Maternal *MTHFR* genotype contributes to the risk of non-syndromic cleft lip and palate

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Recently, we reported a whole genome scan in sib pairs with non-syndromic cleft lip and palate (CLP), highlighting several regions as possible susceptibility loci, one of which is situated on 1p36.¹ This region is of particular interest in CLP as it harbours the gene encoding *MTHFR*, an enzyme fundamental in the metabolism of the biologically active form of folic acid. Dietary folic acid deficiency has been considered as a candidate environmental factor in the aetiology of CLP in several studies. A study of CLP cases born in the Czech Republic suggested that high dose (10 mg) folic acid supplements taken by pregnant mothers could decrease their chance of having a second affected CLP child by 25–65%.² Similar findings were reported in a case-control study in California for a much lower dose supplementation (<1 mg).³ Also, evidence provided by a Hungarian prospective cohort study and a study of the Hungarian Case-Control Surveillance of Congenital Anomalies data set showed that high dose folic acid supplementation during the critical stages of craniofacial development was the most effective at reducing the occurrence of oral clefting.⁴

The *MTHFR* gene has a functional variant owing to the C677T substitution. This leads to a reduced activity of the enzyme after heating and homozygotes for this heat labile variant have raised plasma homocysteine⁵ and an increased risk for NTDs.⁶ Several case-control studies have attempted to implicate this polymorphism in clefting aetiology but results have not been encouraging. Associations have only been found in small studies that lack corroboration when larger groups are tested.^{7–10}

We have established *MTHFR* genotypes in 243 CLP affected subjects and their parents (226 mothers and 210 fathers) in order to determine if genetic susceptibility to folate deficiency may play a role in the development of oral clefts. Families were recruited from the white UK population via the Great Ormond Street Children's Hospital, London. Appropriate ethical approval was obtained from the Great Ormond Street NHS Trust Research Ethics Committee (No 94CG01). TDT analysis was carried out on subjects with heterozygous informative parents but showed no evidence for the association of CLP with the *MTHFR* T allele (table 1). Previous studies regarding the *MTHFR* heat labile variant indicate that TT homozygotes have low normal serum folate and, therefore, genotype frequencies were calculated to ensure Hardy-Weinberg equilibrium among affected subjects and their parents. All proband genotype frequencies obeyed Hardy-Weinberg equilibrium; C and T allele frequencies were 0.66 and 0.34, respectively, and comparable to control population frequencies in independent studies in the UK.^{11–12}

A recent report by Martinelli *et al*¹³ observed an increase in TT homozygotes among mothers of CLP subjects indicating a possible role for the influence of maternal genotype on fetal folate status with a risk ratio of 2.51 (1.00–6.14).¹³ The authors also commented on the high degree of maternal family history of clefting in this cohort with approximately 50% of mothers either themselves being affected or having an affected parent. Observation of maternal genotype frequencies within our

Table 1 Transmission disequilibrium test of the *MTHFR* 677T variant allele

	Transmitted	Not transmitted	χ^2
Maternal	44	52	0.67
Paternal	46	55	0.8
Total	90	107	1.46

Table 2 *MTHFR* genotype frequencies subdivided by normal and affected/carrier parents

	No	<i>MTHFR</i> genotype		
		CC	CT	TT
Mothers	226	103 (45%)	96 (42%)	27 (12%)
Affected	19	6 (31%)	7 (36%)	6 (31%)
Healthy	207	97 (46%)	89 (43%)	21 (10%)
Fathers	210	90 (42%)	93 (44%)	27 (13%)
Affected	25	11 (44%)	9 (36%)	5 (20%)
Healthy	185	79 (43%)	84 (45%)	22 (12%)

cohort showed no distortion from Hardy-Weinberg equilibrium, but maternal family history was considerably lower in our population with 19/226 (8%) mothers also being affected and no others having any known family history. When families were subdivided by parental affection status a significant distortion in Hardy-Weinberg equilibrium ($\chi^2=6.07$, $p=0.018$) was observed with an increased frequency of TT (31%) in the genotypes of affected mothers of affected probands when compared to healthy mothers (12%) (table 2). No such relationship could be found with TT genotype in affected fathers of affected offspring. Overall this gave an odds ratio of 4.09 (1.32–11.57) and relative risk of 3.11 (1.27–6.15) when affected mothers have the TT genotype, which dropped to 1.91 (0.73–5.61) and 1.29 (0.85–1.66), respectively, when heterozygous mothers were also considered as carriers allowing for a dominant model. This effect would not be observed in a TDT study as it involves homozygous parents, who are automatically dropped from TDT analysis, as they are uninformative. Consideration of paternal family history combined with maternal *MTHFR* genotype did not show any effect, indicating that this interaction is strongest when both contributing factors (family history and *MTHFR* TT) are coincident through the mother.

These findings indicate that maternal folate deficiency may be a contributing factor to cleft aetiology when maternal family history is observed and offer a possible explanation for the conflicting results of previous studies investigating *MTHFR* genotype in relation to CLP. Such results would also suggest the importance of collecting parental and grandparental data

- We genotyped 243 parent-case triads for the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism to determine whether this functional variant is responsible for non-syndromic cleft lip and palate in affected subjects.
- We could find no distortion in the transmission frequency of *MTHFR* parental alleles tested. Examination of Hardy-Weinberg equilibrium detected an over-representation of variant *MTHFR* homozygotes among mothers of affected children when the mothers were themselves affected (odds ratio 4.61, 95% CI 1.35-15.77).
- We postulate that these results are direct evidence of a multifactorial interaction in these families involving folate status, *MTHFR* genotype, and another locus. The need for an independent, confirmatory study is apparent.

when evaluating parental effects. Segregation analyses suggest that multigenic inheritance is highly likely in the aetiology of CLP. The results of this study offer molecular evidence of a multifactorial interaction in this disorder. As yet, it is unclear whether these data suggest a direct multiplicative interaction between the *MTHFR* locus and some other locus yet to be determined, or an indirect, cumulative gene-environment interaction with the TT genotype being a marker for low folate status in mothers. The need for further mapping in these families is apparent.

Non-syndromic CLP is a common craniofacial anomaly affecting between 1 in 700 and 1 in 1000 births in the UK and USA. If the results of this preliminary study withstand further investigation in larger populations, it would offer a potential therapeutic intervention of high dose folic acid supplementation in mothers with a clefting family history, particularly if they were genetically determined to have a low folate status.

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