Folate-Related Genes and Omphalocele

James L. Mills,1* Charlotte M. Druschel,2 Faith Pangilinan,3 Kenneth Pass,4 Christopher Cox,1 Rebecca R. Seltzer,3 Mary R. Conley,1 and Lawrence C. Brody3

1 Division of Epidemiology, Statistics and Prevention Research, NICHD, NIH, DHHS, Bethesda, Maryland
2 Congenital Malformations Registry, New York State Department of Health, Albany, New York
3 Genome Technology Branch, NHGRI, NIH, DHHS, Bethesda, Maryland
4 Wadsworth Center, New York State Department of Health, Albany, New York

Women who take folic acid in the periconceptional period greatly reduce their chances of having a child with a neural tube defect (NTD). Using multivitamins may also reduce the risk of having a child with an omphalocele. In this study, we tested single nucleotide polymorphisms in folate-related enzyme genes for association with omphalocele. Polymorphisms in methylenetetrahydrofolate reductase (MTHFR), methylenetetrahydrofolate dehydrogenase (MTHFD1), the reduced folate carrier (SLC19A1), and transcobalamin II (TCN2) were examined in 25 children with euploid omphalocele and 89 matched controls. Omphalocele cases were significantly more likely to carry the T allele of MTHFR 677C→T, a known risk factor for NTDs (odds ratio 3.50, 95% confidence interval 1.07–11.47, P = 0.035). The MTHFD1 R653Q, SLC19A1 R27H, and TCN2 P259R polymorphisms showed no significant association with omphalocele. In this small study, the thermolabile variant of MTHFR, 677C→T, was associated with an increased risk for omphalocele. This variant causes reduced enzyme activity, thus suggesting a mechanism by which multivitamins with folic acid might prevent omphalocele. Additional investigation is required.

Published 2005 Wiley-Liss, Inc.

KEY WORDS: omphalocele; ventral wall defects; folic acid; multivitamins; vitamins; birth defects; MTHFR, MTHFD1; reduced folate carrier; transcobalamin II

INTRODUCTION

It is well known that folic acid, taken periconceptionally, can prevent neural tube defects (NTDs) [MRC, 1991; Cezeil and Dudas, 1992] and there is considerable evidence that folate-related genes play an important part in susceptibility to NTDs. In particular, a variant in methylenetetrahydrofolate homocysteine methyltransferase (commonly known as methylenetetrahydrofolate reductase), MTHFR 677C→T (A222V) has been shown to be a risk factor for NTDs [van der Put et al., 1995; Whitehead et al., 1995; Botto and Yang, 2000]. We have recently reported that mothers who have the trifunctional enzyme—methylenetetrahydrofolate dehydrogenase (5,10 methylenetetrahydrofolate dehydrogenase, 5,10 methylenetetrahydrofolate cyclohydrolase, 10 formyltetrahydrofolate synthetase) variant MTHFD1 R653Q are at significantly increased risk of having children with NTDs [Brody et al., 2002]. Other gene variants including those for transcobalamin II (TCN2) and the reduced folate carrier (SLC19A1, also known as RFC1) have also been associated with increased risk of NTDs [De Marco et al., 2003; Pietrzyk and Bik-Multanowski, 2003] in association with low vitamin B12 levels [Morin et al., 2003], but not in all studies [Afman et al., 2002; Shaw et al., 2002].

Omphalocele is a defect of the anterior abdominal wall that ranges in birth prevalence from 0.8 to 3.9 per 10,000 births [Stoll et al., 2001]. Many cases have chromosomal abnormalities or syndromes, but the etiology of the remaining cases is largely unknown.

Women who use multivitamins, most of which contain folic acid, have been reported to be at reduced risk for having children with omphalocele [Botto et al., 2002]. We hypothesized that folate-related genes may be important in the etiology of omphalocele as well as NTDs. Because non-aneuploid omphalocele cases are rare and it is difficult to find large numbers for study, we present the results of a pilot study addressing this hypothesis.

MATERIALS AND METHODS

Omphalocele was defined as a midline abdominal wall defect limited to an open umbilical ring. The viscera herniate into the base of the umbilical cord and are covered by an amnioperitoneal membrane.

Subjects for this study were selected from all births in New York State for the years 1998 and 1999. Omphalocele cases for this investigation came from the New York State Congenital Malformations Registry (NYSCMR), one of the largest state-wide, population-based birth defects registries in the United States [New York State Department of Health-Congenital Malformations Registry Annual Report]. Cases are ascertained passively via reports from physicians and hospitals. Reporting of major congenital malformations diagnosed from birth through 2 years of age is required by law. For each defect, the reporting physician or hospital is asked to provide a narrative describing the defect. Malformation rates obtained for New York State are similar to those obtained by systems that use active case finding [Salihu et al., 2003]. To help ensure completeness of reporting, a second data source is used. NYSCMR cases are matched to hospital discharge data also filed with the New York State Department of Health. Details on any cases on the NYSCMR list that the hospital did not report are requested directly from the hospital. A recent capture-recapture analysis showed that the NYSCMR was about 87% complete [Honein and Paulozzi, 1999]. A similar estimate of 89% was recently obtained after matching Congenital Malformations Registry reports (New York State Department of
performed well with fairly small samples, as long as the number within each racial/ethnic group. The Mantel–Haenszel test was used for analysis. The samples were stratified by race/ethnicity. In either case, the data can be collapsed to a two-by-two table.

genotypes are assumed to be identical, and a recessive model, in which the effects of the homozygous and heterozygous genotype could be examined together, the Breslow–Day test for homogeneity of odds ratios was applied. Odds ratios were homogeneous for all except the test for a dominant association. Therefore, data for the T allele (dominant test) of MTHFD1 677 and omphalocele, adjusted odds ratio 3.50, 95% confidence interval 1.07–11.47, \( P = 0.035 \). See Table I. The extended rule of five indicated that the chi-square approximation was adequate (see Materials and Methods).

RESULTS

After exclusion of cases with chromosomal abnormalities, there were 37 omphalocele cases without known chromosomal abnormalities identified among the 546,050 births in New York in 1998–1999. Of these, 25 had samples available for DNA testing; 12 did not because the samples had been used up in the neonatal screening process. Nineteen cases had isolated omphalocele; six had other defects: one had cloacal extrophy, one had a cardiac malformation, two had renal defects, and two had Beckwith–Wiedemann syndrome. There were 10 male and 15 female cases. The ethnic distribution was 12 Non-Hispanic White (48%), 7 Non-Hispanic Black (28%), 0 Asian (0%), 5 Hispanic (20%), and 1 other (4%). For all births over the same time period and geographical area, there was a slightly higher proportion of Non-Hispanic Whites (53.4%) and Asians (6.7%), and a lower proportion of Non-Hispanic Blacks (18.8%). The proportion of Hispanics was almost identical (20.5%). As expected because of the matching, the racial distribution of the cases and controls did not differ \( (P = 1.0) \), nor did the proportion of female cases (60%) compared to female controls (61%) \( P = 0.9 \). The mean age of the case mothers (27.8 years) did not differ significantly \( (P = 0.5) \) from the mean age of the control mothers (28.8 years). As the samples were anonymized prior to testing, information on socioeconomic status and other demographic characteristics was not available. There were 59 controls matched as described above to the cases.

To determine whether data from different ethnic groups could be examined together, the Breslow–Day test for homogeneity of odds ratios was applied. Odds ratios were homogeneous for all except the test for a dominant association between omphalocele and TCN2. Therefore, data for the different ethnic/racial groups are presented together in Table I. There was a significant association between the T allele (dominant test) of MTHFR 677 and omphalocele, adjusted odds ratio 3.50, 95% confidence interval 1.07–11.47, \( P = 0.035 \). See Table I. The extended rule of five indicated that the chi-square approximation was adequate (see Materials and Methods). The test for a recessive association was not significant; however, there were only six subjects (one case and five controls) with the TT genotype. When we repeated the analysis excluding the two Beckwith–Wiedemann cases, the association between the T allele (dominant test) and omphalocele had an odds ratio of 2.90, 95% confidence interval 0.89–8.85, \( P = 0.07 \). The MTHFD1, TCN2, and SLC19A1 polymorphisms showed no significant association with omphalocele, either as dominant or recessive effects (Table I). The TCN2 polymorphism did not have homogeneous odds ratios by race/ethnicity (the Breslow–Day test for homogeneity was statistically significant for a test of dominant effect) so the data were examined for each race separately. There was no significant association between this polymorphism and omphalocele for any race/ethnicity.
There was no evidence of an interaction between MTHFR and SLC19A1 (P = 0.77).

**DISCUSSION**

To the best of our knowledge, this is the first study to look for an association between folate related genes and omphalocele. Studying the etiology of omphalocele is difficult because so many cases are aneuploid and must be excluded. Despite this limitation, we were able to demonstrate that the MTHFR CT plus TT genotypes were significantly more common in omphalocele cases than in controls. MTHFR is an important gene in folate metabolism, providing methyl groups for the conversion of homocysteine to methionine, and subsequent methylation reactions. The MTHFR 677 C→T variant has reduced biological activity. The MTHFR 677 C→T polymorphism is also a well known risk factor for NTDs [van der Put et al., 1995; Whitehead et al., 1995; Botto and Yang, 2000] and periconceptional vitamin use greatly reduces the risk of NTDs. Thus, our finding that MTHFR is associated with omphalocele provides biological support for, and a potential mechanism to explain, the observation that women who take multivitamins are at lower risk for having a child with an omphalocele [Botto et al., 2002].

Most studies of MTHFR 677 C→T and NTD have reported an association with the homozygous variant (TT). In contrast, we found that omphalocele is associated with the combined CT and TT group. We have shown recently [Kirke et al., 2004] that the CT genotype is also a statistically significant risk factor for NTDs. Relton et al., 2004 have recently reported that the T allele is a risk factor as well. The meta-analysis by Botto and Yang [2000] also showed that the CT heterozygote was a risk factor for NTDs, but the association did not quite reach statistical significance. The most likely reason that we did not see a TT effect is that we had very few subjects with the TT genotype in this study, limiting our power to find an association for TT alone.

Some limitations of our study should be noted. This is a small study with limited power to rule out associations. Thus, we would not claim that our data totally exclude the possibility that the variants we tested in TCN2, SLC19A1, or MTHFD1 could be related to omphalocele. Although we selected SNPs that are known to alter function, we emphasize that other variants in these genes that were not examined could be risk factors. Because the birth prevalence of omphalocele is low, we pooled genotype data from our cases from different racial/ethnic groups. Because allele frequencies differ by racial/ethnic group, population stratification may be a confounder.

To reduce the risk of population stratification, all cases were matched to controls on race/ethnicity [Wacholder et al., 2002]. The method used to classify race/ethnicity was the same for cases and controls, and was independent of diagnosis. Such a study design greatly reduces the likelihood of stratification artifacts. Moreover, the Mantel–Haenszel test was stratified by race, and the analysis included a test for racial heterogeneity. Data on maternal use of folic acid supplements and dietary folate consumption would have been useful to determine how the risk of omphalocele was affected by folic acid exposure. Collecting such data prospectively on the half million plus pregnancies from which our cases and controls were identified, however, was impossible and ethical constraints prevented us from interviewing the control subjects to gather the data retrospectively. Maternal DNA could not be collected, making it impossible to test for maternal risk factors. Lastly, we tested a number of hypotheses, so positive findings could have occurred by chance. The fact that MTHFR 677 C→T is an established risk factor for birth defects makes this less likely. Our statistical analysis was very conservative; all studies show that the T allele only increases the risk for birth defects. Our statistical testing was two-sided, that is, assuming that the T allele could also prevent birth defects. If we use a one-sided test, our P values for the association between the T allele (dominant test) and omphalocele become P = 0.018 for the entire cohort and P = 0.035 when Beckwith–Wiedemann cases are excluded. We would encourage others to follow up on our findings on the relationship between omphalocele and these folate-related genes.

In summary, this study found that MTHFR 677 CT and TT genotypes were significantly more common in children with omphaloceles than in normal control children. MTHFR 677 C→T causes reduced function in vivo leading to lower red cell folate and higher homocysteine levels. Thus, our finding that MTHFR 677 C→T is associated with omphalocele supports the observation that folic acid containing multivitamins may prevent omphalocele.

**ACKNOWLEDGMENTS**

Dr. Pangilinan is a fellow in the Pharmacology Research Associate Program supported by NIGMS, NIH.

**REFERENCES**

Omphalocele and Folate-Related Genes


