SPINA BIFIDA, FOLATE METABOLISM, AND DIETARY FOLATE INTAKE IN A NORTHERN CANADIAN ABORIGINAL POPULATION

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ABSTRACT

Objectives. Inhabitants of the subarctic region of the Eastern James Bay of Northern Quebec consume a diet low in folate. This is largely secondary to poor access to plant-foods and a preferred diet high in meat, fowl, and fish as in many other northern populations. Furthermore, there is a high frequency of spina bifida in the Cree of the region. It was hypothesized that genetically altered folate metabolism as well as low folate intake contributes to the high frequency of spina bifida. Methods: A case-control study evaluating folate metabolism and the common 677C-T polymorphism of the gene for methylenetetrahydrofolate reductase (MTHFR) in mothers of children with spina bifida, and controls (n=23) of Cree descent from the Eastern James Bay region. These results were compared to a similar Montreal cohort (n=152) who were not of First Nations descent. Dietary intake of folate of 219 women of the Eastern James Bay region was also determined. Results: No Cree mothers of children with spina bifida were homozygous for the 677C-T polymorphism of MTHFR. Although serum cobalamin was significantly higher in Cree mothers, RBC folate was significantly lower than in the Montreal cohort. In addition, plasma homocysteine was significantly lower in the Cree. Dietary intake of folate of women in the same region was substantially lower (100 μg/day) than widely recommended daily intakes.

Conclusions. In this remote Canadian aboriginal community there is no evidence of altered folate metabolism in the mothers of children with spina bifida. Nonetheless, it remains essential that culturally appropriate public health efforts be continued to increase the intake of folic acid in the hope of reducing the high frequency of spina bifida in this population.

Key words. Neural tube defects, Spina Bifida, folate, Homocysteine, Cobalamin, MTHFR, North American Indian, Native Canadian, Eastern James Bay Cree, Diet Survey, Nutrient Intake, Traditional food

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The relationship between folate and birth defects has been studied for more than 20 years (1). There is now overwhelming evidence that the recurrence (2) and occurrence (3) of isolated neural tube defects is significantly reduced with supplemental folic acid (4, 5) This reflects the importance of folate in embryogenesis (6). At least one enzyme of folate metabolism has been implicated as a predisposing genetic factor for neural tube defects when it is present in an unstable state. The 677C-T (A to V) polymorphism of the gene for methylenetetrahydrofolate reductase (MTHFR) has been shown to be present in increased frequency in some studies of patients with neural tube defects and in their mothers (7-9), however, few population isolates with high rates of neural tube defects have been studied (10).

Northern Quebec has been inhabited by ancestors of the modern day Cree for more than 5,000 years. Today the more than 10,000 Cree of Eastern James Bay comprise about 25% of the total Native population of Quebec and inhabit nine coastal and inland communities (11). Their traditional lifestyle is reflected in hunting and fishing practices, and the preferred diet reflects existence in a harsh northern, subarctic environment where main nutrients have traditionally been derived from meat, fowl, and fish, rather than plant foods (12). As with many Canadian northern populations, folate is considered to be a nutrient of concern (13). Although little is known about rates of spina bifida in other Canadian First Nations populations, the apparently high rate of spina bifida in the Cree of the Eastern James Bay Region, (calculated to be 1/260 live births*), impacts significantly on the health care needs of the region.

In conjunction with a larger Canadian study examining specific factors of folate metabolism and their relationship to spina bifida (14), we evaluated folate status and the prevalence of the 677C-T (A to V) polymorphism of the gene for MTHFR in mothers of Cree children affected with spina bifida. In addition, the daily folate intake of Cree women in the region was evaluated to determine whether specific public health efforts should be directed to this population.

* The rate of 1/260 live births with spina bifida was observed between January of 1991 and January of 1996, where there were 6 cases of spina bifida in a cohort of 1,565 live births over the period. This estimate does not include stillbirths or fetal deaths, therefore may be an underestimate. Lumbar/sacral spina bifida accounted for all known cases of neural tube defects.

PATIENTS AND METHODS

All known mothers of children with spina bifida of Cree descent (group 1; n=12) living in the Eastern James Bay region, were ascertained from the Montreal Children's (MCH) Spina Bifida Clinic, and the Waskaganish Health Center for this case-control study after approval of the MCH Institutional Review Board and the Cree Health Board. They lived in 5 different Eastern James Bay villages, although several of the cases could be traced back to 2 founding families in one village, Waskaganish. Ten of 12 cases had sacral or low lumbar level lesions. There were three comparison groups. Mothers of children without spina bifida of Cree descent were ascertained in Waskaganish (group 2; n=11), mothers of children with spina bifida (group 3; n=62), and mothers of children without spina bifida (group 4; n= 90) who were not of Cree descent. Group 2 was ascertained through advertisement in the Waskaganish Health Centre. Cree women participating were not known to be related to the cases, however distant relatedness could not be ruled out because of the small community size. All women participating as controls were mothers of children without spina bifida, or other birth defects.

The ascertainment of Groups 3 and 4 was through the MCH Spina Bifida Clinic and the hospital ambulatory test center, respectively, where the population is known to be approximately one third French speaking, one third English speaking Canadians and the remainder of various ethnic backgrounds and languages (15). Those in Group 4 were mothers of children without spina bifida or other major birth defects. The mean ages of the 4 groups of mothers were not significantly different being 33, 34, 36 and 37, respectively. Mothers taking vitamin supplements containing folate or cobalamin at the time of the study were excluded (n=14, 1 mother of Cree descent, and 13 mothers not of Cree descent). Blood for folate status, cobalamin, plasma homocysteine, and MTHFR genotypes was drawn on participants after individual informed consent was obtained.

Determination of folate, cobalamin, and total homocysteine (tHcy)

Red blood cell folate (RBC folate) and cobalamin were quantitated by routine using an automated system and reagents from Ciba (Ciba Corning Diagnostics Corp., Medfield, MA, USA). All determinations were done at the same laboratory. For plasma tHcy determinations, blood samples were drawn in vacutainers containing EDTA, and kept

on ice until plasma was separated. Plasma was separated by centrifugation and the supernatant was collected and frozen at -20° C until analysis. tHcy was determined by high-pressure liquid chromatography as previously reported (16). The tHcy adduct was detected by fluorescence after precolumn derivation with the thiol-specific reagent 7-fluoro-benzene-2-oxa-1,3-diazole-4-sulphonate (SBD-F) (Wako, TX).

Determination of MTHFR genotypes

DNA was isolated from peripheral leukocytes by extraction with phenol-chloroform after cell lysis in a buffer containing Nonidet-P40 (Boehringer Mannheim, Mannheim, Germany) and stored at -20°C. The presence of the 677C-T polymorphism in MTHFR was determined by PCR followed by restriction digestion with Hinfl, as described (17).

Statistical Analyses

Computer assisted statistical analyses were carried out with Microsoft Excel. Standard summary statistics, ANOVA, t-tests, odds ratios, confidence intervals were used where appropriate. Statistical significance was interpreted as P-values of $p<0.05$.

Folate Intake in Cree women of the Eastern James Bay Region

In a previous study determining traditional and market food use of the Cree of the Eastern James Bay region, one of the authors, TD, carried out a 24-hour dietary recall study in two seasons on Cree women of two Eastern James Bay communities, Eastmain and Wimindji (18) reflective of the dietary practices of the Cree participants in this study. Based on these dietary recalls, for this study, daily intake of folate, in women of 3 age groups (21-40 years n=89,41-60 years n= 82 and over 60 years, n=48 total n=219) was determined. Dietary intakes of folate reflect the mean intake determined from market food only.

RESULTS

MTHFR polymorphism. There were no Cree mothers of children with spina bifida homozygous for the 677 C-T MTHFR polymorphism (0/12) (see Table 2). One control mother of Cree descent (group 2) was homozygous (1/11) for the TT allele. Although the difference does not reach statistical significance, the frequency in Groups 1 and

2 is lower than both non-Cree groups (3 and 4) where 17% and 11% mothers of cases with spina bifida and mothers of control children respectively were homozygous. Although the number of Cree participants is small, and not intended to determine population allele frequency, T allele frequency in the Cree study populations is significantly less than the non-Cree populations in our study (16 and 18% Vs 40 and 31%).

RBC Folate. Table 1 illustrates the RBC folate for the 4 groups of mothers. Mothers of Cree descent of children with spina bifida had lower red cell folate levels (484 nmol/l ±74,) than the control mothers of Cree descent (569±126) ($p<.05$), and each of the groups not of Cree descent (527±236 and 694±308) for case and control mothers respectively. Although no mother of Cree descent had a level that would be considered pathologically low, the largest difference was between the means of group 1 (mothers of Cree descent of children with spina bifida) and group 4 (control mothers not of Cree descent). Two way ANOVA was significant comparing Cree versus non-Cree and cases versus controls ($p=0.0002$).

Cobalamin. As depicted in Table 1, serum cobalamin was significantly higher in the Cree than in the non-Cree groups. The highest levels were seen in the Cree mothers of children with spina bifida (417 pmol/l±105) and this was significantly higher than the mothers of children not of Cree descent (cases: 298 pmol/l± 186 and controls: 350 pmol/l±135 ($p<0.05$). There was no significant difference in se-

Table 1:
RBC folate, serum cobalamin and homocysteine results

	Group 1 Mothers of cases (Cree), n=12	Group 2 Mothers of controls (Cree), n=11	Group 3 Mothers of cases (non-Cree), n=62	Group 4 Mothers of controls (non-Cree), n=90
RBC folate (nmol/l)	484 ± 74 n=12	569 ± 126 n=11	527 ± 236 n=59	694 ± 308 n=90
Serum cobalamin (pmol/l)	417 ± 105 n=12	369 ± 104 n=10	298 ± 186 n=59	350 ± 135 n=88
tHcy (μmol/l)	7.2 ± 1.1 n=11	7.2 ± 1.7 n=11	10.0 ± 3.5 n=61	9.9 ± 4.2 n=90

Values shown are means ± standard deviations

rum cobalamin levels between the Cree mothers of children with spina bifida and those without (p=0.15). Consistent with these results, the 2 way ANOVA (Cree versus non-Cree and cases versus controls) demonstrated a trend toward significance at p=0.06.

Total homocysteine (tHcy). The Cree study groups had the lowest levels of plasma tHcy, at 7.2 μmol/l ± 1.0, 7.2 μmol/l ± 1.7 compared to the non-Cree groups at 10.0 μmol/l ± 3.5, 9.9 μmol/l± 4.5 for group 3 and 4 respectively (Table 1). There was no significant difference between the 2 Cree groups, and between the 2 non-Cree groups; however, the Cree mothers in both the case and control groups had significantly lower plasma homocysteine when compared to the mothers of non-Cree descent (p<.01).

Folate intake in Cree women of the Eastern James Bay Region. Folate intake based on 24 hour recall of market foods of the two seasons, winter and summer, divided according to age group was; age group 1 (21-40 years of age; n=89) 129 μg/day, age group 2 (41-60 years of age n=82) 94 μg/day and age group 3, over 60 years (n=48) 61μg/day. The average daily intake of folate for all women studied in this cohort was 98 μg/day.

DISCUSSION

Rates of neural tube defects (NTD's) vary ethnically and geographically, reflecting the genetic and environmental nature of multifactorial inheritance. Although increased rates of neural tube defects have been reported previously in specific populations, such as in the UK (2), Newfoundland (4), the Sikhs of BC (19) and rural blacks of South Africa (10), increased rates are rarely reported in North American First Nations populations (20). Conversely, Lowry et al (21) reported a lower rate of neural tube defects in American Indians of British

Table 2:
Genotypes and frequencies of methylenetetrahydrofolate reductase 677C-T alleles in spina bifida cases from Northern Canada.

Number of alleles/all	Group 1 Mothers of cases (Cree), n=12	Group 2 Mothers of controls (Cree), n=11	Group 3 Mothers of cases (non-Cree) n=62	Group 4 Mothers of controls (non-Cree), n=90
TT (homozygote)	0/12	1/11	11/62	10/90
CT (heterozygote)	4/12	2/11	27/62	36/90
CC (wildtype)	8/12	8/11	24/62	44/90
Total allele frequency	.16	.18	.40	.31

Columbia compared to the general population (1.06 Vs 1.6 /1,000 births), although a higher rate of congenital heart malformations and facial clefts was noted.

The diets of northern aboriginal populations of arctic and subarctic regions have a high component of meat, fowl and fish rendering them high in protein, cobalamin, and 3-omega fatty acids (22). Reflecting the harsh environment and difficulties inherent to transporting fresh produce to remote locations they are however, low in plant food, thus low in folate. It is well documented that folate is a nutrient of concern for many North American northern aboriginal populations (12, 24-26). Our study supports this view with evidence of low folate intake in women living in the region where a high frequency of spina bifida has been documented. Although folate was measured only from market food, perhaps underestimating the true folate intake, the most commonly eaten traditional foods for this population (for example, goose flesh, whitefish, beaver, rabbit) are poor sources of folate, containing between 3 and 5 micrograms/100 grams, thus adding very little folate to the total amount calculated for market foods. Supportive of the dietary data, RBC folate in the mothers of children with and without spina bifida from the James Bay region is low and serum cobalamin was significantly higher in these women than in the non-Cree women.

Sufficient folate and cobalamin as well as functional MTHFR and methionine synthase are necessary to drive the remethylation pathway of homocysteine to methionine. A deficiency in any one of the factors needed to drive the cycle, would presumably lead to an elevation of homocysteine, which has been speculated to be a risk factor, or at least, a marker of neural tube defects (27, 28). Interestingly, in our study, although the RBC folate was low in Cree mothers, the homocysteine was also unusually low when compared to the other groups. It is possible that the high cobalamin or other as yet undetermined factors may perform a compensatory function, maintaining normal homocysteine metabolism. For example, it has been observed clinically that high doses of parenteral cobalamin reduces hyperhomocysteine in those with end stage renal disease (29). It could also be hypothesized that genetic selection has favored establishing a metabolic mechanism protective for the low folate intake, preventing hyperhomocysteinemia.

Furthermore, the 677C-T mutation, known to predispose to increased homocysteine in its homozygous state and in conjunction

with low folic acid intake is not common in the Cree population studied. The current study and sample size of the Cree was not designed to determine allele frequency in this population. However, it is interesting that a particularly low frequency of the 677C-T allele (6%) has been noted in the Cree's northern neighbors, the Inuit (30). Although it would be tempting to speculate that selection against the thermolabile polymorphism in a low folate environment has contributed to the low allele frequency in both the Cree and the Inuit populations, low allele frequency may also be representative of allele frequency in founding populations. The HuGe review by Botto and Yang (31) reveals great variation in 677C-T allele frequency worldwide, but to our knowledge there has been no correlation with allele frequency and availability of dietary folate.

After more than 20 years of studies, it is now evident that 50-75% of neural tube defects (as well as other birth defects (32-35) can be prevented with the use of periconceptional folate. In studies of alterations in folate metabolism and neural tube defects, only 10-20% of affected mothers and cases have shown definitive evidence of abnormalities, such as the common MTHFR polymorphism (36, 37) and indeed, the well studied 677C-T polymorphism is not common in the Cree individuals evaluated for this study, and particularly is not a maternal predisposing factor to spina bifida in this cohort. Although other common mutations of genes involved in folate metabolism are under study, it remains unclear whether these will prove to be significant risk factors for NTD's (14, 38). Thus a significant number of neural tube defects, considered amenable to prevention with folate, occur without documented abnormalities in folate metabolism. Although dose-response relationships between RBC folate and neural tube defects have been suggested (39), the actual mechanism of such a relationship remains largely elusive. It is possible that low folate intake, independent of altered folate metabolism may be sufficient to increase the risk for neural tube defects. In our population, it is plausible that an unknown genetic predisposition to neural tube defects (based on the fact that most cases arise from two extended Cree families), coupled with low folate intake predisposes these people to a high rate of neural tube defects.

Despite copious information about the protective effects of folate in both scientific and lay literature, there is still evidence that voluntary folate supplementation recommendations are poorly adhered to in Canada (40-42) and elsewhere (43). Furthermore, public health

efforts to fortify flour have been limited because of concern of adverse effects of excessive folate intake, such as the masking of cobalamin deficiency leading to irreversible peripheral neuropathy. This has lead to a minimal level of fortification of flour and of other grain products in North America, which would on average result in an increase of 100 μg/day of folate (44). This would fall short of the consideration of 200 μg/day of folate obtained from diet in the general population (and much less in northern populations), plus 400 μg/day of folic acid supplement shown to increase RBC folate to a level that should effectively reduce the rate of neural tube defects (45, 46).

Although rates of neural tube defects are not well known in northern aboriginal populations, other birth defects such as congenital heart malformations and facial clefts, which may be amenable to the protective effects of folate supplementation, have been frequently shown to be increased (21, 47-49). Although the types of birth defects may differ in northern aboriginal populations, reflecting perhaps distinct genetic predisposing factors, an increased intake of folate, without the concerns of masking dietary cobalamin deficiency, may help reduce rates of birth defects in these remote populations. Because traditional dietary practices in aboriginal populations are known to be protective in many other ways (26, 50), culturally appropriate public health efforts, as well as sufficient folate fortification to meet the specific needs of such populations are essential to reduce rates of birth defects in northern aboriginal populations. Although it is hoped that current public health efforts in the Eastern James Bay Region will increase awareness of the importance of folic acid intake periconceptionally, the effectiveness will only be known with subsequent study.

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