Anterior Encephalocele and Its Association with MTHFR Polymorphisms: A Case-Control Study

Hemonta K. Dutta1 Debasish Borbora2 Mauchumi Baruah3 Kanwar Narain4

1Department of Pediatric Surgery, Assam Medical College & Hospital, Dibrugarh, Assam, India
2Department of Biotechnology, Gauhati University, Guwahati, Assam, India
3Department of Physiology, Assam Medical College, Dibrugarh, Assam, India
4Regional Medical Research Laboratory, ICMR, Lahowal, Dibrugarh, Assam, India

Address for correspondence Hemonta K. Dutta, MBBS, MS, MCh, Department of Pediatric Surgery, Assam Medical College & Hospital, Dibrugarh 786002, Assam, India (e-mail: drhemontad@rediffmail.com).

Keywords
► anterior encephaloceles
► neural tube defects
► polymorphism

Abstract

Background Neural tube defects (NTDs) are thought to be associated with genetic defects and environmental factors. This study aims to determine the association of MTHFR gene polymorphisms and maternal body mass index (BMI) with anterior encephalocele (AE).

Methods Blood samples of 20 patients (out of 41 children) were available for genetic analysis. Genomic DNA was extracted from whole blood samples using Wizard genomic DNA purification kit. The MTHFR C677T and A1298C polymorphisms genotyping protocols were adapted from Cicek et al. Eighty-two age- (1–14 years) and sex-matched apparently healthy children were taken as controls. We assessed the nutritional status of all the volunteers by measuring their BMI and then classified according to WHO BMI cutoff points.

Results Nasofrontal AE was seen mostly among the female cases while among males, nasoethmoidal AE was predominant. We observed a weak association between MTHFR 677CT genotype and AE. In the case of MTHFR A1298C, both the 1298AC and 1298CC genotypes increased the risk of acquiring AE by several folds. Multivariate analysis revealed that both 1298AC and 1298CC genotypes increased the risk of acquiring AE. However, only 1298AC was significantly associated with the risk of AE. The study also showed significantly low BMI among the children and their mothers.

Conclusion There is a strong association between MTHFR A1298C polymorphism and the risk of anterior encephalocele in this community. The C677T polymorphism, however, did not constitute a genetic risk factor in this study. Children with AE also had significantly low BMI.

Introduction

Anterior encephalocele (AE) is a rare congenital anomaly of the central nervous system characterized by protrusion of the intracranial content beyond the normal confinement of the cranium. It is a visible defect at the root of the nose and hence also called as “sincipital” (► Fig. 1). Sporadic in the western world, AE cases are most frequently reported from the developing nations of Southeast Asia where the incidence rate varies between 1 in 3,000 and 1 in 5,000,1 but the

Received November 18, 2016
Accepted July 3, 2017
Published online October 13, 2017

ISSN 2277-954X.
© 2017 Neurological Surgeons’ Society of India

License terms

© THIEME OPEN ACCESS
possible explanation for its "remarkably specific geographical
distribution" is still unknown. Several studies have implicated
 genetic predispositions, nutrition, and environmental factors as
risk factors of neural tube defects (NTDs).2

Two polymorphisms in the 5,10-methylenetetrahydrofolate
reductase (MTHFR) gene—the C677T (rs1801133 C > T) and
A1298C (rs1801131 A > C)—have been frequently studied in
context of NTDs.2–6 MTHFR regulates the folate one-carbon
metabolic pathway. Both the C677T (Ala222Val) and the A1298C
(Glu429Ala) substitutions result in a decreased enzymatic
activity.7 In Assam there is a high incidence of AE, especially
within its tea garden communities.8 However, there are no data
concerning the risk factors of AE for this community. Therefore,
this study was conducted to determine the association of
MTHFR gene polymorphisms with the risk of AE in this region.

Material and Methods

Study Population and Design
Forty-one AE patients, who were surgically operated and
followed up in the Department of Pediatric Surgery,
consented to participate in this matched case-control study.
The cases were classified based on anatomical finding according
to the system described by Suwanwela and Suwanwela9 into
nasofrontal, nasoethmoidal, and naso-orbital encephalocele.
We also recruited 82 age- and sex-matched apparently
healthy kids who had visited the department.

To study the probable risk factors, we administered a
precoded close-ended questionnaire. We accessed the
nutritional status of all the volunteers by measuring their body
mass index (BMI). Parental BMI was calculated by dividing
weight in kilograms by the height in meters squared (kg/m²)
and classified according to the World Health Organization
( WHO) BMI cutoff points. In case of children, the BMI-for-age
percentile was used to interpret “weight status categories.” We
used the Child and Teen BMI calculator (the calculator was
the 41 cases, only 21 cases approved for genetic analysis. One
milliliter of peripheral whole blood was collected from all
volunteers and stored at −80°C until analyzed.

MTHFR Polymorphism Analysis
Genomic DNA was extracted from whole blood samples using
Wizard genomic DNA purification kit (Promega). The MTHFR
C677T and A1298C polymorphisms genotyping protocols were
adapted from Cicek et al.7 A polymerase chain reaction (PCR)
reaction mixture of 25 µl was set up using GoTaq Hot start
Green 2X master mix (Promega) containing nearly 200 ng
template DNA. Primer sequences for MTHFR C677T were
5' GGT CAG AAG CT ATC AGT CT GAG -3' and 5' CTG GGA
AGA ACT CAG CGA ACT CAG -3', which produced a 494 bp PCR
product. The PCR cycles were 2 minutes at 94°C, followed by 30
cycles each of 30 seconds at 94°C, 30 seconds at 62°C, and
30 seconds at 72°C with final extension at 72°C for 7 minutes.
The PCR product was then digested using Hinfl (Promega) at
37°C for 3 hours. When digested products were resolved on a
1.5% agarose gel, wild-type alleles (677C, Ala) resulted in 100-
and 394-bp fragments while the variant alleles (677T, Val)
resulted in 100-, 165-, and 229-bp products (►Fig. 2). The
MTHFR A1298C variant was amplified using primers 5'-
GAG GAG CTG CTG AAG ATG -3' and 5'- CTT TGC CT GTC CAC
AGC ATG -3' and generated a product sized 237 bp. The cycling
conditions were 5 minutes at 95°C, followed by 30 cycles each of
30 seconds at 94°C, 30 seconds at 63°C, and 30 seconds at 72°C
with final extension for 10 minutes at 72°C. The PCR product
was digested using MboII (Promega) at 37°C for an hour. A 15%
acrylamide gel was used to resolve the digested products
in which wild-type alleles (1298A) resulted in 27-, 26-, and
183-bp fragments whereas the variant alleles (1298C) resulted
in 27- and 210-bp fragments (►Fig. 3).

Statistical Analysis
Date were analyzed using IBM SPSS Statistics 20 for Windows
(IBM, SPSS Inc.). Initially a univariate analysis was performed
to identify the putative risk factors. These risk factors were further analyzed using multiple logistic regression to study their independent association with AE. p Value of ≤ 0.05 (two tailed) was considered for statistical significance.

Results

Among the 41 patients who participated in the study, 20 (48.8%) were male and 21 (51.2%) were female. The mean age was 36 months, ranging from 1 to 14 years in both cases and controls. Table 1 lists the cases according to their anatomical locations. Nasofrontal AE was seen mostly among the female cases whereas nasoethmoidal AE was predominant among males. The MTHFR C677T genotype was in Hardy-Weinberg equilibrium (p > 0.8), but the MTHFR A1298C departed from the same (p = 0.0). Data from Table 2 revealed the risk factors associated with AE. The univariate odds ratio (OR) for MTHFR 677CT, MTHFR 1298AC, and 1298CC genotypes were 1.0, 1.65, and 1.55 (95% confidence interval [CI]: 0.8–2.0), respectively. The analysis also indicated that a low maternal BMI significantly increased the risk of acquiring AE.

In the multivariate analysis, there was no association between the MTHFR C677T variant genotypes and AE. The MTHFR 1298AC variant, however, was significantly associated with the risk, 7.75 (95% CI: 1.15–52.24). The MTHFR C677T and A1298C haplotypes were not associated with the risk of AE among all the study patients. An increase in OR during the analysis indicated that low maternal BMI is an independent risk factor of AE.

Discussion

In Assam, there is a high incidence of AE.8 Till date only a few studies have been performed to detect the possible risk factors of AE probably because this anomaly is predominant only in the socioeconomically backward communities of Southeast Asia and Africa.2,3,5,10,11 The present study is the first hospital-based case-control study to be performed in India, which studies the association between MTHFR polymorphisms and BMI with the risk of AE. It clearly indicates that children who carry the MTHFR 1298CC genotype were at a greater risk of having AE. Underweight children were also at a significant risk of developing AE.

Several studies have reported the C677T polymorphism as a significant risk factor of NTD, but in case of AE, these results are conflicting.3,12 In this study, we did not find any association between the MTHFR 677TT genotype and AE, as the frequency of the 677T homozygous mutant genotype among cases was zero. However, the 677CT genotype demonstrated a weak association. This observation is similar to those of studies conducted in Indonesian and Javanese community where the frequency of the 677TT genotype was close to zero.12 Homozygosity for the 677T mutation among mothers increases the risk of recurrent early pregnancy loss (REPL) by two- to threefolds in absence of folate supplementation.13 The National Family Health Survey (NFHS-3) reports that the infant mortality rate in Assam is very high and the consumption of iron folic acid among pregnant mothers at only 13.7%.14 The findings are suggestive of the significant impact of MTHFR polymorphisms in the community.

As AE is a rare event, most of the previous associative studies use a heterogeneous population of NTDs. In a recent review, Zhang et al suggested that MTHFR A1298C is not an independent risk factor of NTD, but its effect could be influenced by low dietary intake of folate and ethnicity.5 This is the first study to report a significant association between the A1298C polymorphism and the development of AE. In the multivariate analysis, both the genotypes, 1298AC and 1298CC, demonstrated a positive association with the risk of AE, but only 1298AC achieved statistical significance. Distinct ethnicity in this region and low folate intake could explain this significant

Table 1 Cases according to anatomical locations of encephaloceles

<table>
<thead>
<tr>
<th>Anatomical location of the hernia</th>
<th>Sex</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasoethmoidal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>% within type of AE</td>
<td>57.1%</td>
<td>42.9%</td>
</tr>
<tr>
<td>Nasofrontal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>% within type of AE</td>
<td>37.5%</td>
<td>62.5%</td>
</tr>
<tr>
<td>Nasoorbital</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>% within type of AE</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>% within type of AE</td>
<td>48.8%</td>
<td>51.2%</td>
</tr>
</tbody>
</table>

Abbreviation: AE, anterior encephalocele.
association. In this study, we used the BMI-for-age percentile system to interpret “weight status categories” to find that underweight children were at a significantly higher risk of having AE as compared with healthy children. Underweight is an indicator of low socioeconomic power\(^\text{15}\) which has been previously associated with AE.\(^\text{11}\)

The present study hints toward a possible association between maternal BMI and the risk of AE. In the univariate analysis (\textit{Table 2}), low maternal BMI (< 18.5) was significantly associated with the risk of AE. Children of obese mothers were also at an elevated risk, but as only a small fraction of mothers in the cohort were obese, its association was not statistically significant. Although these observations were consistent with those of Stothard et al.,\(^\text{16}\) according to Gao et al, maternal underweight, overweight, and obesity were not associated with elevated risks of NTDs.\(^\text{17}\)

In conclusion, this study suggests a strong association between MTHFR A1298C polymorphism and the risk of AE in this community. The C677T polymorphism, however, did not constitute a genetic risk factor in this study. Children who were underweight and born to mothers having a low BMI were at an elevated risk of developing AE.

\textbf{Note}

This study was approved by Institutional Ethics Committee.

\textbf{Funding}

The funding agency had no role in study design, data collection and analysis, interpretation of results, or scripting the report. The authors had full access to the data, and the final decision to submit for publication was taken by the corresponding author.

\textbf{Conflict of Interest}

None.

\textbf{References}


\textbf{Table 2} Analysis of factors associated with AE

<table>
<thead>
<tr>
<th>Category</th>
<th>Cases (n) (%)</th>
<th>Control (n) (%)</th>
<th>Crude OR (95% CI)</th>
<th>(p)-Value</th>
<th>Adjusted OR (95% CI)</th>
<th>(p)-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MTHFR genotypes, (n) (%)(^a)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>677CC</td>
<td>14 (66.7)</td>
<td>31 (73.8)</td>
<td>Ref.</td>
<td>Ref.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>677CT</td>
<td>7 (33.3)</td>
<td>10 (23.8)</td>
<td>1.55 (0.49–3.51)</td>
<td>0.29</td>
<td>2.07 (0.62–6.87)</td>
<td>0.24</td>
</tr>
<tr>
<td>677TT</td>
<td>0 (0.0)</td>
<td>1 (2.4)</td>
<td>0.00</td>
<td>1.0</td>
<td>0.00</td>
<td>1.0</td>
</tr>
<tr>
<td>1298AA</td>
<td>11 (52.4)</td>
<td>33 (78.6)</td>
<td>Ref.</td>
<td>Ref.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1298AC</td>
<td>4 (19.0)</td>
<td>2 (4.8)</td>
<td>6.00 (1.65–21.88)</td>
<td>0.01(^b)</td>
<td>7.75 (1.15–52.24)</td>
<td>0.04(^b)</td>
</tr>
<tr>
<td>1298CC</td>
<td>6 (28.6)</td>
<td>7 (16.7)</td>
<td>2.57 (1.04–6.39)</td>
<td>0.04(^b)</td>
<td>1.54 (1.00–24.74)</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>MTHFR haplotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>677C-1298A</td>
<td>22 (52.4)</td>
<td>58 (69.0)</td>
<td>Ref.</td>
<td>Ref.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>677C-1298C</td>
<td>13 (31.0)</td>
<td>14 (16.7)</td>
<td>2.45 (1.0–6.02)</td>
<td>0.05(^b)</td>
<td>0.94 (0.07–12.44)</td>
<td>0.96</td>
</tr>
<tr>
<td>677T-1298A</td>
<td>4 (9.5)</td>
<td>10 (11.9)</td>
<td>1.06 (0.30–3.71)</td>
<td>0.93</td>
<td>0.84 (0.15–4.78)</td>
<td>0.85</td>
</tr>
<tr>
<td>677T-1298C</td>
<td>3 (7.1)</td>
<td>2 (2.4)</td>
<td>3.96 (0.62–25.28)</td>
<td>0.15</td>
<td>1.37 (0.05–34.55)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

| BMI of mother\(^c\)** | | | | | | |
| 18.5–22.9 | 18 (45.0) | 57 (71.2) | Ref. | Ref. | | |
| < 18.5      | 20 (50.0) | 22 (27.5) | 4.89 (4.13–11.21) | 0.00\(^b\) | 6.30 (2.53–15.70) | 0.00\(^b\) |
| \(\geq 23\) | 2 (5.0)  | 1 (1.3)  | 4.00 (0.52–30.61) | 0.18 | 3.77 (0.42–34.12) | 0.24 |

Abbreviations: AE, anterior encephalocele; BMI, body mass index; CI, confidence interval; OR, odds ratio; Ref., reference.

\(^a\)These risk factors were studied in 21 cases and 42 apparently healthy controls who consented to participate in this study.

\(^b\)Statistically significant.

\(^c\)Insufficient data.
16 Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. JAMA 2009;301(06):636–650