article

Prevalence of aneuploidies in South Carolina in the 1990s

Julianne S. Collins, PhD[1], Rick L. Olson, MD[1], Barbara R. DuPont, PhD[1], Daynna J. Wolff, PhD[2], Robert G. Best, PhD[3], and Roger E. Stevenson, MD[1]

Purpose: Folate insufficiency due to nutritional deficiency or folate processing gene mutations has been proposed as a trisomy 21 risk factor. This study examined the possibility that increased folic acid intake among women of childbearing age may decrease the prevalence of trisomy 21 and other aneuploidies. **Methods:** The prevalence of aneuploidies from 1990 through 1999 was compared with folic acid use in women of childbearing age in South Carolina. **Results:** Folic acid use and the prevalence of all aneuploidies significantly increased during this period. **Conclusion:** Increased folic acid utilization in South Carolina was not associated with decreased prevalence of trisomy 21 or other aneuploidies. ***Genet Med* 2002:4(3):131–135.**

Key Words: *Trisomy 21, Down syndrome, aneuploidy, folic acid, folate*

Abnormal folate metabolism and mutations in two genes essential for folate metabolism, methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR), have been reported in mothers of trisomy 21 patients.[1–3] James et al. found that mothers carrying a 677C→T MTHFR mutation were 2.6 times more likely to have a child with trisomy 21 than control mothers.[1] They also showed that mothers of children with trisomy 21 had significantly higher plasma homocysteine and greater lymphocyte cytotoxicity to methotrexate exposure than control mothers. A follow-up study confirmed that the 677C→T MTHFR mutation was a maternal risk factor for trisomy 21 and that a polymorphism (66A→G) in MTRR also conveyed higher risk (odds ratio = 2.6) for trisomy 21. The presence of both mutations was associated with greater risk (odds ratio = 4.1) of trisomy 21 than either mutation alone.[2] Hassold et al. found a significant increase in the MTHFR 677 C→T polymorphism among mothers of conceptuses with trisomy 18, but no increase among mothers of sex chromosome and other autosomal aneuploidies.[4] These studies led to the suggestion that fortification of cereal grain flours with folic acid and periconceptional folic acid supplements may reduce the incidence of trisomy 21.[1]

During the past decade, there has been a general increase in folic acid availability and use in the United States, primarily in response to the U.S. Public Health Service recommendation that all women of childbearing age supplement their diet with 400 μg folic acid daily to reduce the risk of neural tube defects.[5] Cereal grain flours have been fortified with 140 μg of folic acid per 100 g of milled flour in accordance with a Food and Drug Administration mandate of February 1996.[6] Educational efforts and advertising have promoted foods with natural folate and over 50 breakfast cereals have now added 400 μg of synthetic folic acid per serving.

The National Health and Nutrition Examination Survey has shown that mean serum folate in women of childbearing age was 6.3 ng/mL between 1988 and 1994, which increased to 16.2 ng/mL in 1999.[7] Mean red blood cell folate concentrations increased from 181 ng/mL to 315 ng/mL during this time period. Other studies have confirmed the significant increase in serum and/or red blood cell folate levels in the United States during this time period.[8–10] From information gathered by the South Carolina Neural Tube Defect Prevention Initiative, the estimated rate of folic acid supplementation in South Carolina increased from 8% in 1992 to 35% in 1999.[11] Control mean serum folate levels increased from 9.3 ng/mL in 1992 ($N = 38$) to 16.0 ng/mL ($N = 57$) in 1997. Control mean red blood cell folate concentrations also increased from 255 ng/mL ($N = 37$) to 288 ng/mL ($N = 58$) during this same time period in South Carolina (unpublished data, 2001).

If folate insufficiency predisposes to aneuploidy, the recent increase in folic acid availability should decrease the prevalence of trisomy 21 and other aneuploidies. This study examined the prevalence of trisomy 21 and other aneuploidies in South Carolina during the 1990s, a period of increased utilization of folic acid among women of childbearing age.

MATERIALS AND METHODS

All cases of prenatally and postnatally detected aneuploidy among the estimated 540,130 live births and fetal deaths in South Carolina between January 1990 and December 1999 were included in this study. Most cases had cytogenetic studies at the Greenwood Genetic Center, the Medical University of South Carolina, or the University of South Carolina, which are

From the [1]Greenwood Genetic Center, Greenwood, South Carolina; [2]Medical University of South Carolina, Charleston, South Carolina; [3]University of South Carolina, Columbia, South Carolina.

Julianne S. Collins, PhD, Greenwood Genetic Center, 1 Gregor Mendel Circle, Greenwood, SC 29646.

Received: November 26, 2001.

Accepted: February 11, 2002.

the only laboratories doing cytogenetic studies in South Carolina.

The data from each site included all cytogenetic analyses from amniocentesis, chorionic villus sampling, fetal tissue samples (including both miscarried and aborted fetuses), and peripheral blood samples. Prenatal diagnosis and fetal tissue results for the years 1990 and 1991 were incomplete from one site. There were a limited number of cases performed by labs outside the state, and these were also included if they were available. After institutional review board approval, the data from each site were screened for duplicate samples, identifiers were removed, and the data were combined to form one data set using Microsoft Excel (Redmond, WA).

The cases were grouped by type of aneuploidy: trisomy 13, 18, 21; Turner syndrome; other sex chromosome aneuploidies; other autosomal aneuploidies; and mosaics (>5% of cells studied). Structural chromosome abnormalities such as balanced translocations and inversions were excluded. The data were grouped by year, based on the year of birth or the year the sample was acquired for prenatal diagnoses. The age of the mother at prenatal testing ($N = 439$) and fetal tissue testing ($N = 336$) was known in 775 of 1,385 total individuals. Microsoft Access (Redmond, WA) was used to manage and tabulate the data set. Cochran-Armitage tests for trend were run using SAS version 8.1.[12]

RESULTS

A total of 1,385 aneuploidies were identified in the 10-year period. Of those, 616 had trisomy 21, 72 had trisomy 13, 155 had trisomy 18, 98 had 45 X monosomy, 64 had other sex chromosomal aneuploidies, 264 had other autosomal aneuploidies, and 116 were aneuploidy mosaics. Of these aneuploidies, 600 were found by the Greenwood Genetic Center, 366 were found by the Medical University of South Carolina, 397 were found by the University of South Carolina, and 22 were found by outside laboratories. The distribution of cases by year is given in Table 1.

Because some data (specifically fetal tissue or prenatal testing reports) were missing from one site for the years 1990 and 1991, only years 1992 through 1999 were analyzed for trend tests. The rate of trisomy 21 in South Carolina increased from 0.98 per 1,000 live births and fetal deaths in 1992 to 1.34 per 1,000 live births and fetal deaths in 1999, which resulted in a significant Cochran-Armitage test for trend (P for trend = 0.0029). All aneuploidies increased from 2.01 per 1,000 in 1992 to 4.29 per 1,000 in 1999 (P for trend < 0.0001; see Fig. 1).

Figure 2 shows the change in proportions of cases of trisomy 21 detected by prenatal, postnatal, and fetal tissue studies. In 1992, 33% of cases were detected by prenatal testing, 64% were detected by postnatal testing, and 4% were detected by the testing of fetal tissue. However, in 1999, 33% of cases were detected by prenatal testing, 58% were detected by postnatal testing, and 10% were detected by the testing of fetal tissues. The slight decrease in cases found by postnatal testing is offset by an increase in case detection by fetal tissue testing.

Figure 3 shows the change in proportions of cases of aneuploidies detected by prenatal, postnatal, and fetal tissue studies. In 1992, 38% of cases were detected by prenatal testing, 44% were detected by postnatal testing, and 18% were detected by the testing of fetal tissues. In 1999, 30% of cases were detected by prenatal testing, 25% were detected by postnatal testing, and 45% were detected by the testing of fetal tissues. This finding reflects an increase in fetal tissue testing during the study period.

Maternal ages for 56% ($N = 775$) of the total samples ($N = 1385$) were available, including both prenatal ($N = 439$) and fetal tissue testing ($N = 336$) diagnosed aneuploidy cases. Figure 4 shows the proportion of mothers age 35 or above from

Table 1
Types of aneuploidies detected in South Carolina, 1990–1999

Year	Births[a]	47+13	47+18	47+21	Other	45 X	Other sex	Mosaics	Total
1990[b]	58,461	3	5	58	13	1	2	10	92
1991[b]	57,517	5	4	71	3	6	4	10	103
1992	56,102	2	16	55	8	9	11	12	113
1993	53,715	9	12	50	8	9	2	10	100
1994	51,907	5	15	58	20	4	7	7	116
1995	50,913	11	18	49	13	6	2	15	114
1996	51,105	12	10	52	24	11	11	9	129
1997	52,205	7	25	81	43	12	8	8	184
1998	53,833	11	19	69	60	20	7	15	201
1999	54,372	7	31	73	72	20	10	20	233
Total	540,130	72	155	616	264	98	64	116	1385

[a]Total births and fetal deaths.
[b]Data from 1990 and 1991 are not complete.

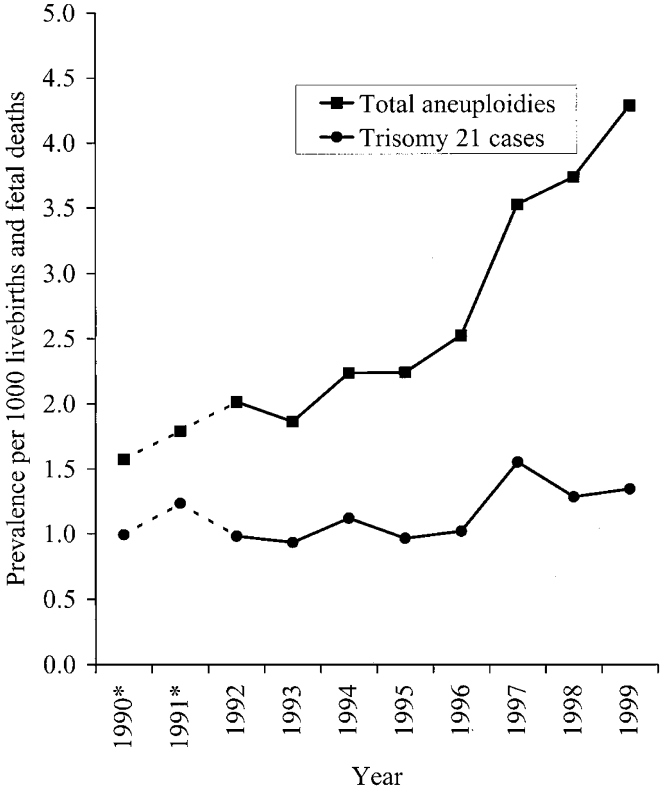

Fig. 1 Prevalence of aneuploidies and trisomy 21 cases per 1,000 live births and fetal deaths from 1990 through 1999. Asterisks indicate that data for all aneuploidies were incomplete for 1990 and 1991. The Cochran-Armitage trend tests have *P* values of <0.0001 for total aneuploidies from 1992 through 1999 and 0.0029 for trisomy 21 cases from 1992 through 1999.

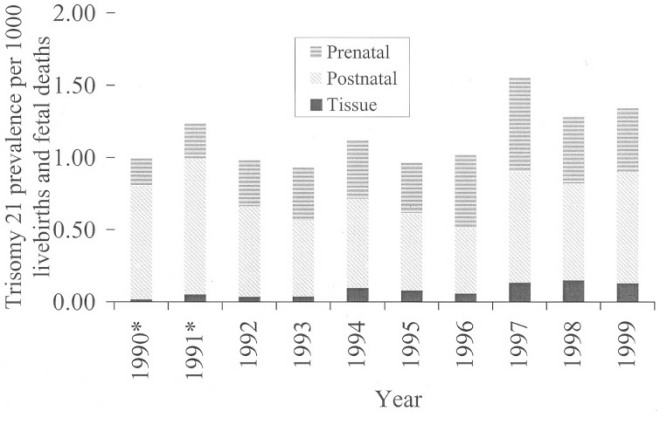

Fig. 2 Cases of trisomy 21 grouped by type of testing (prenatal amniocentesis and chorionic villus sampling, postnatal blood, and fetal tissue testing) and by year. Asterisks indicate that data from 1990 and 1991 were not complete for prenatal and fetal tissue testing.

1990 to 1999, which did not significantly change (*P* for trend = 0.4154) during this time period.

Figure 5 shows the proportion of trisomy 21 and aneuploidy cases found at the three sites as a proportion of the total number of prenatal tests performed. The proportion of trisomy 21 cases remained constant (*P* for trend = 0.4139), whereas the

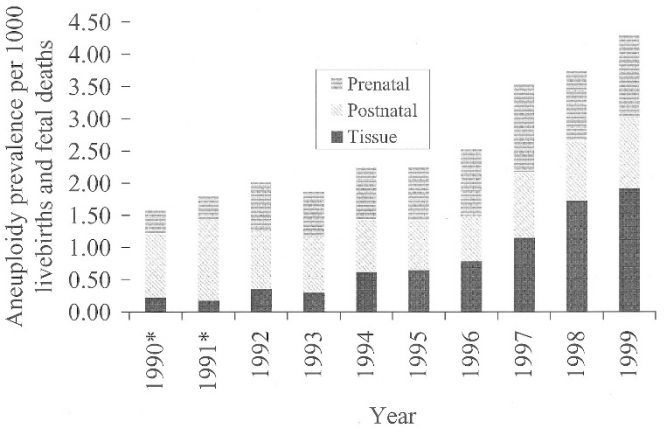

Fig. 3 Total aneuploidies grouped by type of testing (prenatal amniocentesis and chorionic villus sampling, postnatal blood, and fetal tissue testing) and by year. Asterisks indicate that data from 1990 and 1991 were not complete.

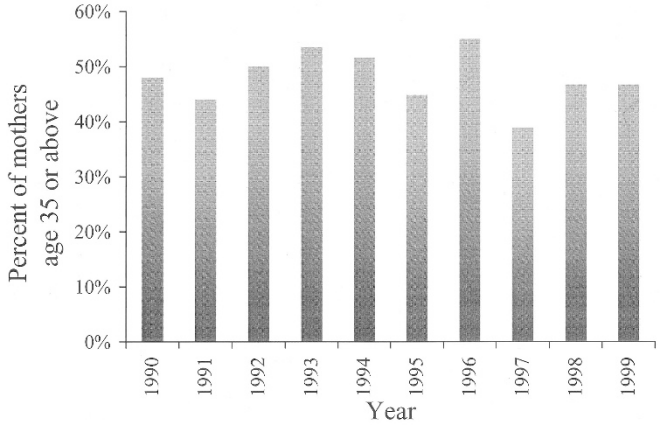

Fig. 4 Percentage of mothers age 35 or over for the years 1990 through 1999 (Cochran-Armitage *P* for trend = 0.4154).

proportion of aneuploidies increased (*P* for trend = 0.0059) during this time period.

DISCUSSION

The mechanism by which maternal folate insufficiency might predispose to aneuploid conceptions is incompletely understood. Several observations suggest that folate insufficiency, due to reduced dietary intake or to impaired metabolism, may lead to hypomethylation of DNA.[13] This in turn has been associated with instability[14] and abnormal segregation of chromosomes in mitosis.[15] Other studies have also shown that folate deficiency directly causes impaired DNA repair and chromosomal breakage.[16–18]

Preserving the stability of DNA and chromosomes is crucial during the prolonged meiotic process in human females. Meiosis begins during fetal development and arrests at birth in prophase I until the completion of meiosis I just before ovulation. The oocyte then arrests at metaphase of meiosis II, and the second division completes when the egg is fertilized.[19] The

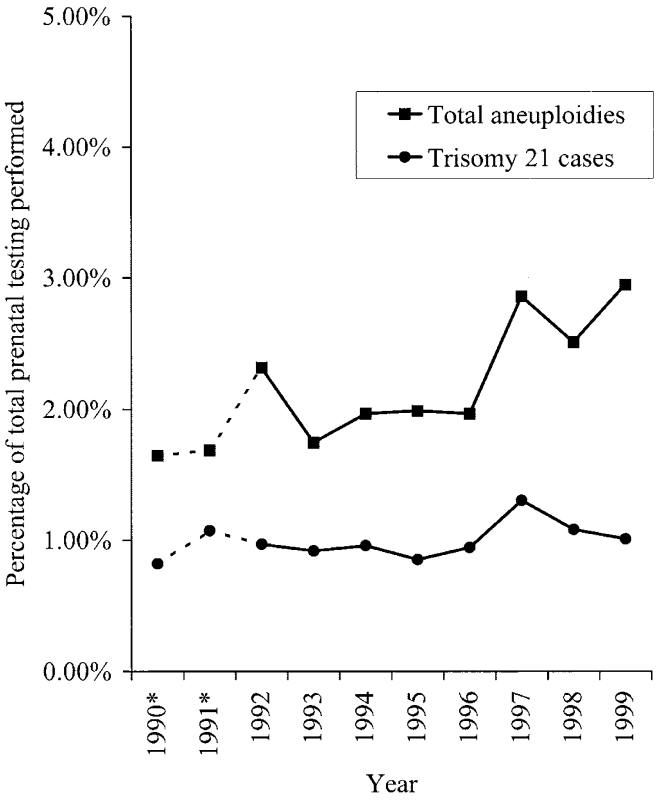

Fig. 5 Aneuploidies found by prenatal testing as a percentage of all prenatal testing done at the three South Carolina laboratories. Asterisks indicate that data from 1990 and 1991 were not complete. The Cochran-Armitage trend tests have *P* values of 0.0059 for total aneuploidies from 1992 through 1999, and 0.4139 for trisomy 21 cases from 1992 through 1999.

meiotic process spans from 10 to 50 years and provides many opportunities for error. However, to date, the exact timing of the majority of the meiosis I errors is unknown. They could occur before the meiotic arrest during fetal development, during the arrest that lasts from 10 to 50 years, or at the resumption of meiosis I around the time of ovulation.

The origin of human trisomy is predominantly due to errors in maternal meiosis I.[20] For trisomy 21, 65% of nondisjunction is due to maternal meiosis I errors and 23% to maternal meiosis II. Interestingly, trisomy 18 has been found to occur in maternal meiosis I in 33% of cases and maternal meiosis II in 56% of cases.[19] The reason for the difference in timing from most other chromosome aneuploidies is unknown. Irrespective, reductions in the rate of maternal meiotic errors might reduce the prevalence of trisomy and other aneuploidies. Increased folic acid consumption has been proposed as a means to reduce these meiotic errors.[1]

Although folate insufficiency due to nutritional deficiency or mutations in folate processing genes has been proposed as a risk factor for trisomy 21, the greater availability of periconceptional folic acid has not been found to have a protective effect against aneuploidies in this study.[1–3] In fact, there was a significant increase in the prevalence of trisomy 21 and other aneuploidies between 1992 and 1999, a time period of increasing reported folic acid use and blood folate levels in South Carolina. Our study calls into question speculation that fortification of cereal grain flours with folic acid and periconceptional folic acid supplements may reduce the incidence of trisomy 21.[1]

It should be noted that prior studies have focused on the association between aneuploidy risk and polymorphisms in MTHFR or MTRR[1,2,4] and plasma homocysteine/methionine levels,[1] and have not studied folic acid intake or folate levels per se. In contrast, we have focused on the relationship between aneuploidy rates and folic acid intake or blood folate levels and have not studied the MTHFR/MTRR polymorphisms or metabolite levels. Hence, it is possible that any predisposition to nondisjunction conveyed by MTHFR/MTRR polymorphisms or homocysteine/methionine levels may be independent of folic acid intake or blood folate levels. Clearly, further study is indicated to help resolve the conflicting findings.

It is possible that increased folic acid supplementation may help aneuploid pregnancies to reach an increased gestational age, allowing the recognition of pregnancies that would have previously gone undetected. This could be a possible explanation for the increasing prevalence of aneuploidies in South Carolina. Hook and Czeizel and others have shown that increasing folate levels may lead to a slight increase in the rate of observed pregnancy loss, which could be due to the increased survival of fetuses normally miscarried before recognition.[21–23] However, a recent study of 23,806 pregnant Chinese women showed no increase in miscarriage due to folic acid consumption.[24]

Advanced maternal age is a known risk factor for aneuploidy.[25] If the proportion of women over age 35 increased during this time period, this could cause an increase in the number of aneuploidies. Because the proportion of mothers age 35 or over did not significantly change (P for trend = 0.4154) during this time period (see Fig. 4), maternal age does not appear to affect the increase in aneuploidy prevalence among those in which maternal age is known. However, a 1-year increase in average maternal age, for example from age 33 to age 34 in a population with 54,000 births per year, could lead to an additional 22 cases of trisomy 21 or 37 additional aneuploidy births in that year.[26,27]

It is recognized that an increase in aneuploidy ascertainment due to increased use of prenatal diagnosis and fetal tissue testing would influence our data. There has been an increase in the number of cases diagnosed by fetal tissue testing (see Fig. 3); however, this does not fully explain the total increase in aneuploidies. In fact, when cases diagnosed by fetal tissue testing are removed, there is still a significant increase in trisomy 21 (P for trend = 0.0173) and total aneuploidy cases (P for trend $<$ 0.0001) between 1992 and 1999.

The addition of the human chorionic gonadotropin and estriol components to the triple screen in 1989 and 1996, respectively, as well as improvements in the use of ultrasound, have changed the detection rate of aneuploidy among women who were otherwise not likely to have had amniocentesis in previous years. This finding is shown by the significant increase in

the proportion of aneuploidies detected by amniocentesis (P for trend = 0.0059; see Fig. 5) during this time period.

This study indicates that increased folic acid availability has been temporally associated with an increase in the occurrence of aneuploidy. Although we cannot clearly identify the cause of the increase in aneuploidies, we can conclude that increased levels of folate do not appear to decrease the prevalence of aneuploidies, specifically trisomy 21.

Acknowledgments

This work was supported in part by grant MH 57840 from National Institutes of Mental Health (J.S.C. and R.E.S.), cooperative agreement U50/CCU 416008 from the Centers for Disease Control, and grant 2001-43 from the South Carolina Department of Disabilities and Special Needs (R.E.S.). Support from the South Carolina Chapter March of Dimes (R.E.S. and R.L.O.) is also acknowledged. We thank Dr. Len Leshin and Dr. Stephanie Sherman, whose ideas inspired our research, as well as Dr. Tom Sadler and Dr. Glen Satten, who made valuable suggestions. We also thank the many people who helped identify patients with aneuploidies from South Carolina: Emily Crocker, Dr. Frank Grass, Bradley Keith, Jane Dean, Fran Annese, Karen Buchanan, Mary Lawrence, Miriam Simmons, Ken Corning, Gail Stapleton, Debbie Bealer, Susan Schroer, Catherine Haddock, Amy Toburen, and Joan Montjoy.

References

1. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, Yi P, Tafoya DL, Swenson DH, Wilson VL, Gaylor DW. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. *Am J Clin Nutr* 1999;70:495–501.
2. Hobbs CA, Sherman SL, Yi P, Hopkins SE, Torfs CP, Hine RJ, Pogribna M, Rozen R, James SJ. Polymorphisms in genes involved in folate metabolism as a maternal risk factors for Down syndrome. *Am J Hum Genet* 1999;67:623–630.
3. Rosenblatt DS. Folate and homocysteine metabolism and gene polymorphisms in the etiology of Down syndrome. *Am J Clin Nutr* 1999;70:429–430.
4. Hassold TJ, Burrage LC, Chan ER, Judis LM, Schwartz S, James SJ, Jacobs PA, Thomas NS. Maternal folate polymorphisms and the etiology of human nondisjunction. *Am J Hum Genet* 2001;69:434–439.
5. Centers for Disease Control. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. *MMWR Morb Mortal Wkly Rep* 1992;41:1–7.
6. Food and Drug Administration. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. *Federal Register* 1996;61:8781–8797.
7. Centers for Disease Control. Folate Status in Women of Childbearing Age - United States, 1999. *MMWR Morb Mortal Wkly Rep* 2000;49:962–965.
8. Jacques PF, Selhub J, Bostom AG, Wilson PWF, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med* 1999;340:1449–1454.
9. Lawrence JM, Petitti DB, Watkins M, Umekubo MA. Trends in serum folate after food fortification. *Lancet* 1999;354:915–916.
10. Caudill MA, Le T, Moonie SA, Esfahini ST, Cogger EA. Folate status in women of childbearing age residing in Southern California after folic acid fortification. *J Am Coll Nutr* 2001;20:129–134.
11. Stevenson RE, Allen WP, Pai GS, Best R, Seaver LH, Dean J, Thompson S. Decline in prevalence of neural tube defects in a high-risk region of the United States. *Pediatrics* 2000;106:677–683.
12. SAS Institute [computer program]. Version 8.1. Cary, NC: SAS Institute; 1999.
13. Jacob RA, Gretz DM, Taylor PC, James SJ, Pogribny IP, Miller BJ, Henning SM, Swendseid ME. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. *J Nutr* 1998;128:1204–1212.
14. Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. DNA hypomethylation leads to elevated mutation rates. *Nature* 1998;395:89–93.
15. Leyton C, Mergudich D, de la Torre C, Sans J. Impaired chromosome segregation in plant anaphase after moderate hypomethylation of DNA. *Cell Prolif* 1995;28:481–496.
16. Titenko-Holland N, Jacob RA, Shang N, Balaraman A, Smith MT. Micronuclei in lymphocytes and exfoliated buccal cells of postmenopausal women with dietary changes in folate. *Mutat Res* 1998;417:101–114.
17. James SC, Basnakian AG, Miller BJ. In vitro folate deficiency induces deoxynucleotide pool imbalance, apoptosis, and mutagenesis in Chinese hamster ovary cells. *Cancer Res* 1994;54:5075–5080.
18. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN. Folate deficiency causes uracil misincorporation into human DNA and chromosomal breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci U S A* 1997;94:3290–3295.
19. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Genet Rev* 2001;2:280–291.
20. Petersen MB, Mikkelsen M. Nondisjunction in trisomy 21: origin and mechanisms. *Cytogenet Cell Genet* 2000;91:199–203.
21. Hook EB, Czeizel AE. Can terathanasia explain the protective effect of folic-acid supplementation on birth defects? *Lancet* 1997;350:513–515.
22. Hook EB. Folic acid: abortifacient or pseudoabortifacient? *Am J Med Genet* 2000;92:301–302.
23. Windham GC, Shaw GM, Todoroff K, Swan SH. Miscarriage and use of multivitamins or folic acid. *Am J Med Genet* 2000;90:261–262.
24. Gindler J, Li Z, Berry RJ, Zheng J, Correa A, Sun X, Wong L, Cheng L, Erickson JD, Wang Y, Tong Q. Folic acid supplements during pregnancy and risk of miscarriage. *Lancet* 2001;358:796–800.
25. Dailey T, Dale B, Cohen J, Munne S. Association between nondisjunction and maternal age and meiosis-II human oocytes. *Am J Hum Genet* 1996;59:176–184.
26. Hook EB, Cross PK, Schreinemachers DM. Chromosomal abnormality rates at amniocentesis and in live-born infants. *JAMA* 1983;249:2034–2038.
27. Hook EB. Rates of chromosome abnormalities at different maternal ages. *Obstet Gynecol* 1981;58:282–285.