ROLE OF FOLIC ACID IN BIRTH DEFECTS PREVENTION
EPIDEMIOLOGIC PERSPECTIVES

Gary M Shaw
Professor & Associate Chair
Dept of Pediatrics
Population Burden-Birth Defects

- 1 in 33 babies born with birth defects (6% in world)
- >100,000/year in US
- 1 in 12 for preterm babies
- ~20% of infant deaths
- ~ 60% of neonatal deaths >1499 gm
Early

Most occur within the first 3 months of pregnancy
The Unknown Fraction

- Chromosomal: 10%
- Single Genes and Familial: 18%
- Uterine Factors: 3%
- Teratogens: 3%

Vitamin deficiencies and neural tube defects

R.W. SMITHELLS, S. SHEPPARD, and C.J. SCHORAH
1976

“These results must be interpreted with caution. If vitamin deficiency is a factor in the genesis of CNS defects, appropriate vitamin supplementation might make a contribution to primary prevention.”
Neural Tube Defects
What is the epidemiologic evidence?
Effect of multivitamins containing folic acid on the risk for neural tube defects, 1980-1999

- '80-Smithells
- '81-S. Wales
- '88-Atlanta
- '89-W. Australia
- '89-CA/Illinois
- '89-Boston
- '90-Cuba
- '91-UK-MRC
- '92-Hungary
- '93-New England
- '95-California
- '99-P.R. China N/S

Risk Ratio With 95% Confidence Intervals

Randomized trials
Non-randomized trials
Observational studies
Reduction in Neural Tube Defects After Fortification

↓15-50% across many registries & countries
Wheat Flour Fortification With Folic Acid: Changes in Neural Tube Defects Rates in Chile

Fanny Cortés, C. Mellado, R.A. Pardo, L.A. Villarroel, and E. Hertrampf

In January 2000, the Ministry of Health mandated the addition of folic acid (FA) to wheat flour in order to reduce the risk of neural tube defects (NTDs). This policy resulted in significant increases in serum and red cell folate in women of fertile age 1 year after fortification. To evaluate the effect of wheat flour fortification on the prevalence of NTDs in Chile, we designed a prospective hospital-based surveillance program to monitor the frequency of NTDs in all births (live and stillbirths) with birth weight ≥500 g at nine public maternity hospitals in Santiago, Chile from 1999 to 2009. During the pre-fortification period (1999–2000) the NTD rate was 17.1/10,000 births in a total of 120,566 newborns. During the post-fortification period (2001–2009) the NTD rate decreased to 8.6/10,000 births in a total of 489,915 newborns, which translates into a rate reduction of 56% (RR: 0.5; 95% CI: 0.4–0.58). For all NTDs, the rate reduction by type of NTD studied was: 50% in anencephaly (RR: 0.5; 95% CI: 0.38–0.67), 42% in cephalocele (RR: 0.58; 95% CI: 0.37–0.89), and 52% in spina bifida (RR: 0.48; 95% CI: 0.38–0.60). Rates showed significant reduction both in stillbirths and live births.

How to Cite this Article:
How does folic acid alter risk of NTDs?
5,10-\textit{MTHFR}

Gene-only effects

meta-analysis, odds ratio=1.8

Botto & Yang 2000
**MTHFR** polymorphism and Spina Bifida risk

Maternal vitamin use (folic acid)

TT vs CC

Odds ratio = 1.2 (0.4-4.0)
MTHFR polymorphism and Spina Bifida risk

Maternal vitamin use (folic acid)  No vitamin use (folic acid)

TT vs CC

Odds ratio = 1.2 (0.4-4.0)  Odds ratio = 1.6 (0.8-3.1)
Neural Tube Defects and Folate Pathway Genes: Family-Based Association Tests of Gene–Gene and Gene–Environment Interactions

Abele L. Boyles,1 Ashley V. Billups,1 Kristen L. Doak,1 Deborah G. Siegel,1 Lorraine Mahfouz,1 Susan H. Sifer,1 Alexander G. Bassuk,2 John A. Kessler,2 Michael C. Reed,2 H. Frederik Nijhout,3 Timothy M. George,2 David S. Enterline,1 John R. Gilbert,1 Merci C. Spear,1 and the NTD Collaborative Group*

1Center for Human Genetics, Duke University Medical Center, Durham, North Carolina, USA; 2Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA; 3Department of Mathematics, and 4Department of Biology, Duke University, Durham, North Carolina, USA; 5Department of Surgery, and 6Department of Radiology, Duke University Medical Center, Durham, North Carolina, USA.

BACKGROUND: Folate metabolism pathway genes have been examined for association with neural tube defects (NTDs) because folic acid supplementation reduces the risk of this debilitating birth defect. Most studies addressed these genes individually, often with different populations providing conflicting results.

OBJECTIVE: Our study evaluates several folate pathway genes for association with human NTDs, incorporating an environmental cofactor: maternal folic acid supplementation.

METHODS: In 304 Caucasian American NTD families with neuropathies or anencephaly, we examined 28 polymorphisms in 11 genes: folate receptor 1, folate receptor 2, white blood cell family 19 member 1, transcobalamin II, methylbenzoate hydroxylase dehydrogenase 1, serine hydroxymethyltransferase 1, 5,10-methylenetetrahydrofolate reductase (MTHFR), 5-methyltetrahydrofolate-homocysteine methyltransferase, homocysteine methyltransferase (BHMT), and cytosinoma beta-thymine.

RESULTS: Only single nucleotide polymorphisms (SNPs) in BHMT were significantly associated in the overall data set; this significance was stronger when mothers took folic acid-containing nutritional supplements before conception. The BHMT SNP rs7735890 was more significant when the data were stratified by preferential transmission of the MTHFR rs1801133 thermodiable T allele from parent to offspring. Other SNPs in folate pathway genes were not significantly associated in some analyses when stratified by maternal supplementation, MTHFR, or BHMT allele transmission.

CONCLUSIONS: BHMT rs7735890 is significantly associated in our data set, whereas MTHFR rs1801133 is not a major risk factor. Further investigation of folate and methionine cycle genes will require extensive SNP genotyping and/or genotyping to identify novel variants, inclusion of environmental factors, and investigation of gene–gene interactions in large data sets.

Review Article

The Search for Genetic Polymorphisms in the Homocysteine/Folate Pathway That Contribute to the Etiology of Human Neural Tube Defects

Anne M. Molloy,1* Lawrence C. Brody,2 James L. Mills,3 John M. Scott,1 and Peadar N. Kirke4

1School of Biochemistry and Immunology, Trinity College, Dublin, Ireland.
2Molecular Pathogenesis Section, Genome Technology Branch, National Human Genome Research Institute, Bethesda, Maryland.
3Division of Epidemiology, Statistics and Prevention Research, Rose Kennedy Shotier National Institute of Child Health and Human Development, Department of Health and Human Services, National Institutes of Health, Bethesda, Maryland.
4Child Health Epidemiology Unit, Health Research Board, Dublin, Ireland.

Received 13 October 2006; Revised 1 December 2006; Accepted 3 December 2006

In this paper, we trace the history of current research into the genetic and biochemical mechanisms that underlie folate-preventable neural tube defects (NTDs). The inspired suggestion by Smithells that common vitamins might prevent NTDs ignited a decade of biochemical investigations—first exploring the nutritional and metabolic factors related to NTDs, then onto the hunt for NTD genes. Although NTDs were known to have a strong genetic component, the concept of common genetic variance being linked to disease risk was relatively novel in 1995, when the first folate-related polymorphism associated with NTDs was discovered. The realization that more genes must be involved started a rush to find polymorphic needles in genetic haystacks. Early efforts entailed the intellectually challenging and time-consuming task of identifying and analyzing candidate single nucleotide polymorphisms (SNPs) in folate pathway genes. Luckily, human genome research has developed rapidly, and the search for the genetic factors that contribute to the etiology of human NTDs has evolved to mirror the increased level of knowledge and data available on the human genome. Large-scale candidate gene analysis and genome-wide association studies are now readily available. With the technical hurdles removed, the remaining challenge is to gather a sample large enough to uncover the polymorphisms that contribute to NTD risk. In some respects the real work is beginning. Although moving forward is exciting, it is humbling that the most important result—prevention of NTDs by maternal folic...
14 genes – 118 SNPs

- BHMT 8
- BHMT2 7
- CBS 9
- DHFR 9
- FOLR1 3
- FOLR2 3
- MTHFD1 10
- MTHFD2 8
- MTHFR 13
- MTR 21
- MTRR 13
- NOS3 3
- RFC1 6
- TYMS 5
Conclusion

......observations do not implicate a particular {polymorphism} folate transport or metabolism gene to be strongly associated with risks for spina bifida .......
A Genetic Signature of Spina Bifida Risk from Pathway-Informed Comprehensive Gene-Variant Analysis

Nicholas J. Marini, Thomas J. Hoffmann, Edward J. Lammer, Jill Hardin, Katherine Lazaruk, Jason B. Stein, Dennis A. Gilbert, Crystal Wright, Anna Lipzen, Len A. Pennacchio, Suzan L. Carmichael, John S. Witte, Gary M. Shaw, Jasper Rine

1 Department of Molecular and Cellular Biology, California Institute for Quantitative Biosciences, University of California, Berkeley, California, United States of America, 2 Department of Epidemiology and Biostatistics and Institute of Human Genetics, University of California San Francisco, San Francisco, California, United States of America, 3 Children's Hospital Oakland Research Institute, Oakland, California, United States of America, 4 VitroPath Genetics, Inc., Foster City, California, United States of America, 5 Department of Energy, Joint Genome Institute, Walnut Creek, California, United States of America, 6 Department of Pediatrics, Stanford University School of Medicine, Stanford, California, United States of America

Abstract

Despite compelling epidemiological evidence that folic acid supplements reduce the frequency of neural tube defects (NTDs) in newborns, common variant association studies with folate metabolism genes have failed to explain the majority of NTD risk. The contribution of rare alleles as well as genetic interactions within the folate pathway have not been extensively studied in the context of NTDs. Thus, we sequenced the exons in 31 folate-related genes in a 480-member NTD case-control population to identify the full spectrum of allelic variation and determine whether rare alleles or obvious genetic interactions within this pathway affect NTD risk. We constructed a pathway model, predetermined independent of the data, which grouped genes into coherent sets reflecting the distinct metabolic compartments in the folate/one-carbon pathway (purine synthesis, pyrimidine synthesis, and homocysteine recycling to methionine). By integrating multiple variants based on these groupings, we uncovered two provocative, complex genetic risk signatures. Interestingly, these signatures differed by race/ethnicity: a Hispanic risk profile pointed to alterations in purine biosynthesis, whereas that in non-Hispanic whites implicated homocysteine metabolism. In contrast, parallel analyses that focused on individual alleles, or individual genes, as the units by which to assign risk revealed no compelling associations. These results suggest that the ability to layer pathway relationships onto clinical variant data can be uniquely informative for identifying genetic risk as well as for generating mechanistic hypotheses. Furthermore, the identification of ethnic-specific risk signatures for spina bifida resonated with epidemiological data suggesting that the underlying pathogenesis may differ between Hispanic and non-Hispanic groups.
Multiple alleles

Mutation loads

Higher in controls!
Nature knows what she is doing, and does it, even when we cannot find out.

- Sir Arthur Stanley Eddington
Autoantibodies against Folate Receptors in Women with a Pregnancy Complicated by a Neural-Tube Defect

Sheldon P. Rothenberg, M.D., Maria P. da Costa, M.D., Jeffrey M. Sequeira, M.S., Joan Cracco, M.D., Jaclyn L. Roberts, M.D., Jeremy Weedon, Ph.D., and Edward V. Quadros, Ph.D.

ABSTRACT

BACKGROUND

In the absence of clinical folate deficiency, periconceptional supplementation with folic acid reduces a woman's risk of having an infant with a neural-tube defect. Since administration to folate receptors induces embryo resorption and malformations in rats, we hypothesized that autoantibodies against folate receptors in women may be associated with pregnancy complicated by a neural-tube defect.

METHODS

Serum from 12 women who were or had been pregnant with a fetus with a neural-tube defect and from 24 control women (20 with current or prior normal pregnancies and 4 who were nulliparous) was analyzed for autoantibodies by incubation with human placental folate receptors radiolabeled with [14C]folic acid. The properties of these autoantibodies were characterized by incubating serum and the autoantibodies isolated from serum with placental membranes, ED27 cells, and KB cells, which express the folate receptors.

RESULTS

Serum from 9 of 12 women with a current or previous affected pregnancy (index subjects) and 2 of 20 control subjects contained autoantibodies against folate receptors (P<0.001). The autoantibodies blocked the binding of [14C]folic acid to folate receptors on placental membranes and on ED27 and KB cells incubated at 4°C and blocked the uptake of [14C]folic acid by KB cells when incubated at 37°C.

CONCLUSIONS

Serum from women with a pregnancy complicated by a neural-tube defect contains autoantibodies that bind to folate receptors and can block the cellular uptake of folic acid. Further study is warranted to assess whether the observed association between maternal autoantibodies against folate receptors and neural-tube defects reflects a causal relation.
Folic Acid During Embryogenesis?

Women produce autoantibodies to folate receptors preventing binding and transport of folic acid to cellular components during embryonic development.

Supplemental folates compete with blocking antibodies and restore cellular folate concentrations.
## Folate AutoAntibody Titers - Mean

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>NTDs</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBP (IgM)</td>
<td>50.4</td>
<td>66.1</td>
<td>0.04</td>
</tr>
<tr>
<td>FR (IgG)</td>
<td>5.7</td>
<td>12.5</td>
<td>0.02</td>
</tr>
<tr>
<td>FR (IgM)</td>
<td>59.0</td>
<td>79.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Cabrera et al. 2008
Nitric Oxide Synthase

- NOS3 variants influence (raise) homocysteine concentrations
- smoking compromises NOS3 activity
- folate intake influences (lowers) homocysteine concentrations
- is clefting risk from NOS3 variants modified by smoking and further modified by vitamin intake (folic acid)?
**NOS3 A922G genotypes, maternal smoking, maternal vitamin use, and Cleft lip/palate risks**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Smoking</th>
<th>Vitamin Use</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variant</td>
<td>Yes</td>
<td>No</td>
<td>4.6</td>
<td>2.0-11.0</td>
</tr>
<tr>
<td>Variant</td>
<td>Yes</td>
<td>Yes</td>
<td>1.6</td>
<td>0.9-2.8</td>
</tr>
<tr>
<td>Wildtype</td>
<td>Yes</td>
<td>No</td>
<td>1.4</td>
<td>0.5-4.0</td>
</tr>
<tr>
<td>Wildtype</td>
<td>Yes</td>
<td>Yes</td>
<td>1.4</td>
<td>0.8-2.6</td>
</tr>
<tr>
<td>Wildtype</td>
<td>No</td>
<td>Yes</td>
<td>Ref</td>
<td>--</td>
</tr>
</tbody>
</table>

Shaw et al. 2004
Folic acid in early pregnancy: a public health success story

Sarah G. Obici,1,2 Richard H. Finnell,1 James L. Mills,3 Gary M. Shaw,4 and Anthony R. Scialli5,6,7
1Department of Obstetrics and Gynecology, George Washington University School of Medicine, Washington, District of Columbia, USA; 2Department of Nutrition, University of Texas, Austin, Texas, USA; 3Epidemiology Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA; 4Department of Pediatrics, Stanford University, Stanford, California, USA; and 5Feira Tech Sciences, Arlington, Virginia, USA

ABSTRACT Folate is a water-soluble B vitamin that must be obtained in the diet or through supplementation. For >50 yr, it has been known that folate plays an integral role in embryonic development. In mice, inactivation of genes in the folate pathway results in malformations of the neural tube, heart, and craniofacial structures. It has been shown that diets and blood levels of women who had a fetus with a neural tube defect are low for several micronutrients, particularly folate. Periconceptional use of folic acid containing supplements decreased recurrent neural tube defects in the offspring of women with a previously affected child and the occurrence of a neural tube defect and possibly other birth defects in the offspring of women with no prior history. Based on these findings, the U.S. Public Health Service recommended that all women at risk take folic acid supplements, but many did not. Mandatory food fortification programs were introduced in numerous countries, including the United States, to prevalence of anencephalic screening. Neural tube defects result from the incomplete closure of the neural tube during the fourth week of gestation (2). The most common neural tube defects are spina bifida, due to incomplete closure of the caudal neural tube, and anencephaly, due to incomplete closure of the rostral end of the neural tube. These malformations are fatal or result in significant lifelong disability.

For >50 yr, it has been known that folate plays an integral role in embryonic development. The investigation into the role of folate in neural tube defects and the use of folic acid supplementation to prevent these and perhaps other malformations has been an example of how scientists in diverse fields have worked together to favorably affect the public health. The Teratology Society is proud that many of these scientists are Society members, and many of the discussions leading to this major public health contribution took place at annual
Alternative interpretations?

"Never ever think outside the box."
Do the folic acid results fit our expectation?
Detracting Evidence or Clues

- Nearly all studies included other vitamins
Effect of multivitamins containing folic acid on the risk for neural tube defects, 1980-1999

'80-Smithells
'81-S. Wales
'88-Atlanta
'89-W. Australia
'89-CA/Illinois
'89-Boston
'90-Cuba
'91-UK-MRC
'92-Hungary
'93-New England
'95-California
'99-P.R. China N/S

Risk Ratio With 95% Confidence Intervals

Randomized trials
Non-randomized trials
Observational studies
### MRC Randomized Trial

<table>
<thead>
<tr>
<th>Condition</th>
<th>Relative Risk</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic Acid only vs. Placebo</td>
<td>0.15</td>
<td>0.05-0.70</td>
</tr>
<tr>
<td>Other Vitamins vs. Placebo</td>
<td>0.60</td>
<td>0.26-1.50</td>
</tr>
</tbody>
</table>
Detracting Evidence or Clues

- Nearly all studies included other vitamins
- Inconsistency across population subgroups
Use of folic acid in periconceptional period among Hispanics and risk for NTDs

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>0.96</td>
</tr>
<tr>
<td>Texas</td>
<td>1.12</td>
</tr>
</tbody>
</table>
Detracting Evidence or Clues

- Nearly all studies included other vitamins
- Inconsistency across population subgroups
- NTDs over time and inclusion of folic acid
NTD Prevalence

Prevalence per 1000 Live Births

Source: Yen et al. AJDC 1992; 146: 857-61
### NTDs and Periconceptional Multivitamin Use - Atlanta

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/68 - 4/72</td>
<td>0.49</td>
</tr>
<tr>
<td>5/72 - 8/76</td>
<td>0.32</td>
</tr>
<tr>
<td>9/76 - 12/80</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Erickson (personal communication)
Detracting Evidence or Clues

- Nearly all studies included other vitamins
- Inconsistency across population subgroups
- Prevalence of NTDs over time and inclusion of folic acid
- Null study findings
Original Contribution

Neural Tube Defects and Maternal Folate Intake Among Pregnanies Conceived After Folic Acid Fortification in the United States

Bridget S. Mosley, Mario A. Cleves, Anna Maria Siega-Riz, Gary M. Shaw, Mark A. Canfield, D. Kim Ward, Martha M. Weiner, and Charlotte A. Hobbs for the National Birth Defects Prevention Study

Initially submitted March 1, 2008; accepted for publication June 11, 2008.

Rates of neural tube defects have decreased since folic acid fortification of the food supply in the United States. The authors' objective was to evaluate the associations between neural tube defects and maternal folate intake among pregnancies conceived after fortification. This is a multicenter, case-control study that uses data from the National Birth Defects Prevention Study, 1998-2003. Logistic regression was used to compute crude and adjusted odds ratios between cases and controls assessing maternal periconceptional use of folic acid and intake of dietary folic acid. Among 180 anencephaly cases, 369 spina bifida cases, and 3,903 controls, 21.1%, 25.2%, and 26.1%, respectively, reported periconceptional use of folic acid supplements. Periconceptional supplement use did not reduce the risk of having a pregnancy affected by a neural tube defect. Maternal intake of dietary folate was not significantly associated with neural tube defects. In this study, conducted among pregnancies conceived after mandatory folic acid fortification, the authors found little evidence of an association between neural tube defects and maternal folate intake. A possible explanation is that folate acid fortification reduced the occurrence of folic-acid-sensitive neural tube defects. Further investigation is warranted to possibly identify women who remain at increased risk of periconceptional neural tube defects.

Abbreviations: B3, 3 months before pregnancy; CI, confidence interval; DFE, dietary folate equivalent; OR, odds ratio; P1, first month of pregnancy.

Editor's note: An invited commentary on this article appears on page 19, and the authors' response is published on page 22.

After 3 decades of epidemiologic research reporting an association between neural tube defects and maternal use of folic acid (1-10), public health organizations developed recommendations and supported interventions to increase folic acid intake among women of reproductive age. In 1992, the US Public Health Service recommended that all women of childbearing age who are capable of becoming pregnant should consume 400 μg of folic acid daily (11). In 1999, the March of Dimes, Centers for Disease Control and Prevention, and National Council on Folic Acid launched the National Folic Acid Educational Campaign. The US Food and Drug Administration had mandated that all enriched cereals and grains contain 140 μg of folic acid per 100 g of grain by January 1998 (12). In 2005, after the National Campaign and mandatory fortification, approximately 33% of women reported taking a daily supplement of folic acid (13), only a modest increase from the 23% reported in 1995 (14). However, median blood folate levels among women of childbearing age increased from 4.8 to 13.0 ng/mL, between 1994 and 2000 (15), with a more recent study (16) reporting median blood folate levels at least 2 times the levels prior to fortification.
### NTDs & Folic Acid – Recent Data

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratios (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spina Bifida</td>
<td>1.1 (0.9, 1.5)</td>
</tr>
<tr>
<td>Anencephaly</td>
<td>1.7 (1.2, 2.4)</td>
</tr>
</tbody>
</table>

Supplements in month before pregnancy

Folic Acid Intake and Spina Bifida in the Era of Dietary Folic Acid Fortification

Katherine Ahrens, Mahsa M. Yazdy, Allen A. Mitchell, and Martha M. Werler

Background: The US Food and Drug Administration mandated that enriched grain products be fortified with folic acid by 1998. We evaluated whether intake of folic acid from supplements and diet was associated with a reduction in spina bifida in the setting of folic acid fortification.

Methods: Data were collected as part of the Iowa Birth Defects Study from 1990 to 2005. Mothers of infants with and without birth defects were interviewed within 6 months of delivery about pregnancy exposure, including details of diet and vitamin intake. Dietary natural folic acid and synthetic folic acid from fortification were combined into a single, weighted measure—dietary folic acid equivalent. Periconceptional folic acid supplementation and dietary folic acid consumption were compared between 205 mothers of spina bifida cases and 637 mothers of nonaffected controls. Relative risks of a spina bifida–affected birth were estimated with odds ratios (ORs) and 95% confidence intervals (CIs).

Results: Spina bifida was not associated with regular folic acid supplementation (>4 days per week) either around the time of conception (adjusted OR = 1.1 [95% CI = 0.74–1.6]) or initiated in early pregnancy (0.79 [0.54–1.1]). After adjustment for confounders, a 0.25 additional months of spina bifida was estimated for each 100 µg increase in daily dietary folic acid equivalent consumed. Conclusion: In the setting of folic acid fortification of grains, our data suggest that folic acid supplementation does not appear to offer further benefit for reducing risk of spina bifida. Rather, the folic acid–associated benefit on spina bifida risk was found with increasing amounts of dietary folic acid consumed, regardless of folic acid supplementation level.

(Epidemiology 2011;22: 731–737)

Since 1998, when the US Food and Drug Administration mandated that enriched grain products be fortified with folic acid, the estimated number of pregnancies affected by neural tube defects (NTDs) has declined by approximately 27%. However, a greater decline was predicted, raising the question of whether at least some of the remaining cases can be prevented through increased periconceptional supplementation or dietary folic acid intake.

Mostly and colleagues recently investigated this issue, using data collected from the National Birth Defects Prevention Study. The authors found that neither periconceptional folic acid supplementation nor dietary folic acid intake was associated with a reduction in the risk of NTDs, including spina bifida. The objective of our study was to examine risks of spina bifida in relation to maternal folic acid supplementation and dietary intake in another large case-control study, focusing on the time period since folic acid fortification began.

METHODS

The Iowa Birth Defects Study was initiated by the Iowa Epidemiology Center in 1976. Infants with birth defects were identified by the study staff from discharge records of participating hospitals serving the areas surrounding Boston, MA; Philadelphia, PA; San Antonio, TX; and Toronto, Canada; in addition, cases have been identified through birth defect registries in Massachusetts and parts of New York State. Unaffected controls have been randomly selected each month from study hospitals’ discharge lists or from statewide birth records. Malformed live-born infants, therapeutic abortions after 12 weeks’ gestation, and fetal deaths after 20 weeks’ gestation were eligible as cases for our study; however, ascertainment of non–live-born cases has not been routine. Only live-born malformed infants were eligible as controls for our study. The Birth Defects Study has been approved by the institutional review boards of Boston University and relevant participating hospitals and centers.

Mothers of eligible cases and controls were telephoned within 6 months of delivery by a research nurse to conduct the computer-assisted interview. After obtaining informed consent, interviews were conducted in either English or Spanish and lasted for approximately 45–60 minutes.

The interview included questions on the following topics: pregnancy intention, medical and obstetric history, illness and medication history during the period 2 months before the last menstrual period (LMP) through the end of the pregnancy, weight and diet before pregnancy, and behavioral risk factors (such as alcohol consumption and smoking) during pregnancy. In addition, the interview obtained demographic information and family history of birth defects. In the
Annual NTD prevalences in central California, 1989-2003

Figure 1 - NTD prevalences

Difference in slopes = 12.8 (4.4, 21.2)

-7.8 (-11.4, -4.2)

5.0 (-2.5, 12.6)
### Effect of Maternal FA Diet on NTD rate in Lrp6 nulls

<table>
<thead>
<tr>
<th>Dietary FA</th>
<th># Observed*</th>
<th>% of Lrp6−/− Embryos with NTD</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>2ppm</td>
<td>7</td>
<td>15</td>
<td>68%</td>
</tr>
<tr>
<td>10ppm</td>
<td>0</td>
<td>15</td>
<td>100%</td>
</tr>
</tbody>
</table>
where

do we go

from

here?
Other Nutrients

- Vitamin $B_{12}$
- Methionine
- Zinc
- Inositol
- Choline
<table>
<thead>
<tr>
<th>Quartile of B12 serum level</th>
<th>“Group 1”</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>3.2 (1.5-6.2)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>2.8 (1.3-6.0)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>1.8 (0.8-4.1)</td>
</tr>
<tr>
<td>4</td>
<td>Reference</td>
<td></td>
</tr>
</tbody>
</table>

Molloy et al. 2009
NTDs & Serum Choline

Shaw et al. Epidemiol 2009
Dr. James Mills and colleagues
Maternal choline concentrations during pregnancy and choline-related genetic variants as risk factors for neural tube defects

James L Mills, Razong Fan, Lawrence C Brody, Aiyi Liu, Per M Ueland, Yifan Wang, Peadar N Kirke, Barry Shane, and Anne M Molloy

ABSTRACT

A beneficial effect of choline could help elucidate the mechanism.

“If we knew what we were doing it wouldn’t be called research” - Einstein

Nested Case-Control Study of One-Carbon Metabolites in Mid-Pregnancy and Risks of Cleft Lip With and Without Cleft Palate

Gary M. Shaw, Stein Emil Vollset, Suzan L. Carmichael, Wei Yang, Richard H. Finnell, Henk Blom, and Per M. Ueland
Diet Quality Index

- "Holistic" approach
- Diet quality > single nutrient?
- 6 (+): grains, vegetables, fruits, folate, iron, calcium
- 2 (-): % calories from fats; sweets

Carmichael et al. 2011
Questions

• Are the folate-sensitive NTDs removed from the population?
• Folic acid works – but how?
  - shed light on development
  - response vary by genetic background?
Questions

• Are the folate-sensitive NTDs removed from the population?
• Folic acid works – but how?
  - shed light on development
• Unintended effects:
  - twinning?
  - Miscarriage ↑?
Questions

- Are the folate-sensitive NTDs removed from the population?
- Folic acid works – but how?
  - shed light on development
- Unintended effects:
  - twinning?
  - miscarriage?
  - birth defects?
  - cancers?
  - childhood asthma?
- Other nutrients are important – but which ones?
“it is better to have an approximate answer to the right question than an exact answer to the wrong one” - Tukey
## Collaborators

<table>
<thead>
<tr>
<th>Stanford University</th>
<th>UT Austin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suzan Carmichael</td>
<td>Richard Finnell</td>
</tr>
<tr>
<td>Wei Yang</td>
<td>Huiping Zhu</td>
</tr>
<tr>
<td>Chen Ma</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Children’s Hospital Oakland</th>
<th>Free University</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edward Lammer</td>
<td>Henk Blom</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CDC</th>
<th>University of Bergen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sonja Rasmussen</td>
<td>Per Ueland &amp; Stein Emil Volset</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>U of Arkansas</th>
<th>U of California San Francisco</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charlotte Hobbs</td>
<td>John Witte</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>UC Berkeley</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nick Marini</td>
<td></td>
</tr>
<tr>
<td>Jasper Rine</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CA Dept of Public Health</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Shabbir Ahmad &amp; Fred Lorey</td>
<td></td>
</tr>
</tbody>
</table>