statistically significant difference between the two groups (OR = 1.500, 95\%CI: 1.061~2.120).
| Genotypes | Cases (N = 152) | Controls(N = 169) | OR(95%CI)       | P value |
|-----------|----------------|------------------|----------------|---------|
| rs1950902(401A > G) | | | | |
| AA       | 12             | 10               | Ref            |         |
| AG       | 52             | 59               | 0.734(0.293–1.840) | 0.509   |
| GG       | 88             | 100              | 0.733(0.302–1.780) | 0.492   |
| AG + GG  | 140            | 159              | 0.734(0.308–1.750) | 0.484   |
| A allele | 76             | 79               | Ref            |         |
| G allele | 228            | 259              | 0.915(0.637–1.314) | 0.630   |
| rs2236225(1958G > A) | | | | |
| GG       | 65             | 93               | Ref            |         |
| GA       | 74             | 70               | 1.513(0.959–2.384) | 0.074   |
| AA       | 12             | 6                | 2.862(1.022–8.015) | 0.039   |
| GA + AA  | 86             | 76               | 1.619(1.040–2.520) | 0.032   |
| G allele | 204            | 256              | Ref            |         |
| A allele | 98             | 82               | 1.500(1.061–2.120) | 0.021   |
| rs2236224(2136 + 31G > A) | | | | |
| CC       | 54             | 80               | Ref            |         |
| CT       | 79             | 78               | 1.500(0.942–2.391) | 0.087   |
| TT       | 19             | 11               | 2.559(1.128–5.804) | 0.022   |
| CT + TT  | 98             | 89               | 1.631(1.041–2.556) | 0.032   |
| C allele | 187            | 238              | Ref            |         |
| T allele | 117            | 100              | 1.489(1.072–2.068) | 0.017   |
| rs11849530(2458-2060A > G) | | | | |
| AA       | 54             | 66               | Ref            |         |
| AG       | 80             | 85               | 1.150(0.718–1.844) | 0.561   |
| GG       | 18             | 18               | 1.222(0.580–2.577) | 0.598   |
| AG + GG  | 98             | 103              | 1.163(0.739–1.831) | 0.514   |
| A allele | 188            | 217              | Ref            |         |
| G allele | 116            | 121              | 1.107(0.803–1.525) | 0.536   |
Moreover, *MTHFD1* rs2236224 polymorphism was associated with increased risk of NTDs. In homozygous comparison (TT vs. CC), children having TT genotype showed an increased risk of NTDs compared with individuals with CC genotype (OR = 2.559, 95%CI: 1.128 ~ 5.804). Under the dominant model (CT + TT vs. CC), children who had CT and TT genotypes were likely to affect NTDs compared with healthy controls (OR = 1.631, 95%CI: 1.041 ~ 2.556). Under allele model (T vs. C), T allele carriers were shown to be correlated with increased risk of NTDs compared with carrier with C allele (OR = 1.489, 95%CI: 1.072 ~ 2.068). Besides, rs1950902 and rs11849530 polymorphisms were not associated with the susceptibility of NTDs (all P > 0.05).

**Meta-analysis of the correlation between MTHFD1 1958G > A polymorphism and NTDs risk**

Due to the limitation of sample size, we conducted a meta-analysis of association between *MTHFD1* 1958G > A polymorphism and the risk of NTDs based on previous studies. A total of seven case-control studies were identified according to the inclusion criterion [10–16]. The characteristics of selected studies are summarized in Table 4. As shown in Table 5, *MTHFD1* 1958G > A polymorphism was significantly associated with increased the risk of NTDs (AA vs. GG: OR = 1.665, 95%CI = 1.195 ~ 2.318; GA vs. GG: OR = 1.326, 95%CI = 1.109 ~ 1.585; GA + AA vs. GG: OR = 1.414, 95%CI = 1.194 ~ 1.674; AA vs. GA + GG: OR = 1.409, 95%CI = 1.048 ~ 1.896; A vs. G: OR = 1.307, 95%CI = 1.113 ~ 1.534).
| First author | Year | Country | Ethnicity | Group | Genotype distributions | Allele frequencies | $P_{\text{HWE}}$ for control |
|--------------|------|---------|-----------|-------|------------------------|-------------------|---------------------------|
|              |      |         |           |       | GG AG AA G A          |                   |                           |
| Hol [10]     | 1998 | Netherland | Caucasian | case  | 32 55 16 119 87       |                   |                           |
|              |      |          |           | control | 100 172 63 372 298 | 0.469             |                           |
| De Marco [11] | 2006 | Italy | Caucasian | case  | 25 74 43 124 160     |                   |                           |
|              |      |          |           | control | 143 251 129 537 509 | 0.367             |                           |
| van der Linden [12] | 2007 | Netherland | Caucasian | case  | 31 58 14 120 86      |                   |                           |
|              |      |          |           | control | 71 98 34 240 166 | 0.985             |                           |
| Doudney [13] | 2009 | England | Caucasian | case  | 95 133 58 323 249    |                   |                           |
|              |      |          |           | control | 71 82 33 224 148 | 0.276             |                           |
| Marini [14]  | 2011 | America | Caucasian | case  | 50 86 36 186 158     |                   |                           |
|              |      |          |           | control | 49 69 20 167 109 | 0.587             |                           |
| Prasoon [15] | 2016 | India | Asian     | case  | 20 44 56 84 156      |                   |                           |
|              |      |          |           | control | 50 83 47 183 177 | 0.298             |                           |
| Meng [16]    | 2015 | China | Asian     | case  | 31 58 33 120 124     |                   |                           |
|              |      |          |           | control | 37 48 15 122 78 | 0.930             |                           |
| Current study | 2017 | China | Asian     | case  | 65 74 12 204 98      |                   |                           |
|              |      |          |           | control | 93 70 6 256 82 | 0.098             |                           |
Table 5
Meta-analysis results of association between the MTHFD1 1958G > A polymorphism and NTDs risk

| Genetic models       | No. of studies | Heterogeneity test | Statistical model | Meta-analysis results |
|----------------------|----------------|-------------------|-------------------|-----------------------|
|                      |                | \(P\) value | \(I^2\) (%) |                     | OR(95%CI) | \(P\) value |
| Co-dominant model    |                |             |               |                       |           |             |
| AA vs. GG            | 8              | 0.065      | 47.4          | Random model          | 1.665(1.195 ~ 2.318) | 0.003       |
| GA vs. GG            | 8              | 0.902      | 0.0           | Fix model             | 1.326(1.109 ~ 1.585) | 0.002       |
| Dominant model       |                |             |               |                       |           |             |
| GA + AA vs. GG       | 8              | 0.520      | 0.0           | Fix model             | 1.414(1.194 ~ 1.674) | 0.000       |
| Recessive model      |                |             |               |                       |           |             |
| AA vs. GA + GG       | 8              | 0.041      | 52.1          | Random model          | 1.409(1.048 ~ 1.896) | 0.023       |
| Multiplicative model |                |             |               |                       |           |             |
| A vs. G              | 8              | 0.045      | 51.3          | Random model          | 1.307(1.113 ~ 1.534) | 0.001       |

Discussion

NTDs are a multifactorial disease and its precise mechanism remains unknown. Epidemiologic studies had confirmed the pathogenesis of NTDs was mainly involved in genetic and environmental factors. A number of studies had demonstrated that variants of several genes involved in the folate-dependent one-carbon metabolism had been shown to be associated with the risk of NTDs [17–18]. Besides, NTDs are associated with polymorphisms involved in planar cell polarity (PCP) signaling pathway [19–21]. Of these, the most important is the gene encoding MTHFD1, which plays an important role in one-carbon metabolism by providing folate cofactors for DNA synthesis and for cellular methylation reactions.

The aim of this study was to explore the association between MTHFD1 polymorphisms and the risk of NTDs. A total of 321 subjects including 152 NTDs cases and 169 healthy controls were enrolled in this study. Related studied showed that low S-adenosylmethionine (SAM), concentration in combination with increased S-adenosylhomocysteine (SAH) concentration had been supposed to reduce methylation capacity [22]. Moreover, many epidemiological studies further confirmed that mothers with NTDs offspring had lower folate and higher homocysteine than those with normal offspring [23]. In our study, comparing the clinical characteristics between case and control groups, there was a significant difference in homocysteine, SAM, SAH and SAM/SAH, which indicated that these factors might be responsible for the occurrence of NTDs. Meanwhile, we selected four tag-SNPs (rs1950902, rs2236225, rs2236224 and rs11849530) to further evaluate the correlation between MTHFD1 polymorphisms and the risk of NTDs. Moreover, we also found that...
A-A (rs1950902-rs2236225) and T-A (rs2236224-rs11849530) haplotypes could increase the risk of NTDs, but C-A (rs2236224-rs11849530) haplotype might reduce the risk of NTDs.

To date, the *MTHFD1* rs2236225 is a functional exonic SNP that has been extensively studied in several studies, but the results were inconsistent. Carroll et al reported no evidence concerning correlation between the rs2236225 polymorphism and the risk of NTDs [24]. But Van der Linden et al indicated that G1958A mutation was an important risk factor of NTDs [12]. There could be several factors resulting in the conflicting conclusions among individual study. The most likely reasons were the sample size of each study and selected different populations in each study. In our study, we found the rs2236225 polymorphism was significantly associated with increased risk of NTDs. In order to comprehensively evaluate the effect of *MTHFD1* rs2236225 polymorphism on NTDs, we conducted a meta-analysis based on previous studies. Meta-analysis results showed a significant association between *MTHFD1* rs2236225 polymorphism and the risk of NTDs, which further confirmed the reliability of our findings. Besides, rs2236224 polymorphism is another common genetic variation in *MTHFD1*, we also found *MTHFD1* rs2236224 mutation was associated with increased the risk of NTDs. Etheredge AJ et al reported that the risk of NTDs was significantly increased for infant with *MTHFD1* rs2236224 polymorphism, and the results are consistent with ours[25].

**Conclusions**

In conclusion, our results revealed that the SNPs of rs2236225 and rs2236224 in *MTHFD1* gene were associated with increased NTDs risk in Han population of Northern China. The meta-analysis in our study further supported the conclusion. Due to the limitation of this study, further study with a large sample and functional study should be conducted to get a deeper insight into the etiology of NTDs.

**Abbreviations**

NTDs: Neural tube defects; *MTHFD1*: Methylene tetrahydrofolate dehydrogenase1; SNPs: single nucleotide polymorphisms; LD: linkage disequilibrium; PCP: planar cell polarity; SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine.

**Declarations**

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**Availability of data and materials**
The dataset and analyses are available from the corresponding author on reasonable request.

**Authors’ contributions**

F-YL, L-Y and S-JB analysed data and drafted the manuscript. Z-LS was responsible for clinical diagnosis. W-L and W-XT were responsible for experimental studies. Z-XF and Z-J were responsible for data collection. O-YS and C-CQ participated in the design and coordination of this study in addition to revising and critiquing the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

This study was approved by the Tianjin Children’ Hospital Ethics Committee. The guardian (parents) of the patient consented to both participation and publication of the case.

**Consent for publication**

Informed consent was obtained from the guardian (parents), who agreed to join this study, and using the medical information for scientific research and publication.

**Competing interests**

The authors declare that they have no competing interests.

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**Figures**

![Figure 1](image)

**Figure 1**

LD information and LD blocks of MTHFD1 gene region. Right figure: color and LD value are shown with D'; left figure: color and LD value are shown with r2. LD block were estimated by Confidence Intervals implemented in Haploview.