ONLINE MUTATION REPORT

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

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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 between 2000 and 2002 from seven centres in Thailand (Nakornratchasima, Nan, Uthaithanee, Maehongsorn, Trang, Srakae, 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.

Key points

- Previous data have shown an association between the 677C>T polymorphism in the MTHFR gene in either non-syndromic cleft lip with or without cleft palate (CL/P) patients or their mothers and an increased risk of the anomaly, but this finding remains controversial. No studies have investigated 1298A>C, the second most common polymorphism in MTHFR, in CL/P patients and their parents.

- We investigated 109 CL/P patients, 67 of their mothers, 45 of their fathers, and 202 controls for the 677C>T and 1298A>C polymorphisms. We found no association between any of the patients’ genotypes and CL/P.

- However, a significantly higher frequency of the compound heterozygous 677CT and 1298AC genotype was detected in mothers of CL/P patients with an odds ratios of 4.43 (95% confidence interval 1.33 to 15.10).

- These results indicate an effect of the maternal genotype, rather than in the affected subjects.

The control samples were blood donors with no oral clefts in Bangkok and Nakornratchasima 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 677C>T and 1298A>C polymorphisms was performed by restriction digestion of PCR products with Hinfl and MboII, respectively.

Statistical analysis

Standard chi-square and p values were calculated by a program available at http://quantrm2.psych.ohio-state.edu/kris/chsq/chsq.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 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.

N on-syndromic cleft lip with or without cleft palate (CL/P) is one of the most common congenital anomalies world wide. It has a prevalence of approximately 1/1000 among white populations and 1/600 among Thai newborns. Environmental and genetic factors have been implicated in CL/P and several different loci and genes have been associated with them.

Maternal folic acid supplementation during early pregnancy may reduce the risk for oral clefts, but this is controversial. One of the mechanisms by which low folate levels predispose some subjects to oral clefts could be the presence of polymorphisms in the genes encoding enzymes of the folate pathway, such as 5,10-methylene tetrahydrofolate reductase (MTHFR, MIM 236250). MTHFR catalyses the reduction of 5,10-methylene tetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate and the carbon donor for the remethylation of homocysteine to methionine. Two polymorphisms, 677C>T and 1298A>C, in the MTHFR gene have been shown to have reduced MTHFR activity.

The 677C>T transition, producing an alanine to valine amino acid substitution within the catalytic domain of the MTHFR enzyme, has been associated with many disorders and conditions including neural tube defects, vascular disease, migraine, smoking behaviour, and oral clefts. However, the last is controversial. Recent studies reported an association between the maternal polymorphism and the anomalies, but this again is not a consistent finding.

No studies have investigated 1298A>C, the second most common polymorphism in MTHFR resulting in a glutamate to alanine substitution, in CL/P patients and their parents. We therefore carried out a case-control study to determine whether the two MTHFR polymorphisms in Thai patients with CL/P or their parents were associated with an increased risk of the anomaly.
We have established MTHFR genotypes in 109 CL/P patients, their parents, and controls. The frequency of the 677T allele in our controls was 12%, 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%).

No 677T-1298C haplotype was detected. This finding agrees with an observation that a subject with the 677TT genotype always had a 1298AA genotype and a subject with the 1298C genotype always had a 677CC genotype (table 4). In addition, it supports the recent finding that the 677T occurred on a founder haplotype of 1298C.

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 also showed no evidence for the association. This finding is consistent with a previous report. However, when we analysed maternal genotypes and CL/P in their offspring, we found the odds ratio calculated for mothers having the 677TT genotype always had a 1298AA genotype and a subject with the 1298C genotype always had a 677CC genotype (table 4). In addition, it supports the recent finding that the 677T occurred on a founder haplotype of 1298C.

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increasing the risk of CL/P alone. Another explanation would be a limited number of mothers with the 677TT/1298AA genotype in our study owing to its low frequency. Although not statistically significant, a higher risk is associated with the 677TT/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.17 18 27 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.28

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. Although neither the 677C>T polymorphism nor the 1298A>C polymorphism alone contribute to the risk of CL/P alone. Another explanation would be a limited number of mothers with the 677TT/1298AA genotype in our study owing to its low frequency. Although not statistically significant, a higher risk is associated with the 677TT/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.

<table>
<thead>
<tr>
<th>MTHFR 677/1298 genotype</th>
<th>Observed frequency*†</th>
<th>Odds ratio (95% CI):‡</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CL/P patients (n=109)</td>
<td>Mothers (n=67)</td>
</tr>
<tr>
<td>CC/AA</td>
<td>0.30 (33)</td>
<td>0.25 (17)</td>
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<td>CC/AC</td>
<td>0.41 (45)</td>
<td>0.37 (23)</td>
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<td>0.06 (4)</td>
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<tr>
<td>CT/AA</td>
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<td>0.16 (11)</td>
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<tr>
<td>CT/AC</td>
<td>0.03 (3)</td>
<td>0.12 (8)</td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>TT/CC</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

*The number of subjects is indicated in parentheses. †NO = not observed. ‡The 95% CI is not determined owing to the invalidity of the Cornfield 95% confidence limits for odds ratio.

**Table 4 Prevalence and calculated odds ratios with 95% CI of the MTHFR polymorphisms in patients with CL/P, their parents, and controls**

**ACKNOWLEDGEMENTS**

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