Folate and One-Carbon Metabolism Gene Polymorphisms and Their Associations With Oral Facial Clefts

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Folate metabolism plays a critical role in embryonic development. Prenatal folate supplementation reduces the risk of neural tube defects and probably oral facial clefts. Previous studies of related metabolic genes have associated polymorphisms in cystathionine-beta-synthase (CBS) and 5,10-methylenetetrahydrofolate reductase (MTHFR) with cleft risk. We explored associations between genes related to one-carbon metabolism and clefts in a Norwegian population-based study that included 362 families with cleft palate only (CPO) and 191 families with cleft lip with or without cleft palate (CL/P). We previously showed a 39% reduction in risk of CL/P with folic acid supplementation in this population. In the present study we genotyped 12 polymorphisms in nine genes related to one-carbon metabolism and looked for associations of clefting risk with fetal polymorphisms, maternal polymorphisms, as well as parent-of-origin effects, using combined likelihood-ratio tests (LRT). We also stratified by maternal periconceptional intake of folic acid (>400 µg) to explore gene–exposure interactions. We found a reduced risk of CL/P with mothers who carried the CBS C699T variant (rs234706); relative risk was 0.94 with one copy of the T allele (95% CI 0.63–1.4) and 0.50 (95% CI 0.26–0.96) with two copies (P = 0.008). We found no evidence of interaction of this variant with folate status. We saw no evidence of risk from the MTHFR C677T variant (rs1801133) either overall or after stratifying by maternal folate intake. No associations were found between any of the polymorphisms and CPO. Genetic variations in the nine metabolic genes examined here do not confer a substantial degree of risk for clefts.

Key words: alleles; cleft lip; cleft palate; dietary supplements; folic acid; metabolism; humans; single nucleotide polymorphisms


INTRODUCTION

Folic acid clearly reduces the risk of neural tube defects [MRC Vitamin Study Research Group, 1991; Berry, 1998; Botto et al., 1999]. Whereas early work showed that folic acid could reduce the risk of facial clefts in mouse models [Munger, 2002], epidemiologic studies of facial clefts have provided inconsistent results [Shaw et al., 1995, 2006; Hayes et al., 1996; Werler et al., 1999; Wong et al., 1999; Itikala et al., 2001; Munger et al., 2004; van Rooij et al., 2004]. A recent meta-analysis of 17 prospective and case–control studies supported a protective effect of folic acid supplementation on cleft lip with or without
cleft palate (CL/P) and perhaps cleft palate only (CPO) [Badovinac et al., 2007]. Our own study of facial clefts in Norway provided evidence that dietary supplements with folic acid reduced the risk of isolated CL/P by 39%, but with no apparent effects on CPO [Wilcox et al., 2007]. Genetic polymorphisms in the folate and one-carbon metabolism pathway (Fig. 1) could be associated with risk of oral facial clefts (especially CL/P) possibly interacting with low maternal folic acid intake.

**MATERIALS AND METHODS**

**Participants**

Of 300,000 babies delivered in Norway from 1996 to 2001, 676 were referred for oral facial cleft corrective surgery. Twenty-four babies who died after birth or whose mothers did not speak Norwegian were excluded. From the 652 remaining, 573 (377 CL/P cases and 196 CPO cases) agreed to participate in the study (88%). The number of cases dropped slightly at each subsequent analytic step (Table I). Control families were collected using the same exclusions (763 of 1,022 randomly sampled live births). We used these controls only to estimate Hardy–Weinberg equilibrium (HWE) and population minor allele frequencies (MAFs). Future references to the “full” data set include case families only. All parents provided informed consent. Further details of the overall study design and implementation have been previously published [Wilcox et al., 2007].

**Genotyping**

Genotyping of cases, controls, and their available first-degree relatives was performed using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) multiplex method [Meyer et al., 2004]. We examined 13 frequently-studied polymorphisms in nine genes related to one-carbon metabolism (Table II): solute carrier family 19, member 1 (SLC19A1); transcobalamin II (TCN2); methylenetetrahydrofolate dehydrogenase 1 (MTHFD1); 5,10-methenyltetrahydrofolate dehydrogenase 1 (MTHFD1); 5,10-methylenetetrahydrofolate reductase (MTHFR); 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR); 5-methyltetrahydrofolate-homocysteine methyltransferase.

**TABLE I. Case Family Datasets**

<table>
<thead>
<tr>
<th>Datasets</th>
<th>Cleft lip with or without cleft palate</th>
<th>Cleft palate only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participating families</td>
<td>377</td>
<td>196</td>
</tr>
<tr>
<td>Index cases genotyped</td>
<td>367</td>
<td>194</td>
</tr>
<tr>
<td>&lt;4 Mendelian inconsistencies</td>
<td>362</td>
<td>191</td>
</tr>
<tr>
<td>&lt;400 µg supplemental folate</td>
<td>512</td>
<td>160</td>
</tr>
<tr>
<td>&gt;400 µg supplemental folate</td>
<td>50</td>
<td>31</td>
</tr>
</tbody>
</table>

Number of families participating, index case successfully genotyped for at least one polymorphism, less than four Mendelian inconsistencies, and subdivided at 400 µg supplementation.
TABLE II. MALDI-TOF MS Genotyping of Folate and Homocysteine Pathway Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>rs no.</th>
<th>Type</th>
<th>Location</th>
<th>Call rate</th>
<th>MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHMT</td>
<td>G742A</td>
<td>rs7343890</td>
<td>Nonsynonymous</td>
<td>5: 78457715</td>
<td>0.983</td>
<td>0.20</td>
</tr>
<tr>
<td>CBS</td>
<td>844ins68</td>
<td>n/a</td>
<td>Nonsynonymous</td>
<td>22: 29350060</td>
<td>0.980</td>
<td>0.13</td>
</tr>
<tr>
<td>CBS</td>
<td>G699T</td>
<td>rs234706</td>
<td>Synonymous</td>
<td>22: 29350060</td>
<td>0.983</td>
<td>0.33</td>
</tr>
<tr>
<td>MTHFD1</td>
<td>A1958A</td>
<td>rs2236225</td>
<td>Nonsynonymous</td>
<td>14: 63978598</td>
<td>0.984</td>
<td>0.43</td>
</tr>
<tr>
<td>MTHFR</td>
<td>A1298C</td>
<td>rs1801131</td>
<td>Nonsynonymous</td>
<td>1: 11777065</td>
<td>0.985</td>
<td>0.33</td>
</tr>
<tr>
<td>MTHFR</td>
<td>G677T</td>
<td>rs1801135</td>
<td>Nonsynonymous</td>
<td>21: 43358419</td>
<td>0.978</td>
<td>0.33</td>
</tr>
<tr>
<td>MTR</td>
<td>A2750G</td>
<td>rs1805087</td>
<td>Nonsynonymous</td>
<td>21: 43358419</td>
<td>0.978</td>
<td>0.33</td>
</tr>
<tr>
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<td>rs1801394</td>
<td>Nonsynonymous</td>
<td>21: 43358419</td>
<td>0.978</td>
<td>0.33</td>
</tr>
<tr>
<td>PON1</td>
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<td>rs602</td>
<td>Nonsynonymous</td>
<td>21: 43358419</td>
<td>0.978</td>
<td>0.33</td>
</tr>
<tr>
<td>PON1</td>
<td>T163A*</td>
<td>rs854560</td>
<td>Nonsynonymous</td>
<td>21: 43358419</td>
<td>0.978</td>
<td>0.33</td>
</tr>
<tr>
<td>SLCT19A1</td>
<td>G80A</td>
<td>rs1051266</td>
<td>Nonsynonymous</td>
<td>21: 43782222</td>
<td>0.984</td>
<td>0.43</td>
</tr>
<tr>
<td>TCN2</td>
<td>A67G</td>
<td>rs9606756</td>
<td>Nonsynonymous</td>
<td>21: 43782222</td>
<td>0.984</td>
<td>0.43</td>
</tr>
<tr>
<td>TCN2</td>
<td>C77G</td>
<td>rs1801198</td>
<td>Nonsynonymous</td>
<td>21: 43782222</td>
<td>0.984</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Gene symbol, polymorphism, rs number, type of polymorphism, genomic location (based on Ensembl release 54—October 2007), genotyping call rate, and control population minor allele frequencies (MAF) of the genotyped polymorphisms.

*This SNP had a low call rate, was involved in many Mendelian inconsistencies, and was out of Hardy–Weinberg equilibrium so it was not included in the association analyses. More than three alleles have been reported for this SNP and it was removed from Ensembl in release 44.

methyltransferase reductase (MTRR); betaine-homocysteine methyltransferase (BHMT); cystathionine beta synthase (CBS); and paraoxonase 1 (PON1).

PON1 rs854560 had a lower genotyping call rate (92% vs. 98% for the other polymorphisms). Furthermore, it generated a high level of Mendelian inconsistencies and was not in HWE. For these reasons we excluded PON1 rs854560 from subsequent analyses. Of 194 genotyped CPO cases, genetic information was unavailable for 2 mothers (1%) and 12 fathers (6%). For 367 CL/P cases, genetic information was unavailable for 13 mothers (4%) and 26 fathers (7%).

Folic Acid Supplementation

Soon after delivery, mothers were mailed questionnaires regarding their vitamin intake during each of the 6 months prior to and the first 3 months of pregnancy (questionnaire available online http://www.niehs.nih.gov/research/atniehs/labs/epi/studies/sci/question.cfm). Mothers who reported taking a folic acid supplement or multivitamin during this time were asked for the product name. Women were also asked to send an empty pill bottle or product label to the study office for verification of the amount of folic acid. Women were considered supplemented if they took folic acid for at least 1 month during a 3-month window beginning 1 month before the last menstrual period and continuing through the first 2 months of pregnancy. This covers the crucial time period when the facial structures of the embryonic lip and palate are fusing.

Further details on assignment of folic acid dose are provided elsewhere [Wilcox et al., 2007]. Daily intake of 400 µg or more of folic acid was associated in these data with a 39% reduction in CL/P. No protective effect was seen with lower doses. Also, no protective effect was seen in CPO. We explored interactions of variations in one-carbon metabolism genes with folic acid intake by stratifying women into less than 400 µg/day and more than 400 µg/day.

Statistical Analyses

Familial genotypes inconsistent with Mendelian inheritance were identified with MEGA2 [Mukhopadhyay et al., 2005]. Of the 561 successfully genotyped families, eight had four or more inconsistencies. These were considered higher than expected due to random assay error alone, suggesting that samples may have been mislabeled. The whole families were excluded in subsequent analyses. All markers were tested for HWE using a Pearson’s chi-squared test with one degree of freedom. Disequilibrium can indicate poor assay performance (as in PON1 rs854560) in addition to other possibilities, such as recent admixture.

The remaining 12 polymorphisms were analyzed for child genotype effects, maternal genotype effects, and parent-of-origin (POO) effects using a combined likelihood ratio test (LRT) that utilizes unaffected siblings when parental genotypes are missing [Rampersaud et al., 2007]. The Combined LRT for Candidate Gene Studies, Version 1.0 (www.chg.duke.edu/research/lrt.html) was implemented with SAS (version 9.1.2, Cary, NC) software. Based on the maximum-likelihood-based method for analysis of triad data originally described by Weinberg et al. [1998], this implementation allows unaffected siblings from incompletely genotyped families to contribute to the LRT of association. POO effects are fitted by an iterative method relying on the expectation-maximization (EM) algorithm. When one or both parents are unavailable, sibling genotypes are utilized by the expectation step of the algorithm. No phenotypic information from these individuals is used. The LRTs for child’s genotype risk and maternal genotype risk are 2-df tests, whereas the POO test involves a single parameter and is a 1-df
Wald 95% confidence intervals were calculated from the model coefficient and standard error estimates and exponentiated to determine the relative risk with 95% CIs. We did not undertake a formal test of gene–environment interaction in this dataset as it is not currently implemented with this software. All polymorphisms were tested for child genotype, maternal genotype, and POO in the full dataset as well as in the two subsets stratified by total daily folate supplementation (less than 400 or >400 µg).

RESULTS

The relative risks of CL/P and CPO associated with one or two copies of all polymorphisms in the full data set are shown in Figure 2. Among CL/P, the only LRT reaching statistical significance was for the maternal effect of CBS rs234706 variant (LRT P value = 0.008). Figure 3 includes the relative risks of CL/P and P values for the three LRTs (child, mother, POO) in each of the full dataset and two subsets for the four SNPs that had a P value less than 0.05 on any LRT: CBS rs234706 (Fig. 3A), MTHFR rs1801133 (Fig. 3B), BHMT rs3733890 (Fig. 3C), and TCN2 rs1801198 (Fig. 3D). All three groups showed a protective association with two copies of the CBS rs234706 T variant. Whereas one copy of the T allele in the full dataset was not protective (0.94, 95% CI 0.63–1.4), two copies did show a protective effect (0.5, 95% CI 0.26–0.96). For the <400 µg group, one copy had a relative risk of 0.96 (95% CI 0.62–1.5) and two copies had a risk of 0.61 (95% CI 0.3–1.2). The folic acid-supplemented group showed the same trend (one copy 0.9, 95% CI 0.32–2.5; two copies 0.18, 95% CI 0.03–1.04). Thus, while offspring of mothers homozygous for this CBS variant may have some decreased risk of CL/P, its association was not appreciably modified by maternal folate supplementation.

We did not observe an increased risk of CL/P with the maternal C677T MTHFR T allele of rs1801133 in the full data set (one maternal copy 0.62, 95% CI 0.40–0.93; two copies 0.51, 95% CI 0.26–1.01; Fig. 3B). In the subset of families where mothers took less than 400 µg of folic acid, the MTHFR T allele was protective for the maternal genotype (LRT P = 0.037, one copy 0.60, 95% CI 0.39–0.92; two copies 0.44, 95% CI 0.21–0.95). We saw no evidence for a protective effect in the subset who took more than 400 µg of folate supplementation (one copy...
0.84, 95% CI 0.26–2.6; two copies 0.90, 95% CI 0.16–4.9).

In the subset of 50 families with CL/P and folic acid supplementation, LRT's with \( P \) values less than 0.05 were found for three SNPs. For CBS rs234706, the LRT of child's genotype was 0.02, but the relative risks showed no dose dependent trend for this variant (one copy 0.33, 95% CI 0.11–1.1; two copies 1.3, 95% CI 0.39–4.2). The test of BHMT rs3733890 produced a \( P \) value of 0.04 for maternal genotype, again with no trend according to the number of variant alleles (one copy 1.3, 95% CI 0.44–3.8; two copies 0.78, 95% CI 0.24–2.2).

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**Fig. 2.** (Continued)

**FIG. 3.** Relative risks of CL/P with 95% CIs and LRT \( P \) values in the full, <400, and >400 mg folate datasets. The four SNPs where any LRT \( P \) value was less than 0.05 are shown: (A) CBS rs234706, (B) MTHFR rs1801133, (C) BHMT rs3733890, and (D) TCN2 rs1801198.
FIG. 3. (Continued)
DISCUSSION

These data from a population-based case-control study in Norway provide the largest sample size to date for the analysis of genes related to folate and homocysteine metabolism and oral facial clefts. In this population, folic acid supplements in early pregnancy reduced the risk of CL/P but not CPO [Wilcox et al., 2007], irrespective of genotype. We provide data here on associations of 12 polymorphisms in nine of the one-carbon pathway genes including stratification by maternal periconceptional folate supplementation. Previous studies of these genes in clefts are summarized in Table III.

Folic acid derivatives provide essential single-carbon units for nucleic acid synthesis and methylation reactions—both of which are necessary for cell division, proper gene expression, and maintenance of chromosome structure during fetal development [Morrison et al., 1998]. Polymorphisms in SLC19A1 or TCN2 could impair one-carbon metabolism by limiting the cellular availability of folate or vitamin B12. Reactions within the folate metabolic cycle are complex, with multiple enzymes converting folate into several chemical forms. Genes encoding proteins that perform or support these conversions include MTHFD1, MTHFR, MTR, and MTRR. MTHFR polymorphisms are widely studied in coronary artery disease, neural tube defects, and facial clefts [selected recent publications: Bennouar et al., 2007; Chevrier et al., 2007; Munoz et al., 2007]. The folate cycle is coupled to one-carbon metabolism by the MTR-mediated conversion of homocysteine to methionine. This conversion is also performed by BHMT in a non-folate dependent reaction [van der Linden et al., 2006]. Homocysteine can also be converted to cystathionine by CBS [Morrison et al., 1998], or generated from homocysteine thiolactone by PON1 [Jakubowski et al., 2000].

In this study, mothers with a synonymous SNP, rs234706, in CBS had slight protection from CL/P that was not influenced by periconceptional folate supplementation (Fig. 3A). These results were modest and not concordant with a previous study of this gene in clefts [Rubini et al., 2005]. CBS performs the rate-limiting step in the transsulfuration pathway that degrades homocysteine [Finkelstein, 1990], and this SNP was associated with a decrease in the total homocysteine/cystathionine ratio [Fredriksen et al., 2007]. While the SNP is a silent transition at codon position 233, it may be in linkage disequilibrium with transcriptional regulatory elements [Aras et al., 2000].

Our study provides modest evidence for a protective effect of the C677T allele of MTHFR in CL/P risk (Fig. 3B). This SNP causes an alanine at codon 222 in the catalytic region of the enzyme to be changed to a valine [Frosst et al., 1995]. Enzymatic function is lower in the thermolabile MTHFR protein encoded by this polymorphism, and it has been associated with higher levels of plasma homocysteine and lower levels of folate and betaine [Frosst et al., 1995; Fredriksen et al., 2007]. It is not readily apparent how this variant would provide protection from CL/P in the offspring of mothers who carry it. Some studies

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**Table III. Previous Studies of Folate Gene Associations With Oral Facial Clefts**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of cleft studies</th>
<th>Number with a positive association</th>
<th>Number including folate supplementation</th>
<th>Number with evidence of gene and folate effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC19A1</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TCN2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>MTHFD1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>MTHFR</td>
<td>19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>MTHFR meta-analysis</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>MTR</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>i</td>
</tr>
<tr>
<td>MTRR</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>i</td>
</tr>
<tr>
<td>BHMT2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CBS</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PON1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Performs the same enzymatic step as BHMT.

<sup>b</sup>Including an analysis of a subset of samples from this study.

<sup>c</sup>Based on 10 case-control studies included in the 19 studies above.

<sup>d</sup>Mostowska et al. [2006], Pei et al. [2006], Shaw et al. [2003], Shi et al. [2004], Vieira et al. [2005].

<sup>e</sup>Mostowska et al. [2006].

<sup>f</sup>Beaty et al. [2002], Blanton et al. [2000], Chevrier et al. [2007], Gaspar et al. [2004], Grunert et al. [2002], Jugessur et al. [2003], Martinelli et al. [2001], Mills et al. [1999], Mostowska et al. [2006], Nurk et al. [2004], Pezzetti et al. [2004], Prescott et al. [2002], Shaw et al. [1998], Shi et al. [2004], Shotelersuk et al. [2003], Tolarova et al. [1998], van Rooij et al. [2003], Vieira et al. [2005], Zhu et al. [2006].

<sup>g</sup>Verkleij-Hugoort et al. [2007].

<sup>h</sup>Mostowska et al. [2006], Mostowska et al. [2006].

<sup>i</sup>Mostowska et al. [2006].

<sup>j</sup>Zhu et al. [2005].

<sup>k</sup>Rubini et al. [2005].
have suggested protection from CL/P by the MTHFR T allele in mothers [van Rooij et al., 2003; Gaspar et al., 2004; Nurk et al., 2004] and children [Shaw et al., 1998; Grunert et al., 2002; Shottelersuk et al., 2003; Gaspar et al., 2004], although none reached statistical significance. A meta-analysis including these studies found no effect on CPO (OR 1.0, 95% CI 0.9–1.2) and a slightly increased risk of CL/P (OR 1.2, 95% CI 0.9–1.5).

A similar protective effect of the T allele has been consistently seen in colorectal cancer [Chen et al., 1996; Ma et al., 1999; Slattery et al., 1999; Le Marchand et al., 2002, 2005; Ulvik et al., 2004; Jiang et al., 2005]. One possible explanation is that when folate is low, the wild-type enzyme depletes 5,10-CH₂THF for methylation at the expense of pyrimidine synthesis leading to uracil misincorporation and DNA strand breaks. Thus the TT genotype would slow the conversion of 5,10-CH₂THF to 5-CH₃THF and close this “window of opportunity” for DNA damage [Brockton, 2006].

In this study the protective effect of the mother's or child's T allele was strongest when mothers did not take supplemental folate, as seen in a previous analysis of a subset of these data [Jugessur et al., 2003]. The T allele was also found to be protective against spina bifida occulta and anencephaly [Relton et al., 2003], while it is considered a risk factor for myelomeningocele in some populations [van der Put et al., 1995; de Franchis et al., 1998; O'Leary et al., 2005]. Studies of simulated data suggest that reversals in allelic association could also be due to multi-locus effects or inter-locus correlations [Lin et al., 2007].

Although based on a small sample size, there was a significant LRT of BHMT rs3733890 in the >400 µg folate supplemented subset (Fig. 3C). This polymorphism was also associated with risk in NTD cases whose mothers took supplements with folic acid around conception [Boyles et al., 2006]. The suggestion of a POO effect for TCN2 in >400 µg folate supplemented CL/P cases was based only on a few families (Fig. 3D).

Protection from CPO by folate supplementation is weak, if present at all [Badovinac et al., 2007; Wilcox et al., 2007]. We found no evidence of association between CPO and the polymorphisms studied here. Segregation analyses of CL/P have shown a significant genetic component to clefts but are inconclusive as to the mode of inheritance [Lidral and Moreno, 2005]. An extensive Danish study found evidence of genetic heterogeneity in CL/P [Marazita et al., 1984] making it difficult to identify single gene or environment risk factors associated with risk in all affected people.

If we had corrected for the multiple tests performed, even with a method less stringent than a Bonferroni correction, it is unlikely that even our lowest P-value of 0.008 would remain significant. We also did not perform haplotype-based analysis in the three genes with two polymorphisms. Based on the single polymorphism results there was little reason to believe that such tests would strengthen our results, and this would have further increased the overall number of tests.

Our study fell well short of capturing all the genetic variation in these genes. Expanded genotyping with haplotype tagging SNPs could provide more comprehensive results. Also this set of nine genes does not cover the entire metabolic pathway. For example, mouse knockouts of folate binding protein 1 have major craniofacial defects [Tang and Finnell, 2003]; therefore the human homolog, folate receptor 1, could harbor risk-conferring polymorphisms. Furthermore, the protective effect of folic acid supplementation could act via other developmental pathways. Several WNT signaling pathway genes are regulated by folate and must function properly for normal fetal growth and differentiation [Krapels et al., 2006].

Future research must capture more of the genetic variation in the folate and homocysteine pathway with haplotype tagging SNPs and additional genes. More direct analytical methods for evaluating gene–gene and gene–gene–environment interactions could be more powerful than SNP-by-SNP approaches. Haplotype-based association testing of gene–gene and gene–environment interactions may provide insight into the complex relationship between folate supplementation and the risk of oral facial clefts.

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REFERENCES


