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Letter to the Editor

Maternal Vitamin Use, Infant C677T Mutation in MTHFR, and Isolated Cleft Palate Risk

To the Editor:

We recently reported the lack of an increased risk of cleft lip with or without cleft palate among infants homozygous for a variant form (C677T/C677T genotype) of the 5,10 methylenetetrahydrofolate reductase (MTHFR) gene [Shaw et al., 1998a]. This variant form codes for a thermolabile enzyme with reduced activity and has been linked to elevated plasma homocysteine levels in C677T homozygous individuals [Frost et al., 1995]. Several groups have also investigated an association between homozygosity for MTHFR mutations and risk for neural tube defects in individuals (e.g., see Kirke et al. [1996] and Shaw et al. [1998b]).

Based on epidemiologic leads that maternal vitamin use in early pregnancy reduces the risks of cleft palate with or without cleft lip and cleft palate alone [Khoury et al., 1989; Shaw et al., 1995], as well as on MTHFR's involvement with folate metabolism, we hypothesized that infants homozygous for the C677T genotype would be at increased risk for clefting owing to the effects of lower MTHFR enzymatic activity. We further hypothesized that elevations in maternal serum folate levels resulting from periconceptional supplementation with folic acid could improve the activity of the poorly functioning MTHFR enzyme, and that clefting risk among infants homozygous for C677T would be lower among those whose mothers periconceptionally used supplements containing folic acid, compared to the group whose mothers did not. Although we did not observe evidence consistent with these hypotheses in our recently reported results pertaining to cleft lip with or without cleft palate [Shaw et al., 1998a], we thought it prudent to investigate whether the association between MTHFR and cleft palate would be the same, given that cleft lip with or without cleft palate and cleft palate alone have been suggested to be etiologically different [Wyszynski and Beaty, 1996].

Data were derived from a large population-based case-control study of fetuses and liveborn infants among a cohort of 1987–89 California births (n = 548,844) [Shaw et al., 1995, 1996]. Women were interviewed in English or Spanish and asked about the types of vitamin supplements they used (prenatal vitamins, multivitamins, vitamin A, folic acid, and other types) for each month during a 4-month time period, which included 1 month before to 3 months after conception. Vitamin users were defined as those women who used vitamin supplements containing folic acid in the period 1 month before to 2 months after conception. Analyses involved 1) case infants diagnosed with isolated (no other major anomaly present) cleft palate whose mothers completed a telephone interview (141 of 160 eligible) *and* whose DNA samples were available from stored newborn screening blood specimens (128 of 141); and 2) control infants, without a congenital anomaly, selected randomly from all infants born alive in the same geographic area and time period as cases, whose mothers completed a telephone interview (734 of 972 eligible) *and* whose DNA samples were available (652 of 734). DNA was extracted from blood specimens by standard laboratory procedures and was amplified using polymerase chain reaction (PCR). Genotyping for the MTHFR mutation was performed by dideoxy DNA fingerprinting of the PCR products [Barber et al., 1998]. Genotyping was performed blinded to subject's case or control status and to maternal multivitamin use status.

To minimize the number of samples for genotyping, the 652 controls were further randomly reduced to 400, 383 of whom were successfully genotyped. Of the 128 isolated cleft palate case infants, 117 were successfully genotyped.

Cases and controls were genotyped TT if homozygous for the C677T allele, CT if heterozygous for the C677T allele, and CC if homozygous for the C677 (wildtype) allele. For cleft palate case infants, 11 (9.4%) were genotyped TT, 50 (42.7%) CT, and 56 (47.9%) CC. For control infants, 49 (12.8%) were genotyped TT, 178 (46.5%) CT, and 156 (40.7%) CC. Odds ratios, reflecting the risk of cleft palate, were 0.6 (95% confidence interval, 0.3–1.3) and 0.8 (0.5–1.2) for infants with TT versus CC and infants with CT versus CC genotypes, respectively. These odds ratios were not substantially dif-

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ferent for male or female infants with TT versus CC genotypes.

Compared to the CC genotype, the odds ratios for cleft palate among infants with the TT genotype were 0.4 (0.2–1.1) for those infants whose mothers were vitamin users, and 0.9 (0.2–3.3) for those infants whose mothers were not users of vitamins containing folic acid in the period 1 month before to 2 months after conception. Among those heterozygous, i.e., genotype CT, cleft palate risks (compared to those with the CC genotype) were essentially the same, irrespective of maternal vitamin use.

These results, similar to our results for cleft lip with or without cleft palate [Shaw et al., 1998a], do not indicate an increased risk of cleft palate alone among infants homozygous for the MTHFR C677T genotype, nor do they indicate an interaction between this infant genotype and maternal supplemental vitamin use on the occurrence of cleft palate. In this study, we genotyped infants rather than their mothers for the MTHFR variant. It is possible that the reduced risk of clefting associated with intake of vitamins relates to correction of a maternal metabolic defect, rather than that of the fetus. Continued investigation of candidate genes in both mother and infant encoding folate-related enzymes, or proteins associated with folate absorption and transport, should be helpful in understanding the mechanism underlying the association between early pregnancy supplementation with folic acid and subsequent reduction in risk for several congenital anomaly phenotypes among newborns.

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