Does the Interaction between Maternal Folate Intake and the Methylenetetrahydrofolate Reductase Polymorphisms Affect the Risk of Cleft Lip with or without Cleft Palate?

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Periconceptional folic acid supplementation may reduce the risk of cleft lip with or without cleft palate (CL(P)). Polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene reduce availability of 5-methyltetrahydrofolate, the predominant circulating form of folate. To determine the effect of MTHFR C677T and MTHFR A1298C genotypes and haplotypes on CL(P) risk and the interaction with maternal periconceptional dietary folate and folic acid supplement intake, the authors conducted a case-control triad study in the Netherlands (1998–2000) among 179 CL(P) and 204 control families. Infant and parental MTHFR C677T and MTHFR A1298C genotypes and haplotypes were not associated with CL(P) risk in the case-control and transmission disequilibrium test analyses. Mothers carrying the MTHFR 677TT genotype and who either did not use folic acid supplements periconceptionally or had a low dietary folate intake, or both, had an increased risk of delivering a CL(P) child (odds ratio (OR) = 5.9, 95% confidence interval (CI): 1.1, 30.9; OR = 2.8, 95% CI: 0.7, 10.5; OR = 10.0, 95% CI: 1.3, 79.1, respectively). No supplement use, low dietary folate intake, and maternal MTHFR 1298CC genotype increased the risk of CL(P) offspring almost sevenfold (OR = 6.5, 95% CI: 1.4, 30.2). Thus, the detrimental effect of low periconceptional folate intake on the risk of giving birth to a CL(P) child was more pronounced in mothers with the MTHFR 677TT or MTHFR 1298CC genotype.

Abbreviations: CI, confidence interval; CL(P), cleft lip with or without cleft palate; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio.

Cleft lip with or without cleft palate (CL(P)) is a frequently occurring congenital malformation characterized by closure defects of the lip, alveolus, and/or palate. The suggested multifactorial etiology of nonsyndromic CL(P) is still not understood (1). In addition to genetic predisposition, environmental risk factors may play an important role in the...
pathogenesis of these birth defects. Several intervention and case-control studies have proposed that maternal periconceptional use of multivitamins containing folic acid protects against CL(P) occurrence and recurrence (2–6). Folate is a one-carbon donor; as such, it is involved in the biosynthesis of purines and pyrimidines and in homocysteine remethylation, which produces methyl groups for methylation of DNA, proteins, and lipids. Therefore, folate is important for the expression of several genes essential for cellular multiplication and differentiation during embryogenesis.

Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate metabolism. This enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate and the methyl donor for the remethylation of homocysteine into methionine. The gene encoding the MTHFR enzyme is known to have at least two functional polymorphisms: C677T and A1298C. The homozygous MTHFR 677TT genotype results in a thermolabile enzyme with reduced activity. The consequence of this polymorphism is that the concentrations of folate in serum, plasma, and red blood cells decrease and plasma homocysteine concentrations increase mildly (7, 8). A second polymorphism in the MTHFR gene, an A-to-C substitution at nucleotide 1298, also results in decreased MTHFR activity but is not associated with higher homocysteine or lower plasma folate levels (9).

Studies in which the MTHFR C677T polymorphism has been tested for an association with CL(P) risk show conflicting results (10–12). Shaw et al. (10) first demonstrated a small, but nonsignificant effect of the interaction between the MTHFR C677T polymorphism and maternal periconceptional multivitamin use. However, they did not clarify the independent role of folic acid in supplements or the dietary intake of folate. To our knowledge, only one study has investigated the MTHFR A1298C variant in relation to CL(P) risk but found no association (13).

In this study, we investigated the independent genetic association between maternal, paternal, and infant MTHFR C677T and MTHFR A1298C genotypes and haplotypes and nonsyndromic CL(P) risk by conducting a case-control comparison and a transmission disequilibrium test in a family-based design. The latter design is more valid for genetic associations than is the case-control design, since it cannot be biased because of population stratification (14). Furthermore, the gene-environment interaction of maternal and infant MTHFR genotypes with periconceptional folic acid supplementation and dietary folate intake by the mother was studied by using cases and controls.

**MATERIALS AND METHODS**

**Study population**

A case-control triad study was carried out in the Netherlands between 1998 and 2000. The CL(P) families were recruited in collaboration with the nine largest cleft palate teams in the country. A clinician in each center, for example, a clinical geneticist, plastic surgeon, pediatrician, or orthodontist, used a standard form to record the malformations in children with an orofacial cleft. These forms were registered in a database developed on behalf of the Dutch Association for Cleft Palate and Craniofacial Anomalies (15). Criteria for inclusion were that the infant had a nonsyndromic CL(P), was not adopted, and was 9–18 months of age at the time of the study and that the mother, father, and infant were Dutch Caucasians. Seventy-six percent of those invited to participate were included in this study, which resulted in 179 CL(P) triad families. Successful DNA analyses were performed for 130 CL(P) infants, 170 mothers, and 158 fathers.

Control families were first enrolled by the case mothers; for example, they were friends, acquaintances, and neighbors (44 percent). However, we were not able to reach our desired sample size by using this recruitment strategy. Therefore, we approached mothers by using posters and leaflets in nurseries and infant welfare centers in the city of Nijmegen and surrounding areas (56 percent). The selection criteria we used for controls were similar to those for CL(P) families except that the infants had no congenital malformations. In total, 204 control triad families were recruited, and successful DNA analyses were performed for 150 infants, 189 mothers, and 177 fathers.

The Medical Ethical Committees of the participating hospitals approved the study protocol. In addition, written informed consent was obtained from every participant before entering the study.

**Data collection**

All mothers filled out a general and a food frequency questionnaire about 14 months after birth of the index child. At the same time, DNA was collected from the mother, father, and infant by either a blood sample or a buccal swab.

The questionnaires were sent to the mothers, who filled them out at home by themselves. After they were returned, they were checked for completeness and unclear answers by the principal investigator and a research assistant. From the general questionnaire we extracted information on maternal age at delivery, educational level, and periconceptional use of folic acid supplements. Educational level was categorized as low (primary/lower vocational/intermediate secondary/intermediate vocational education) or high (higher secondary/higher vocational or university education). Information on folic acid supplements was collected with open-ended questions and consisted of dosage, content (folic acid only or multivitamins), frequency of intake, and specification of in which weeks the supplements were taken before and during pregnancy. In this study, we classified a “folic acid supplement user” as a daily user of folic acid in the form of a multivitamin supplement containing folic acid or as a single tablet, starting 4 weeks before until 8 weeks after conception, conforming to the recommended period in the Netherlands (16). Incidental users and women who started to use folic acid supplements later than 4 weeks before conception were categorized as “nonusers.”

Information on maternal dietary intake was obtained by using a validated food frequency questionnaire (17, 18). Our assumption was that information on usual dietary intake collected 14 months after birth is comparable to dietary intake 24 months before, covering the periconceptional
period of the pregnancy, as stated by Devine et al. (19). Moreover, this assumption was strengthened by the fact that the season of the periconceptional period and the moment at which the questionnaire was filled out were comparable. Mothers were not asked to recall their periconceptional dietary habits because we expected that they might report their habits during the second and third trimesters of pregnancy. The food frequency questionnaire accounted for at least 90 percent of the population mean intake of food groups and nutrients of interest. Average daily intake of folate and total energy was estimated by multiplying the frequency of consumption of the food items by portion size and nutrient content per gram. Total energy intake was calculated by using the 1996 computerized Dutch food composition table (20). Folate data were derived from a table based on a recent analysis of Dutch foods in which a validated high performance liquid chromatography technique was used (21).

Data on periconceptional folic acid supplement use were missing for two CL(P) mothers and six control mothers because their answers were unclear. In addition, dietary folate intake could not be determined for 16 CL(P) mothers and nine control mothers because they were late in returning the food frequency questionnaire.

Laboratory methods

The C677T polymorphism in the MTHFR gene alters an alanine into a valine residue. This mutation creates an HinFl site, allowing restriction enzyme analysis (7). The MTHFR A1298C polymorphism results in a glutamate-to-alanine substitution. This change abolishes an MboII restriction site. We investigated the prevalence of both polymorphisms by using polymerase chain reaction (PCR) of genomic DNA and restriction enzyme digestion, followed by agarose gel electrophoresis, as described previously (9).

To perform the family-based transmission disequilibrium test, 86 case triads (mother, father, and CL(P) infant) were completely genotyped for the MTHFR C677T polymorphism and 76 case triads for the MTHFR A1298C polymorphism. Fifty-five triads were completely genotyped for both polymorphisms.

Statistical analyses

For both MTHFR polymorphisms, we used a case-control design to calculate the odds ratios for CL(P) risk, and the corresponding 95 percent confidence intervals, for mothers, fathers, and infants with the homozygous (677TT or 1298CC) or the heterozygous (677CT or 1298AC) genotype relative to those with the wild-type (677CC or 1298AA) genotype. The allele frequencies of the MTHFR polymorphisms were compared between the CL(P) and control families, and CL(P) risk was estimated by using odds ratios and 95 percent confidence intervals. The composite distribution of the MTHFR genotype was compared between the cases and controls by using the chi-square test. The putative risk alleles 677TT and 1298CC and risk haplotypes 677T/1298A and 677C/1298C were tested for linkage and linkage disequilibrium with CL(P) by using a family-based study design and analysis. The frequency of transmission of these risk alleles and haplotypes from heterozygous parents was determined. In case triads, the transmission frequency of an allele or haplotype responsible for an increased risk of CL(P) should exceed 50 percent. Statistical significance of deviation from 50 percent transmission was determined by using McNemar’s test (14).

Because the distribution of dietary folate intake was skewed positively, we applied natural logarithmic transformations. Folate was energy adjusted by regressing the folate intake for each subject on total energy intake (22). The energy-independent residuals of this analysis were standardized to the predicted folate intake at the average energy intake (9.176 kJ/day) in our population. The mothers were divided into low and high dietary folate intake groups by using the median of the energy-adjusted folate intake of the total group as a cutoff point.

Gene-environment interaction effects were calculated for maternal periconceptional folic acid supplement use and dietary folate intake with the MTHFR C677T and MTHFR A1298C genotypes of mother and infant in the case-control comparison by using odds ratios and 95 percent confidence intervals, after adjustment for maternal educational level.

RESULTS

The characteristics of the study groups are presented in table 1. Educational level was lower for CL(P) mothers compared with controls. Twelve percent more control mothers than CL(P) mothers used folic acid supplements daily during the recommended periconceptional period. Ninety-eight percent of the folic acid users used a daily dose of 400–500 µg, and 93 percent of all users used a tablet containing only folic acid. CL(P) mothers had a lower dietary intake of folate compared with control mothers. Although the former were less educated, maternal educational level appeared not to confound the association between intake of folic acid supplements or dietary folate and risk of delivering a child with CL(P).

MTHFR C677T and MTHFR A1298C polymorphisms

Genetic analysis revealed homozygosity for the MTHFR C677T polymorphism (TT) in six (6 percent) of the CL(P) infants versus four (3 percent) of the controls (table 2), not resulting in a significantly raised odds ratio for the TT genotype compared with the wild type (CC). In addition, we did not find an increased risk of CL(P) for the heterozygous genotype compared with the wild type. Among mothers of CL(P) offspring and mothers of controls, there was a small difference in frequency of the TT genotype, 10 percent and 7 percent, respectively, similar to that for the father’s TT genotype, 9 percent and 6 percent, respectively, but these differences did not substantially affect the risk of CL(P) in the offspring. The frequency of the 677T allele was 27 percent in CL(P) infants and 24 percent in controls, which resulted in an odds ratio of 1.2 (95 percent confidence interval [CI]: 0.8, 1.8). Compared with mothers and fathers of CL(P) offspring, the 677T allele frequencies for mothers and fathers of controls were similar.
We observed no differences in distribution of the \textit{MTHFR} \textit{A1298C} genotypes among case and control infants, their mothers, and their fathers. Frequencies of the \textit{1298C} allele were similar between CL(P) and control infants, between CL(P) and control mothers, and between CL(P) and control fathers. The distributions of the above-described genotypes were all in Hardy-Weinberg equilibrium. In our group of 634 subjects in whom both MTHFR \textit{C677T} and MTHFR \textit{A1298C} polymorphisms were analyzed successfully, only six recombinant genotypes were observed (0.9 percent): five subjects had the \textit{677CT/1298CC} genotype, and one had the \textit{677TT/1298AC} genotype. The frequencies of the composite \textit{MTHFR} genotype distribution were not different between the CL(P) and control groups for the infants, mothers, or fathers (data not shown).

The \textit{T} allele of the \textit{MTHFR C677T} gene was transmitted from a heterozygous parent to the CL(P) offspring 42 percent of the time (table 3). The \textit{C} allele of the \textit{MTHFR A1298C} gene was transmitted 35 times (52 percent). No linkage disequilibrium could be demonstrated for either \textit{MTHFR} polymorphism with CL(P). When we assumed that there were no recombinants among those with the \textit{677CT/1298AC} genotypes, the \textit{677T/1298A} or the \textit{677C/1298C} haplotype of the parents was not transmitted significantly more often to a CL(P) child than the expected 50 percent of the time (table 3).

\begin{table}[h]
\centering
\caption{General characteristics of the cleft lip with or without cleft palate and the control groups, the Netherlands, 1998–2000} \label{tab:1}
\begin{tabular}{lcc}
\hline
& CL(P)$^\dagger$ & Controls $^\ddagger$
\hline
Age in years (mean (SD$^\ddagger$))
\begin{itemize}
\item Mother, at delivery & 31.0 (3.9) & 31.6 (3.6)
\item Infant, at time of study$^\ddagger$ & 1.2 (0.2) & 1.2 (0.4)
\end{itemize}
Low maternal educational level$^\ddagger$ (%) & 57.4 & 43.6$^{**}$
\hline
Periconceptional intake of
\begin{itemize}
\item Folic acid supplements$^\ddagger$ (%) & 32.1 & 44.3$^*$
\item Dietary folate (µg/day)$^\ddagger$ (median (range)) & 186 (80–376) & 199 (96–753)$^{**}$
\end{itemize}
\hline
\end{tabular}
\begin{flushleft}
$^*$ $\chi^2$ test, $p < 0.03$; $^{**}$ Wilcoxon test, $p < 0.01$.
$\dagger$ CL(P), cleft lip with or without cleft palate; SD, standard deviation.
$\ddagger$ n = 130 for CL(P) infants, n = 150 for control infants.
$^\ddagger$ Primary/secondary vocational/intermediate secondary/intermediate vocational education.
$\ddagger$ n = 168 for CL(P) mothers, n = 183 for control mothers.
$\ddagger$ n = 154 for CL(P) mothers, n = 180 for control mothers.
\end{flushleft}
\end{table}

\begin{table}[h]
\centering
\caption{\textit{MTHFR} \textit{C677T} and \textit{MTHFR A1298C} genotypes in infants, mothers, and fathers in association with the risk of cleft lip with or without cleft palate, the Netherlands, 1998–2000} \label{tab:2}
\begin{tabular}{lcccc}
\hline
\textit{MTHFR} genotype & CL(P)/controls (no.) & OR$^*$ & 95\% CI$^*$ & MTHFR genotype & CL(P)/controls (no.) & OR & 95\% CI
\hline
\textbf{Infants}
\begin{itemize}
\item \textit{677 TT} & 6/4 & 1.9 & 0.5, 7.2 & \textit{1298 CC} & 12/11 & 1.4 & 0.6, 3.4
\item \textit{677 CT} & 45/54 & 1.1 & 0.6, 1.8 & \textit{1298 AC} & 34/43 & 1.0 & 0.6, 1.8
\item \textit{677 CC} & 54/70 & 1.0 & Referent & \textit{1298 AA} & 48/61 & 1.0 & Referent
\end{itemize}
\begin{itemize}
\item Total & 105/128 & & & Total & 94/115 & &
\end{itemize}
\hline
\textbf{Mothers}
\begin{itemize}
\item \textit{677 TT} & 15/12 & 1.3 & 0.6, 3.1 & \textit{1298 CC} & 16/16 & 1.3 & 0.6, 2.9
\item \textit{677 CT} & 55/74 & 0.8 & 0.5, 1.3 & \textit{1298 AC} & 52/67 & 1.0 & 0.6, 1.7
\item \textit{677 CC} & 78/84 & 1.0 & Referent & \textit{1298 AA} & 57/76 & 1.0 & Referent
\end{itemize}
\begin{itemize}
\item Total & 148/170 & & & Total & 125/159 & &
\end{itemize}
\hline
\textbf{Fathers}
\begin{itemize}
\item \textit{677 TT} & 12/9 & 1.5 & 0.6, 3.8 & \textit{1298 CC} & 17/17 & 1.1 & 0.5, 2.2
\item \textit{677 CT} & 58/72 & 0.9 & 0.6, 1.5 & \textit{1298 AC} & 48/55 & 0.9 & 0.6, 1.5
\item \textit{677 CC} & 64/73 & 1.0 & Referent & \textit{1298 AA} & 67/71 & 1.0 & Referent
\end{itemize}
\begin{itemize}
\item Total & 134/154 & & & Total & 132/143 & &
\end{itemize}
\hline
$^*$ \textit{MTHFR}, methylenetetrahydrofolate reductase; CL(P), cleft lip with or without cleft palate; OR, odds ratio; CI, confidence interval.
\end{tabular}
\end{table}
MTHFR, Folate Intake, and CL(P)

Interaction of folate and MTHFR C677T and MTHFR A1298C genotypes

Mothers who did not use folic acid supplements periconceptionally and carried the MTHFR 677TT genotype showed almost a sixfold increased risk of delivering a CL(P) offspring compared with supplement users who carried the wild-type genotype (odds ratio (OR) = 5.9, 95 percent CI: 1.1, 30.9) (table 4). No folic acid supplement use in combination with carrying the maternal MTHFR 1298CC genotype also revealed an increased risk of CL(P) birth (OR = 2.2, 95 percent CI: 0.7, 6.5), but this effect was not as strong as it was for the 677TT genotype, and the confidence interval included 1. Comparable results were observed for maternal nonsupplement users with an infant who carried the MTHFR 677TT genotype. No interaction was found for the infant’s MTHFR A1298C polymorphism.

Table 5 shows that mothers who had the MTHFR 677TT genotype and whose periconceptional intake of dietary folate was low had an increased risk of delivering a CL(P) child compared with mothers who carried the wild-type genotype and had a high dietary folate intake (OR = 2.8, 95 percent CI: 0.7, 10.5). No such differences in infants’ genotypes at either marker were seen. On the other hand, mothers who had the homozygous MTHFR A1298C polymorphism and a low dietary folate intake appeared to have a higher risk of delivering a CL(P) offspring compared with mothers who carried the wild-type genotype and had a high dietary folate intake (OR = 2.5, 95 percent CI: 0.8, 7.9). Moreover, after combining maternal MTHFR 677TT genotype with no maternal periconceptional folic acid supplement use and low dietary folate intake, we calculated a 10-fold increased risk of a CL(P) offspring (OR = 10.0, 95 percent CI: 1.3, 79.1). A similar result was found for maternal MTHFR 1298CC genotype (OR = 6.5, 95 percent CI: 1.4, 30.2). These different genotypic distributions, in combination with maternal folic acid supplement use and dietary folate intake, were not observed among infants.

DISCUSSION

This study suggests a gene-environment interaction between maternal periconceptional folic acid supplement use and dietary folate intake and the MTHFR 677TT and MTHFR 1298CC genotypes of the mother on the risk of delivering CL(P) offspring. The results of both the case-control comparison and the family-based transmission disequilibrium test analysis revealed no association of the MTHFR 677TT genotype or the 677T allele with CL(P) risk. Earlier case-control studies conducted in California, Ireland, and Brazil found comparable negative results (10, 11, 23). We previously indicated involvement of the 677TT genotype in Argentine infants (24) and others in Italian mothers (12) in CL(P) risk. These two studies showed a very high proportion of the 677TT genotype in both CL(P) cases and their mothers, 17 percent and 21 percent, respectively, compared with 6–10 percent in our population. The differences in genotype frequencies could merely reflect genetically distinct populations. It is striking to note that our family-based study and none of the other family studies performed thus far have found evidence to support a major role for MTHFR C677T in the development of CL(P) (12, 23, 25), while this study design is the most reliable for detecting an association between DNA sequence differences and a specific abnormality (14).

We found no association or linkage between the MTHFR A1298C polymorphism and CL(P) risk, neither separately nor in combination with the MTHFR C677T polymorphism. Our findings confirm the results of Beaty et al. (13).

Our study suggests that maternal periconceptional use of folic acid supplements is an independent preventive factor for CL(P), which has been proposed by others as well (3–6). Because periconceptional folic acid supplement use was based on retrospective questionnaire information, random misclassification might have occurred, leading to attenuation of our findings. Differential misclassification by contrast is not likely, because participants were unaware of the specific hypotheses of this study. Our reference group of non-folic-acid users included 35 percent of mothers who irregularly.
used supplements or initiated use later than the recommended start of at least 4 weeks before conception. Because as many CL(P) as control mothers belonged to this latter group, our findings therefore were not distorted.

In addition, our data indicate that low maternal periconceptional intake of dietary folate is a risk factor itself. This finding is in contrast to the study by Bower et al. (26), although those authors studied a combination of birth defects. Data on dietary intake were collected by using a semiquantitative food frequency questionnaire. Relative validity was assessed by comparing the data collected from this questionnaire with those drawn from 24-hour dietary recalls, which were repeated 12 times. Correlation coefficients between the two methods for food groups predominantly contributing to folate intake were 0.70 for meat, 0.78 for bread, 0.56 for fruit, and 0.31 for vegetables. For total folate intake, the validity was acceptable and comparable to that for other food frequency questionnaires (17).

We do not expect that, because of the recruitment of controls, selection bias occurred with respect to genotypes; the Dutch Caucasians included were derived from an ethnically homogeneous population. However, the frequency of the MTHFR 677TT genotype in control infants (3 percent) in our study was rather low compared with the range of 4–9 percent reported in a meta-analysis of Dutch controls (27). Unintended selection may have occurred related to the MTHFR genotype distribution, possibly because of the low proportion of eligible control infants for whom genotype data were available. This possibility might have biased the results of the association of the genotypes with CL(P) risk, although even with a low percentage of the 677TT genotype in control infants, no indication of an association between

<table>
<thead>
<tr>
<th>MTHFR genotype</th>
<th>Folic acid supplement use*</th>
<th>CL(P)</th>
<th>Controls</th>
<th>OR†, CI†</th>
</tr>
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<tbody>
<tr>
<td>Infant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.9</td>
<td>3.5</td>
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</tr>
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</tr>
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<td>23</td>
<td>15.8</td>
<td>1.0</td>
</tr>
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</table>

* MTHFR, methylenetetrahydrofolate reductase; CL(P), cleft lip with or without cleft palate; OR, odds ratio; CI, confidence interval.
† Adjusted for maternal educational level.
MTHFR 677TT and CL(P) risk was found. In addition, the interaction of the MTHFR 677TT genotype with folic acid supplement use and/or dietary folate intake cannot have been biased, since distribution of the MTHFR C677T genotypes was not related to intake of folate from supplements or diet.

Control mothers were more educated than CL(P) mothers possibly because well-educated people are more willing to participate than are those with a lower educational level. Selection bias is not expected to have occurred. Use of folic acid supplements and dietary folate intake were not different between mothers with low and high levels of education, and adjustment for maternal education in the interaction of folate intake and MTHFR polymorphisms in association with CL(P) risk hardly changed the results.

We investigated gene-environment interaction of folic acid supplement use and MTHFR polymorphisms and demonstrated a more than fivefold increased risk of delivering a CL(P) child when the maternal 677TT genotype was present together with not using folic acid supplements peri-conceptionally. A comparable, yet inconclusive result was obtained regarding the 677TT genotype of the infant and the 1298CC genotype of the mother. Low dietary folate intake in combination with a 677TT or 1298CC genotype in the mother was associated with an almost threefold increased risk of delivering a CL(P) infant. The CL(P) risk for

| MTHFR genotype | Infant 677 TT | Low | 4 | 4.1 | 4 | 3.2 | 1.4 | 0.3, 6.1 |
| | | High | 2 | 2.0 | 0 | 0.0 | NC* | NC |
| | Infant 677 CT | Low | 20 | 20.4 | 23 | 18.5 | 1.2 | 0.5, 2.7 |
| | | High | 19 | 19.4 | 28 | 22.6 | 1.0 | 0.5, 2.2 |
| | Infant 677 CC | Low | 27 | 27.6 | 31 | 25.0 | 1.1 | 0.5, 2.4 |
| | | High | 26 | 26.5 | 38 | 30.7 | 1.0 | Referent |
| Mother 677 TT | Low | 7 | 5.1 | 4 | 2.5 | 2.8 | 0.7, 10.5 |
| | High | 8 | 5.8 | 8 | 4.9 | 1.7 | 0.6, 4.9 |
| Mother 677 CT | Low | 29 | 21.2 | 41 | 25.1 | 1.0 | 0.5, 2.0 |
| | High | 22 | 16.1 | 30 | 18.4 | 1.2 | 0.6, 2.5 |
| Mother 677 CC | Low | 41 | 29.9 | 31 | 19.0 | 1.8 | 0.9, 3.6 |
| | High | 30 | 21.9 | 49 | 30.1 | 1.0 | Referent |
| Infant 1298 CC | Low | 7 | 8.1 | 6 | 5.4 | 1.5 | 0.4, 5.3 |
| | High | 4 | 4.7 | 5 | 4.5 | 1.2 | 0.3, 5.2 |
| Infant 1298 AC | Low | 13 | 15.1 | 15 | 13.4 | 1.1 | 0.4, 2.8 |
| | High | 17 | 19.8 | 28 | 25.0 | 1.0 | 0.4, 2.3 |
| Infant 1298 AA | Low | 24 | 27.9 | 24 | 21.4 | 1.5 | 0.7, 3.3 |
| | High | 21 | 24.4 | 34 | 30.3 | 1.0 | Referent |
| Mother 1298 CC | Low | 10 | 8.9 | 6 | 3.9 | 2.5 | 0.8, 7.9 |
| | High | 4 | 3.6 | 9 | 5.9 | 0.7 | 0.2, 2.7 |
| Mother 1298 AC | Low | 23 | 20.6 | 31 | 20.2 | 1.1 | 0.5, 2.4 |
| | High | 24 | 21.4 | 33 | 21.6 | 1.2 | 0.6, 2.5 |
| Mother 1298 AA | Low | 26 | 23.2 | 31 | 20.3 | 1.3 | 0.6, 2.7 |
| | High | 25 | 22.3 | 43 | 28.1 | 1.0 | Referent |

*MTHFR, methylenetetrahydrofolate reductase; CL(P), cleft lip with or without cleft palate; OR, odds ratio; CI, confidence interval; NC, not countable.
† The cutoff point for low and high dietary folate intake was defined by the median folate intake of the total group of 357 mothers, calculated after log transformation and energy adjustment. The actual median folate intake in the low intake group was 167 µg/day and 215 µg/day in the high intake group.
‡ Adjusted for maternal educational level.
maternal carriers of the 677TT or the 1298CC genotype was most pronounced (10- and sixfold, respectively) among mothers not using folic acid supplements and having a low periconceptional dietary folate intake. Many of the MTHFR-folate comparisons involved relatively small numbers of study subjects, and some of the elevated risks were consistent with random variation; that is, some confidence intervals included 1.0. It might be possible that some risks associated with folic acid supplement use or dietary folate intake were not truly modified by the MTHFR genotypes. Therefore, we have to be cautious with the interpretation of these results.

DNA analyses were not successful for all members of the CL(P) and control triads studied, resulting in a smaller sample number for each group when we studied the MTHFR polymorphisms and thus in less power to draw firm conclusions. There appeared to be no selective dropping out in the gene-environment interactions, given that, compared with the total group, the difference in folic acid supplement use and dietary folate intake between CL(P) and control mothers was almost similar for those mothers and infants who were successfully genotyped for the MTHFR C677T or MTHFR A1298C polymorphisms. The only exception was that the difference in maternal dietary folate intake for those infants genotyped for the MTHFR C677T polymorphism was smaller compared with the total group. It is not clear whether the finding of no interaction between dietary folate intake and the infant’s MTHFR C677T polymorphism is caused by this selective dropout or is true.

Our study indicates that MTHFR C677T and MTHFR A1298C polymorphisms only increase the risk of delivering a CL(P) child for mothers with a low periconceptional folate intake. In other words, this study suggests that it is possible to overcome the effect of the reduced enzyme activity, as a result of the MTHFR polymorphisms, by increasing maternal folate intake by supplement use and/or in the diet. Frosst et al. (7) hypothesized that folate is able to stabilize the impaired MTHFR enzyme. This hypothesis is supported by studies demonstrating decreased homocysteine levels in MTHFR 677TT women after a period of low-dose folic acid supplementation (28, 29).

The MTHFR genotypes of the mother appeared to be more important than those of the infant or the father. This finding seems logical because the mother provides the environment for the embryo during its development, and the embryo is totally dependent on the folate status of the mother. When the MTHFR enzyme activity of the mother is reduced because of one of the polymorphisms, less 5,10-methylenetetrahydrofolate will be converted to 5-methyltetrahydrofolate and the amount to be transmitted to the embryo will decrease. When the amount of 5-methyltetrahydrofolate transferred from the mother to the embryo is already low, the additional influence of MTHFR enzyme activity of the embryo seems negligible.

The pathogenic mechanism by which a decrease in folate exerts its detrimental effect is not well understood. A few hypotheses exist. When the concentration of 5-methyltetrahydrofolate is reduced, remethylation of homocysteine into methionine consequently will be diminished, and fewer methyl groups will be available for DNA methylation. Hypomethylation can change the transcription and suppression of genes involved in formation of the lip, alveolus, and/or palate. Another hypothesis is that an elevated homocysteine level is teratogenic (30–32).

In conclusion, MTHFR C677T and MTHFR A1298C polymorphisms are not independent risk factors for CL(P). However, low periconceptional folate intake increases the risk of CL(P) in the offspring, and this risk is even more pronounced in mothers carrying the MTHFR 677TT or MTHFR 1298CC genotype.

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