Maternal 677CT/1298AC genotype of the MTHFR gene as a risk factor for cleft lip

V Shotelersuk, C Ittiwut, P Siriwan, A Angspatt

SUBJECTS AND METHODS

The study sample consisted of 109 CL/P patients, 67 of their mothers, 45 of their fathers, and 202 controls. Of 109 patients, 14 were familial. All of them were studied under the auspices of the Thai Red Cross, a national charity organisation devoted to providing clinical care for the poor. Subjects were recruited from Nakornratchasima, Nan, Uthaithanee, Maehongsorn, Trang, Srakaew, and Bangkok. As preoperative evaluations, every patient was examined by a geneticist (VS) for any presence of associated anomalies suggestive of syndromic variants. In addition, a family history and epidemiological data were obtained and will be reported elsewhere. After receiving their informed consent, blood samples for DNA analysis were obtained at the time of blood typing and haematocrit determination. Only one patient in families with more than one affected subject was included in the case group. All syndromic cases were excluded. Only the cases of non-syndromic CL/P (normal growth, normal development, and no other major anomalies) were analysed in this report.

The control samples were blood donors with no oral clefts in Bangkok and Nakornratrachaseema collected in the same period. The study was approved by the institutional review board in Thailand.

DNA was extracted by standard procedures and was amplified using the polymerase chain reaction (PCR). Genotyping for the MTHFR 677CT and 1298AC polymorphisms was performed by restriction digestion of PCR products with HinfI and MboII, respectively.

Statistical analysis

Standard chi-square and p values were calculated by a program available at http://quantum2 psy.ohio-state.edu/kris/chisq/chisq.htm. Haplotype frequencies were estimated by the EH program downloaded from http://linkage.rockefeller.edu/ott/eh.htm. The transmission disequilibrium test (TDT) analysis was carried out on subjects with heterozygous informative parents. Data from families with one parent missing were excluded. Haplotypes of subjects homozygous for at least one polymorphism are readily predicted from their genotypes. All of the 677CT/1298AC genotypes were predicted to be 677C-1298C, 677T-1298C, and 677T-1298A haplotypes because the 677T-1298C haplotype has not been identified. The TDT data were analysed using a k = 1/k correction (where k is the number of alleles). Odds ratios and 95% confidence intervals (95% CI) were calculated from the Epi Info 2000 program, to estimate the relative risk of the different genotype combinations.
RESULTS

The observed frequencies of the 677C, 677T, 1298A, and 1298C alleles in affected subjects, their mothers, their fathers, and controls are given in table 1. The observed distribution of genotypes among controls was compared with that expected according to Hardy-Weinberg equilibrium: no difference was found. The observed distribution of alleles in affected subjects, their mothers, their fathers, and controls are given in table 1. The observed frequencies of the 677C, 677T, 1298A, and 1298C alleles in patients with CL/P, their parents, and controls from the EH program are shown in table 2.

Table 1 Frequencies of MTHFR alleles in patients with CL/P, their parents, and controls

<table>
<thead>
<tr>
<th>MTHFR allele</th>
<th>CL/P patients (n=109)</th>
<th>Mothers (n=67)</th>
<th>Fathers (n=45)</th>
<th>Controls (n=202)</th>
</tr>
</thead>
<tbody>
<tr>
<td>677C*</td>
<td>0.89 (193)</td>
<td>0.83 (111)</td>
<td>0.87 (78)</td>
<td>0.88 (334)</td>
</tr>
<tr>
<td>677T*</td>
<td>0.11 (25)</td>
<td>0.17 (23)</td>
<td>0.13 (12)</td>
<td>0.12 (50)</td>
</tr>
<tr>
<td>χ² (p value)</td>
<td>0.11 (0.74)</td>
<td>1.97 (0.16)</td>
<td>0.06 (0.81)</td>
<td>-†</td>
</tr>
<tr>
<td>1298A*</td>
<td>0.72 (158)</td>
<td>0.69 (93)</td>
<td>0.79 (71)</td>
<td>0.73 (296)</td>
</tr>
<tr>
<td>1298C*</td>
<td>0.28 (60)</td>
<td>0.31 (41)</td>
<td>0.21 (19)</td>
<td>0.27 (108)</td>
</tr>
<tr>
<td>χ² (p value)</td>
<td>0.05 (0.83)</td>
<td>0.75 (0.39)</td>
<td>1.22 (0.27)</td>
<td>-†</td>
</tr>
</tbody>
</table>

*The number of subjects is indicated in parentheses.
†Reference category.

Table 2 Calculated frequencies of the MTHFR haplotypes in patients with CL/P, their parents, and controls from the EH program

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>CL/P patients</th>
<th>Mothers</th>
<th>Fathers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>677C-1298A</td>
<td>0.61</td>
<td>0.52</td>
<td>0.66</td>
<td>0.61</td>
</tr>
<tr>
<td>677C-1298C</td>
<td>0.28</td>
<td>0.31</td>
<td>0.21</td>
<td>0.27</td>
</tr>
<tr>
<td>677T-1298A</td>
<td>0.11</td>
<td>0.17</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>677T-1298C</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>χ²**</td>
<td>0.14</td>
<td>3.46</td>
<td>1.26</td>
<td>0.14</td>
</tr>
<tr>
<td>p</td>
<td>0.986</td>
<td>0.494</td>
<td>0.805</td>
<td>0.986</td>
</tr>
</tbody>
</table>

*χ² = 2 (L - T (ln(L) - ln(T)) - ln(L) - ln(T)).
†Reference category.

We found no association between any of the patients’ genotypes and CL/P. TDT analysis was carried out on subjects with heterozygous informative parents but it showed no evidence for association of CL/P with any of the haplotypes, as shown in table 3. In addition, the TDT analysis of each individual polymorphism did not show any association between the variant and CL/P (data not shown).

Analyses were carried out to estimate the risk associated with each genotype. The results calculated as odds ratios and 95% CI are reported in table 4. Interestingly, a significantly higher frequency of the 677CT/1298AC genotype was observed in the mothers of CL/P patients compared to controls, with an odds ratio of 4.43 (95% CI 1.33 to 15.10). There were approximately 12% (eight of 67) of mothers with such a genotype; therefore, folate supplementation in a pregnant woman’s diet may benefit these 12% of Thai children who are susceptible to CL/P owing to the 677CT/1298AC genotype in their mother. No such relationship could be found with any genotypes in fathers of affected offspring.

This observation is in agreement with two recent independent association studies showing that there is an association between a maternal polymorphism, 677C>T, in the MTHFR gene in mothers of affected subjects and an increased risk of CL/P with risk ratios of 2.51 (1.00 to 6.14) and 4.09 (1.32 to 11.57). A previous study showed that the activity of the MTHFR enzyme in subjects with 677CT/1298AC (47.7%) is higher than that of 677TT/1298AA (24.8%). However, we found that the detected risk of the 677TT/1298AA mothers was no higher than that of the 677CT/1298AC mothers. Although the higher risk is associated with the 677TT/1298AA mothers compared to the normal 677CC/1298AA controls (odds ratio = 3.08), it is not statistically significant (95% CI 0.30 to 31.28). A possible interpretation is that the lower activity of the enzyme increases the susceptibility to fetal loss or to giving birth to children with multiple associated malformations, without which is comparable to the frequencies of 14% in the control population reported in independent studies in Thailand. Our results agree with a previous observation that the polymorphism was found in every population tested and its frequency among Asians is quite similar to that of Europeans, but higher than in Africans. The frequency of the 1298C allele in our controls was 27%, which is similar to that of white populations (27–33%).

Table 3 TDT analysis in CL/P patients

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Transmitted</th>
<th>Untransmitted</th>
<th>χ²**</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>677C-1298A</td>
<td>17</td>
<td>14</td>
<td>-†</td>
<td>-†</td>
</tr>
<tr>
<td>677C-1298C</td>
<td>12</td>
<td>14</td>
<td>-†</td>
<td>-†</td>
</tr>
<tr>
<td>677T-1298A</td>
<td>6</td>
<td>9</td>
<td>-†</td>
<td>-†</td>
</tr>
<tr>
<td>677T-1298C</td>
<td>0</td>
<td>0</td>
<td>-†</td>
<td>-†</td>
</tr>
</tbody>
</table>

*χ² were analysed using a k−1/4 correction (where k is the number of alleles). Degree of freedom = (number of rows - 1) × (number of columns - 1) = 1×2 = 2.
increasing the risk of CL/P alone. Another explanation would be a limited number of mothers with the 677TT/1298AA genotypes in our study owing to its low frequency. Although not statistically significant, a higher risk is associated with the 677TT/1298AA fathers compared to the normal 677CC/1298AA controls (odds ratio = 4.65), which is also greater than that observed for the 677TT/1298AA mothers. Further studies with more numbers of fathers are needed to determine the association.

Three previous studies, however, showed no association between the maternal 677TT genotype and CL/P.\(^7\)\(^8\)\(^17\)\(^21\) Our results do not contradict these reports because the three studies did not determine the genotype at the 1298 position. Moreover, the inconsistent result could be caused by the difference in populations studied with diverse genetic backgrounds and environmental factors. In addition, the timing of sample collections, whether it was before or after folic acid fortification of foods, started in the USA in 1998, could affect the frequencies of the polymorphisms in subjects with some anomalies.\(^26\)

These findings indicate a possible involvement of the folate pathway in the causation of CL/P and support an influence of the maternal genotype, rather than an effect of the embryo’s genotype. The 677C>T polymorphism converts an alanine to a valine residue at position 222 making the MTHFR thermolabile with reduced activity to 65% in heterozygotes and 30% in homozygotes. The 677C>T polymorphism contributes to the risk of non-syndromic cleft lip and palate.

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