American Journal of Medical Genetics 80:196–198 (1998)

Infant C677T Mutation in MTHFR, Maternal Periconceptional Vitamin Use, and Cleft Lip

Gary M. Shaw,[1]* Rima Rozen,[2] Richard H. Finnell,[3] Karen Todoroff,[1] and Edward J. Lammer[4]

[1]*California Birth Defects Monitoring Program, Emeryville, California*
[2]*Departments of Human Genetics, Pediatrics and Biology, McGill University, Montreal Children's Hospital, Montreal, Canada*
[3]*Department of Veterinary Anatomy and Public Health, College of Veterinary Medicine, Texas A & M University, Austin, Texas*
[4]*Division of Medical Genetics, Children's Hospital, Oakland, California*

Studies have reported an association between homozygosity for a variant form of the methylenetetrahydrofolate reductase (MTHFR) gene and risk for neural tube defects. Because of MTHFR's involvement with folate metabolism and evidence that maternal use of a multivitamin with folic acid in early pregnancy reduces risk for cleft lip with or without cleft palate (CLP), we hypothesized that infants homozygous for the C677T genotype would be at increased risk for CLP because of lower MTHFR enzymatic activity. Data were derived from a large population-based, case-control study of fetuses and liveborn infants among a cohort of 1987 to 1989 California births. The analyses involved 310 infants with isolated CLP whose mothers completed a telephone interview and whose DNA was available from newborn screening blood specimens and involved 383 control infants without a congenital anomaly whose mothers completed a telephone interview and whose DNA was available. 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 (wild-type) allele. Odds ratios for CLP were 0.89 (0.55 to 1.4) and 0.78 (0.56 to 1.1) for infants with TT versus CC and infants with CT versus CC genotypes, respectively. Compared with the CC genotype, the odds ratios for CLP among infants with the TT genotype were 0.74 (0.39 to 1.4) for those infants whose mothers were users and 1.4 (0.54 to 3.6) for those infants whose mothers were not users of multivitamins containing folic acid periconceptionally. The two estimates were not statistically heterogeneous (P = 0.30). Our results did not indicate increased risks for CLP among infants homozygous for the C677T genotype, nor do they indicate an interaction between infant C677T genotype and maternal multivitamin use on the occurrence of CLP. Am. J. Med. Genet. 80:196–198, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: birth defects; gene-environment interaction; vitamins

INTRODUCTION

Several studies have reported an association between fetal homozygosity for a variant form (C677T/C677T genotype) of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene and risk for neural tube defects (NTDs) in individuals [Kirke et al., 1996]. This variant form codes for a thermolabile enzyme with reduced activity and has been linked to elevated plasma homocysteine levels in C677T individuals [Frosst et al., 1995]. It has been hypothesized that maternal folic acid supplementation prevents NTDs by partially correcting this lower MTHFR activity of the variant form of the enzyme. Additionally, evidence has emerged to suggest that maternal use of a multivitamin with folic acid in early pregnancy results in reduced risks for other congenital anomalies such as cleft lip with or without cleft lip palate (CLP) [Shaw et al., 1995]. The underlying process, however, by which maternal vitamin use facilitates a reduction in infant CLP risk is unknown. Because of MTHFR's involvement with the metabolism of folate, we hypothesized that infants homozygous for the C677T genotype would be at increased risk for CLP because of lower MTHFR enzymatic activity. We additionally hypothesized that elevations in maternal serum folate levels resulting from periconceptional

Contract grant sponsor: Cigarette and Tobacco Surtax Fund of California Tobacco-Related Disease Research Program, University of California; Contract grant number: 1RT466 and 3RT0413.

*Correspondence to: Dr. Gary M. Shaw, California Birth Defects Monitoring Program, 1900 Powell Street, Suite 1050, Emeryville, CA 94608.

Received 24 November 1997; Accepted 21 July 1998

© 1998 Wiley-Liss, Inc.

supplementation of folic acid could improve the activity of the poorly functioning MTHFR enzyme and that CLP risk among infants homozygous for C677T would be lower among those whose mothers used periconceptional multivitamin supplements containing folic acid compared with the group whose mothers did not. Thus, we investigated whether such an interaction existed between C677T genotype and maternal multivitamin use on the risk of CLP.

MATERIALS AND METHODS

Data were derived from a large population-based, case-control study of fetuses and liveborn infants among a cohort of 1987 to 1989 California births (n = 548,844) [Shaw et al., 1995; Shaw et al., 1996]. The current analyses were restricted to: 1) those infants diagnosed with isolated (no other major anomaly present) CLP (cases) whose mothers completed a telephone interview (348 of 412 eligible) and whose DNA was available from stored newborn screening blood specimens (310 of 348) and 2) those infants (controls) 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 was available (652 of 734). To minimize the number of samples for genotyping, the 652 controls were randomly reduced to 400, 383 of whom were genotyped.

Interviews were completed in English or Spanish approximately 3.5 years after the date of delivery for cases and 3.6 years for controls. Women were asked about the types of vitamin supplements they used (prenatal vitamins, multivitamins, vitamin A, folic acid, and other types). For each supplement and for each month during a 4-month time period (1 month before conception to 3 months afterwards) women were asked about the frequency of use and the quantity taken. If a woman used a vitamin supplement whose folic acid content could not be determined, the folic acid intake from that supplement was considered to be zero.

DNA was extracted from filter papers by standard laboratory procedures and was amplified using the polymerase chain reaction (PCR). Genotyping for the MTHFR mutation was performed by restriction digestion of PCR products with *Hin*fI [Frosst et al., 1995]. Genotyping was performed blinded to subjects' case or control status and to maternal multivitamin use status.

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. Analyses were performed to estimate CLP risk among infants with either the TT or CT genotypes, compared with infants with the CC genotypes. These analyses were additionally stratified on the basis of maternal multivitamin use. Maternal multivitamin use was considered affirmative if a multivitamin containing folic acid was used in the period 1 month before conception through 2 months afterwards (approximately 90% of women who used vitamins reported daily use). We chose the latter time cutoff because it encompasses the most relevant period of lip and palate morphogenesis, which is usually completed by 60 days.

RESULTS

Overall, case and control infants had similar percentages of TT and CT genotypes (Table I). With respect to race/ethnic background, the percentages of cases versus controls with the TT genotype were: 13.1% versus 10.8% for non-Hispanic whites; 13.6% versus 18.9% for Hispanics; 12.5% versus 15.4% for Blacks; and 8.7% versus 2.3% for "other" groups combined. The odds ratios for CLP among infants with either the TT or CT genotypes were not elevated compared with the CC genotype for all race/ethnic groups combined nor for most of the specific race/ethnic groups. We did observe

TABLE I. Infant MTHFR Genotype and Risk (Odds Ratio) for Cleft Lip With or Without Cleft Lip Palate by Race/Ethnic Group

	Cases		Controls			
Infant MTHFR genotype	No.	%	No.	%	Odds ratio	95% Confidence interval
All Race/ethnic groups						
CC	143	46.1	156	40.7	Reference	—
TT	40	12.9	49	12.8	0.89	0.55–1.4
CT	127	41.0	178	46.5	0.78	0.56–1.1
Hispanic						
CC	31	35.2	35	31.5	Reference	—
TT	12	13.6	21	18.9	0.65	0.28–1.5
CT	45	51.1	47	42.3	1.1	0.58–2.0
White, Non-Hispanic						
CC	93	48.7	84	36.2	Reference	—
TT	25	13.1	25	10.8	0.90	0.48–1.7
CT	73	38.2	116	50.0	0.57	0.38–0.86
Black						
CC	6	75.0	8	61.5	Reference	—
TT	1	12.5	2	15.4	0.67	0.01–16.2
CT	1	12.5	3	23.1	0.44	0.01–7.6
Other, race/ethnic group						
CC	13	56.5	28	68.3	Reference	—
TT	2	8.7	1	2.4	4.3	0.41–45.1
CT	8	34.8	12	29.3	1.4	0.41–5.0

TABLE II. Infant MTHFR Genotype, Maternal Use of Multivitamins Containing Folic Acid, and Risk (Odds Ratio) for Cleft Lip With or Without Cleft Lip Palate Among All Race/Ethnic Groups

Infant MTHFR genotype/maternal vitamin use[a]	Cases no.	Controls no.	Odds ratio	95% Confidence interval
CC/Yes	73	102	Reference	–
TT/Yes	19	36	0.74	0.39–1.4
CC/None	41	27	Reference	—
TT/None	17	8	1.4	0.54–3.6
CC/Yes	73	102	Reference	—
CT/Yes	73	124	0.82	0.54–1.2
CC/None	41	27	Reference	—
CT/None	36	30	0.79	0.40–1.6

[a]Defined as maternal use of vitamin supplement containing folic acid in the period 1 month before through 2 months after conception. Women who began use in the third month post-conception have been excluded from analyses.

an elevated point estimate in the "other" race/ethnic group, but this estimate was very unstable owing to sparse data.

Compared with the CC genotype, the odds ratios for CLP among infants with the TT genotype were 0.74 (0.39 to 1.4) for those infants whose mothers were users and 1.4 (0.54 to 3.6) for those infants whose mothers were not users of vitamins containing folic acid in the period 1 month before to 2 months after conception (Table II). Although the difference between the risk estimates is in the hypothesized direction, i.e., higher risk associated with the TT genotype, the two estimates were not statistically heterogeneous ($P = 0.30$), nor was the observed risk of 1.4 substantially above 1.0. In addition, among those heterozygous (genotype CT), risk estimates were essentially the same irrespective of maternal vitamin use. Data were too sparse to adequately assess risks for the TT genotype by maternal vitamin use strata for each race/ethnic group with the possible exception of non-Hispanic whites. For non-Hispanic whites, the odds ratios for CLP among infants with the TT genotype (compared with CC) were 0.64 (0.30 to 1.4) for those whose mothers used vitamins and 1.8 (0.30 to 7.9) for those infants whose mothers did not use vitamins. These two estimates were not statistically heterogeneous ($P = 0.35$).

DISCUSSION

Our results do not indicate an increased risk for CLP among infants homozygous for the C677T genotype nor do they indicate an interaction between infant C677T genotype and maternal supplemental vitamin use on the occurrence of CLP. Because we did not observe an interaction between maternal vitamin use and infant genotype, it is possible that the reduced risk associated with vitamins relates to correction of a maternal metabolic defect rather than that of the fetus. In NTD studies, the potential role of maternal MTHFR genotype has been suggested [van der Put et al., 1996]. A possible alternative interpretation of our findings is that the mutant genotype is associated with a reduced risk rather than an increased risk for CLP. We observed a lower odds ratio for the TT infant genotype among mothers who used vitamins. A similar lowered risk was observed for the TT genotype and colorectal cancer; this lowered risk was observed only in individuals who were folate-sufficient [Ma et al., 1997]. The suggested mechanism in the colorectal study was that an inhibition of MTHFR activity would result in higher levels of the MTHFR substrate, 5,10-methylenetetrahydrofolate, which is required for conversion of dUMP to dTMP. Increased levels of 5,10-methylenetetrahydrofolate might decrease uracil misincorporation into DNA, which results in DNA damage.

The mechanism underlying the risk reduction associated with maternal folic acid supplementation remains an important question for the etiology of CLP and other congenital anomalies. Investigation of candidate genes encoding other specific folate-related enzymes, or proteins associated with folate absorption and transport, may be revealing.

ACKNOWLEDGMENTS

This work was partially supported by the Cigarette and Tobacco Surtax Fund of California Tobacco-Related Disease Research Program, University of California, 1RT466 and 3RT0413.

REFERENCES

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R (1995): A candidate genetic risk factor for vasular disease: A common mutation in methylenetetrahydrofolate reductase. Nat Genet 510:111–113.

Kirke PN, Mills JL, Whitehead AS, Moloy A, Scott JM (1996): Methylenetetrahydrofolate reductase mutation and neural tube defects. Lancet 348:1037–1038.

Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens CH, Rozen R (1997): Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 57:1098–1102.

Shaw GM, Lammer EJ, Wasserman CR, O'Malley CD, Tolarova MM (1995): Risks of orofacial clefts in children born to women using multivitamins containing folic acid periconceptionally. Lancet 345:393–396.

Shaw GM, Wasserman CR, Lammer EJ, O'Malley CD, Murray JC, Basart AM, Tolarova MM (1996): Orofacial clefts, parental cigarette smoking, and tranforming growth factor-alpha gene variants. Am J Hum Genet 58:551–561.

van der Put NMJ, van den Heuvel LP, Steegers-Theunissen RPM, Trijbels FMJ, Eskes TKAB, Mariman ECM, den Heyer M, Blom HS (1996): Decreased methylenetetrahydrofolate reductase activity due to the 677C-T mutation in families with spina bifida offspring. J Mol Med 74:691–694.