Maternal *MTHFR* genotype and haplotype predict deficits in early cognitive development in a lead-exposed birth cohort in Mexico City[1–4]

J Richard Pilsner, Howard Hu, Robert O Wright, Katarzyna Kordas, Adrienne S Ettinger, Brisa N Sánchez, David Cantonwine, Alicia L Lazarus, Alejandra Cantoral, Lourdes Schnaas, Martha Maria Téllez-Rojo, and Mauricio Hernández-Avila

ABSTRACT

Background: Maternal folate nutritional status and prenatal lead exposure can influence fetal development and subsequent health. The methylenetetrahydrofolate reductase (*MTHFR)* gene is important for folate metabolism, and 2 common polymorphisms, C677T and A1298C, reduce enzymatic activity; C677T is present at high penetrance in Mexican populations.

Objective: The objective of this study was to examine potential links between maternal and child *MTHFR* polymorphisms and child neurodevelopment in a lead-exposed population.

Design: Data regarding *MTHFR* polymorphisms C677T and A1298C, peri- and postnatal lead measures, and Bayley Mental Development Index at 24 mo of age (MDI-24) scores were available for 255 mother-child pairs who participated in the ELEMENT (Early Life Exposures in Mexico to Environmental Toxicants) study during 1994–1995.

Results: In covariate-adjusted regression models, maternal *MTHFR* 677 genotype predicted MDI-24 scores, in which each copy of the maternal *MTHFR* 677T variant allele was associated with lower MDI-24 scores ($\beta = -3.52$; 95% CI: -6.12, -0.93; $P = 0.004$). Maternal *MTHFR* haplotype also predicted MDI-24 scores (mean ± SE: 93.3 ± 1.2 for 677C-1298A compared with 89.9 ± 0.8 for 677T-1298A; $P < 0.05$). MDI-24 scores were not associated with maternal *MTHFR* 1298 genotype or child *MTHFR* genotypes. We did not observe significant *MTHFR* genotype × lead interactions with respect to any of the subject biomarkers of lead exposure.

Conclusions: The maternal *MTHFR* 677T allele is an independent predictor of poorer child neurodevelopment at 24 mo. These results suggest that maternal genetic variations in folate metabolism during pregnancy may program offspring neurodevelopment trajectories. Further research is warranted to determine the generalizability of these results across other populations. *Am J Clin Nutr* 2010;92:226–34.

INTRODUCTION

Nutritional status and environmental factors play a critical role in neurodevelopment (1, 2). The mammalian central nervous system is the most susceptible biological system to developmental injury from exogenous insults because of its complexity and reliance on numerous timed processes, such as neuronal differentiation, cell migration, and myelin deposition (3). Developmental lead exposure in animal models target numerous processes for cognitive neurodevelopment, such as long-term potentiation (4), neurogenesis (5), and expression profiles of glutamate receptor subunits (6). In addition, several epidemiologic studies have shown that maternal body burdens of lead during pregnancy can impair the neurobehavioral development of children and manifest as cognitive delays and a lower intelligence quotient (IQ) later in life (7–9). Of particular interest for neurodevelopment is the interplay between environmental toxicants, such as developmental lead exposure, and critical nutrients.

Adequate maternal folate nutritional status is a central contributor to fetal development and subsequent offspring health, especially concerning its role in reducing the incidence of neural tube defects (10). To meet the high daily requirement of folate for one-carbon reactions during fetal development, this micronutrient is actively transported across the placenta against

[1] From the Department of Epidemiology (JRP and ALL), the Robert Wood Johnson Health and Society Program (JRP), the Department of Environmental Health Sciences (HH and DC), and the Department of Biostatistics (BNS), School of Public Health, University of Michigan, Ann Arbor, MI; the Department of Environmental Health, Harvard School of Public Health, and the Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (HH, ASE, and ROW); Children's Hospital Boston, Boston MA (ROW); the Department of Nutritional Sciences, Pennsylvania State University, University Park, PA (KK); the Division of Program Evaluation and Biostatistics, Center for Evaluation Research and Surveys, National Institute of Public Health, Cuernavaca, Morelos, Mexico (AC and MMT-R); the Division of Public Health, National Institute of Perinatology Isidro Espinosa de los Reyes, Mexico City, Mexico (LS); and the Ministry of Health, Mexico City, Mexico (MH-A).

[2] The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the US National Institute of Environmental Health Sciences (NIEHS), the National Institutes of Health, or the US Environmental Protection Agency (EPA).

[3] Supported by grants R01 ES007821, R01 ES014930, R01 ES013744, P30 ES00002; P42 ES05947, K23 ES000381, and K01 ES014907 from the NIEHS, grant 4150M9405 from the Consejo Nacional de Ciencia y Tecnología and CONSERVA, Department of Federal District, México. Additional support for the interpretation of results and authorship of this publication was made possible by NIEHS grant P01 ES012874 and a STAR (Science to Achieve Results) Research Assistance Agreement no. RD-83172501 awarded by the EPA. The Robert Wood Johnson Health & Society Program provided support to JRP.

[4] Address correspondence to JR Pilsner, Department of Epidemiology, School of Public Health, University of Michigan, 3638 SPH Tower, 109 Observatory, Ann Arbor, MI 48109. E-mail: rpils@umich.edu.

Received October 20, 2009. Accepted for publication April 26, 2010.

First published online May 26, 2010; doi: 10.3945/ajcn.2009.28839.

 © 2010 American Society for Nutrition

a concentration gradient such that folate concentrations are typically 3 times higher in the umbilical cord than in the maternal circulation (11). Consequently, maternal folate status and/or polymorphisms in folate-metabolizing genes may affect nutrient delivery to the fetus and subsequent neurodevelopment.

The methylenetetrahydrofolate reductase (*MTHFR)* gene is critical to folate metabolism and contains 2 common missense polymorphisms, C677T and A1298C, each of which renders the enzyme functionally impaired (12–14). *MTHFR* is located at the major branch point of one-carbon metabolism, where folate in the 5,10,-methlyenetetrahydrofolate (5,10-methyleneTHF) isoforms can be used for nucleotide synthesis or, alternatively, can be reduced to 5-methylTHF and thereby commit folate solely to biomethylation reactions. Individuals who carry the *MTHFR* 677 *TT* variant genotype have reduced plasma and red blood cell folate concentrations, elevated plasma homocysteine concentrations (in individuals with low blood folate concentrations) (15–17), and differential coenzymatic forms of folate in red blood cells (18). In Mexican populations, the prevalence of the *MTHFR* C677T variant is reported to be one of the highest worldwide (19).

There are relatively few studies on the influence of maternal folate status and/or the *MTHFR* genotype on child neurocognitive development, especially with regards to the adverse effects of environmental lead exposure. In the central Philippines, folate deficiency was reported to increase the susceptibility to lead-associated cognitive declines in children (20). To gain a better understanding of other potential neurodevelopmental risk factors, the current study examined the influence of maternal and child *MTHFR* genotypes on child neurodevelopment at 24 mo of age. In addition, we examined potential interactions between the *MTHFR* genotype and prenatal lead concentrations that concern child neurodevelopment.

SUBJECTS AND METHODS

Sample population

The ELEMENT (Early Life Exposures in Mexico to Environmental Toxicants) study is a group of sequentially enrolled epidemiologic birth-cohort studies with the aim of investigating the influence of cumulative maternal lead burden on fetal and infant development. For the current study, which used data and biological samples from the first birth cohort, maternal/child pairs were recruited between 1994 and 1995 from 3 hospitals in Mexico City (Mexican Social Security Institute, Manuel Gea Gonzalez Hospital, and National Institute of Perinatology) which serve low-to-moderate-income populations. Exclusion criteria were as follows: factors that could interfere with maternal calcium metabolism; medical conditions that could cause low birth weight ($<$2000 g); logistic reasons that would interfere with data collection (households located outside the metropolitan area); delivery of a premature neonate ($<$37 wk) or an infant with an Apgar score at 5 min of $\leq$6; conditions that required placement in a neonatal intensive care unit; a physician's diagnosis of multiple fetuses; the intention not to breastfeed; preeclampsia; psychiatric, kidney, or cardiac diseases; gestational diabetes; a history of repeated urinary infections; a family or personal history of kidney-stone formation; a seizure disorder requiring daily medication; the ingestion of corticosteroids; or $>$140 mm Hg systolic or $>$90 mm Hg diastolic blood pressure. Of the initial 1382 mothers who remained eligible, 617 agreed to participate and continued in the birth-cohort study. Of these, DNA was extracted from 412 umbilical cord samples. Our data set consisted of 256 mother-child pairs who had complete *MTHFR* 677 and *MTHFR* 1298 genotype data and Bayley Mental Development Index at 24 mo of age (MDI-24) scores.

Blood lead measurements

Umbilical cord venous blood samples were collected in trace metal-free tubes at delivery. Blood samples were analyzed with an atomic absorption spectrometry instrument (Perkin-Elmer 3000; Perkin-Elmer, Chelmsford, MA) at the metals laboratory of the American British Cowdray Medical Center (Mexico City, Mexico). External blinded quality-control samples were provided throughout the study period by the Maternal and Child Health Bureau (Rockville, MD) and the Wisconsin State Laboratory of Hygiene Cooperative Blood Lead Proficiency Testing Program (Madison, WI).

Bone lead measurements

Maternal bone lead was measured noninvasively around 1 mo postpartum with a spot-source ^{109}Cd K-X-ray fluorescence instrument constructed at Harvard University and installed in a research facility in the American British Cowdray Medical Center. The physical principles, technical specifications, and validation of this and other similar K-X-ray fluorescence instruments have been described in detail elsewhere (21). For this study, 30-min measurements were taken at the midshaft of the left tibia (cortical bone) and the left patella (trabecular bone). Analysis of means and standard deviations of phantom-calibrated measurements did not disclose any significant shift in accuracy or precision. As a quality-control measure, any tibia lead measurements with an uncertainty $>$10 μg/g or any patella lead measurements with an uncertainty $>$15 μg/g were excluded as a routine practice (22). Only 3% of tibia lead concentrations and 6% of patella lead concentrations were excluded because of measurement uncertainty.

Measurements of childhood development and potential confounders

The Bayley Scales of Infant Development (BSID-II) is a revision and restandardization of the BSID, the most widely used test of infant development, and our protocol for its implementation has been described previously by our group (7). The revised scale can be used to assess the development of children between the ages of 1 and 42 mo. Scores have been shown to be sensitive to a variety of prenatal, perinatal, and postnatal insults, including lead exposure (8, 23, 24). The BSID-II has also been used in numerous cross-cultural studies of lead and child development (25, 26). A Spanish version of the BSID-II was developed and validated by our research group before this study. MDI-24 scores were used as the primary child-development endpoint in this study. An interviewer-administered survey collected at 1 mo postpartum provided information on sociodemographic characteristics, reproductive history, and other factors that may constitute potential cofounders of the relation between lead and child development. Maternal IQ was assessed

by using the Information, Comprehension, Similarities, and Block Design components of the Wechsler Adult Intelligence Score, which has been translated into Spanish and used in Mexico (7).

DNA extraction and genotyping

DNA extraction was performed in the Harvard-Partners Center for Genetics and Genomics. High-molecular-weight DNA was extracted with commercially available PureGene Kits (Gentra Systems, Minneapolis, MN) from the white blood cells of archived umbilical cord blood samples that were collected at delivery. A TaqMan platform (Applied Biosystems, Carlsbad, CA) was used to genotype the *MTHFR* single-nucleotide polymorphisms C677T (rs1801133) and A1298C (rs1801131) as described elsewhere (27). The combinations of wild-type genotypes are abbreviated as follows: for example, 677 *CC* and 1298 *AA* is abbreviated *CCAA*, and 677 *CT* and 1298 *AC* is abbreviated *CTAC*. Allele distribution was previously shown to be in Hardy-Weinberg equilibrium for this study population (27).

Dietary assessment

Food-frequency questionnaire

Maternal dietary folate intake was estimated by using a semiquantitative food-frequency questionnaire (FFQ) administered at 1 mo postpartum by trained interviewers. The FFQ was developed on the basis of a previously validated instrument (28, 29) and subsequently adapted and validated for use in Spanish-speaking, adult women (30, 31). The FFQ queried usual frequency over the previous year of consuming 116 foods and beverages that are typical in Mexican diets with 10 response options that ranged from never to monthly, weekly, and daily intakes. Food folate intakes were then calculated on the basis of a nutrient database that comprised reference data from the US Department of Agriculture and the National Institute of Nutrition in Mexico (30).

Folate bioequivalence

The bioavailability of folic acid derived from dietary supplements is much higher than from food folate (32, 33). To account for bioavailability differences, all folate data were converted to dietary folate equivalents (DFEs) as calculated by the following formula: DFE = micrograms of food folate + 1.7 × micrograms of folic acid. Low folate intake was classified as $<$520 DFEs, which is the estimated average requirement (EAR) for pregnant women (34).

Dietary supplement use

Information for dietary supplementation use was collected as part of the FFQ, which determined the brand, frequency, and duration of prenatal supplement use over the previous year. The average daily intake of folic acid was calculated by the number of days that supplement use was reported, the number of pills per day, and the serving size from the product label (35).

Ethics

The study protocol was approved by the Ethics Committee of the National Institute of Public Health of Mexico, the participating hospitals, the Brigham and Women's Hospital, the Harvard School of Public Health, and the University of Michigan. All participating mothers received a detailed explanation of the study intent, research procedures, and counseling on how to reduce environmental lead exposure.

Statistics

Descriptive statistics for characteristics of the study sample were calculated separately for participating or nonparticipating individuals. Excluded individuals were those who were missing either *MTHFR* genotype data or child MDI-24 scores. Differences by participation status were tested by using Wilcoxon's rank-sum or chi-square tests for quantitative and categorical variables, respectively. Linear regression models were used to describe the relation between *MTHFR* genotype and child MDI-24 scores. In adjusted models, covariates were included on the basis of biological plausibility or those previously shown to be associated with child MDI scores in a previous study of prenatal lead exposure and child cognition (7). The included covariates were as follows: maternal age, maternal IQ, marital status, parity, gestational age, inadequate folate intake, and umbilical cord lead concentrations. Regression diagnostics were performed on all models to evaluate multicollinearity and violations of assumptions of the linear regression model. To test for potential interactions between the *MTHFR* genotype and bone and blood lead measures, interaction terms (*MTHFR* 677 × lead and *MTHFR* 1298 × lead) were included in separate analyses that predicted MDI-24 scores. Interaction terms between the *MTHFR* genotype and low folate intake were also investigated. Data were analyzed with SAS 9.1 software (2002–2003; SAS Institute, Cary, NC).

PHASE version 2.1.1 software (University of Washington, Seattle, WA) was used to construct haplotype estimates and allele frequencies for *MTHFR* C677T and A1298C. This program requires an input file with information on genotypes organized by the individual. The set was run in 100 iterations with a burn-in interval of 100 and a thinning interval of 1. The PHASE program uses a type of Markov chain–Monte Carlo algorithm to find the appropriate sample of individuals given the genotypes. The repetition of this process over enough intervals conveys an approximate sample, constructs a Markov chain with a stationary distribution, and conveys possible haplotype reconstructions (36, 37). Linear regression models were used to examine the relation between *MTHFR* haplotypes (n = 510) and child MDI-24 scores.

RESULTS

The characteristics of study participants (n = 255) and subjects who were excluded (n = 374) because of missing MDI-24 scores and/or *MTHFR* genotype data are presented in **Table 1**. Subjects who were excluded from the analyses and subjects who were included in the analyses were similar in such variables as maternal age, body mass index, IQ, and nutritional intake, child birth measures, and lead biomarkers. The use of dietary supplements containing folic acid was reported by 17.3% (n = 44) of the participating mothers. In study participants, supplement users had a higher mean of total folate intake than did non-supplement users (mean ± SD: 492.9 ± 241.5 compared with 372.3 ± 138.6 DFEs/d, respectively; $P < 0.001$). With the use

TABLE 1
Characteristics of subjects by participation status[1]

Variable	Participating (n = 255)		Nonparticipating (n = 374)	
	Values	n	Values	n
Mothers				
Age (y)	24.6 ± 5.1[2]	255	24.5 ± 5.1	374
BMI (kg/m^2)	23.6 ± 3.5	205	23.5 ± 3.8	291
Education (y)	9.4 ± 3.1	255	9.1 ± 3.2	374
IQ	84.3 ± 23.5	253	85.1 ± 24.3	229
Parity	1.9 ± 1.1	255	2.1 ± 1.3	372
Total protein (g/d)	79.9 ± 31.6	253	76.1 ± 29.6	370
Folate intake (DFEs/d)	393.3 ± 167.0	253	369.6 ± 161.1	370
Supplement users (%)	17.3	44	15.8	59
Food intake (DFEs/d)	389.6 ± 185.7	44	399.5 ± 153.6	59
Total intake (DFEs/d)	492.9 ± 241.5	44	495.1 ± 244.2	59
Low folate intake (%)[3]	65.9	29	67.8	40
Non–supplement users (%)	82.0	209	81.2	311
Food intake (DFEs/d)	372.3 ± 138.6	209	345.8 ± 127.2	311
Total intake (DFEs/d)	372.3 ± 138.6[4]	209	345.8 ± 127.2	311
Low folate intake (%)[3]	87.1[5]	182	91.0	283
Vitamin B-12 (μg/d)	6.6 ± 3.0	253	6.0 ± 2.7	370
Tibia lead (μg/g)	10.5 ± 10.4	248	9.7 ± 9.9	366
Patella lead (μg/g)	14.7 ± 13.7	239	14.9 ± 16.6	348
Children				
Birth weight (g)	3144.3 ± 432.3	254	3128.3 ± 411.4	372
Birth length (cm)	50.3 ± 2.3	251	50.4 ± 2.4	368
Gestational age (wk)	39.2 ± 1.5	254	39.1 ± 1.5	365
MDI-24 scores	91.6 ± 14.1	255	92.0 ± 13.9	84
Male (%)	45.3	115	45.3	168
Umbilical cord blood lead (μg/dL)	6.7 ± 3.6	221	6.6 ± 3.6	298

[1] IQ, intelligence quotient; DFEs, dietary folate equivalents; MDI-24, Bayley Mental Development Index at 24 mo of age.
[2] Mean ± SD (all such values).
[3] <520 DFEs/d, which is the estimated average requirement for pregnant women.
[4,5] Significantly different from supplement users: [4]$P < 0.0001$ (t test), [5]$P < 0.0005$ (chi-square test).

of the folate EAR for pregnant women of 520 DFEs/d, 65.9% of supplement users and 87.1% of nonusers were classified as having low folate intake ($P < 0.001$).

Genotype frequencies for the C677T and A1298C polymorphisms in children and mothers are shown in **Tables 2** and **3**. For the C677T polymorphism, ≈15% of subjects carried the wild-type *CC* genotype, whereas >38% of subjects carried the variant *TT* genotype. Conversely, the majority of subjects (>77%) were carriers of the wild-type 1298C *AA* genotype, whereas only 1% of subjects carried the variant *CC* genotype. The frequencies of the 9 genotype combinations resulting from the C677T and A1298C polymorphisms are also presented in Tables 2 and 3. No subjects were homozygous recessive for both *MTHFR* genotypes (*TTCC*) or homozygous recessive for one *MTHFR* genotype and heterozygous for the other (*TTAC* and *CTCC*). The most common genotypes of children and mothers were *TTAA* (39.2% and 38.4%, respectively) and *CTAA* (31.8% and 34.9%, respectively). The majority of children and mothers were carriers of at least one 677T variant allele (85.5% and 86.2%, respectively).

The unadjusted mean MDI-24 scores with maternal and child *MTHFR* 677 genotypes are shown in **Figure 1**. In mothers, we observed a *MTHFR* 677 genotype-dependent decrease in MDI-24 scores such that a higher number of copies of the 677T variant allele was associated with lower MDI-24 scores (P for trend = 0.01). Children born to mothers carrying the 677 *TT*

TABLE 2
Child *MTHFR* genotype frequencies

677	1298 *AA*	1298 *AC*	1298 *CC*	Total
		% (n)		% (n)
CC	6.67 (17)	7.1 (18)	0.8 (2)	14.5 (37)
CT	31.8 (81)	14.5 (37)	—	46.3 (118)
TT	39.2 (100)	—	—	39.2 (100)
Total	77.7 (198)	21.6 (55)	0.8 (2)	255

TABLE 3
Maternal *MTHFR* genotype frequencies

677	1298 *AA*	1298 *AC*	1298 *CC*	Total
		% (n)		% (n)
CC	8.2 (21)	4.3 (11)	1.2 (3)	13.7 (35)
CT	34.9 (89)	12.9 (33)	—	47.8 (122)
TT	38.4 (98)	—	—	38.4 (98)
Total	81.6 (208)	17.3 (44)	1.2 (3)	255

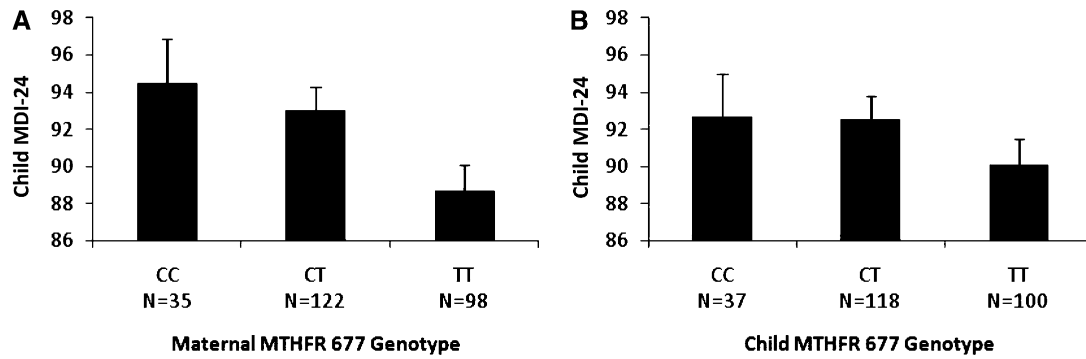

FIGURE 1. A: Unadjusted mean scores of the Bayley Mental Development Index at 24 mo of age (MDI-24) by maternal *MTHFR* 677 genotype (*P* for trend = 0.01; *TT* compared with *CC* or *CT*; $P < 0.05$). B: Unadjusted mean MDI-24 scores by child *MTHFR* 677 genotype (*P* for trend = 0.22).

genotype had mean (±SE) MDI-24 scores 5.8 points lower (88.7 ± 1.4) than the MDI-24 scores of children of mothers carrying the 677 *CC* genotype (94.5 ± 2.4; *P* = 0.04). For the maternal *MTHFR* 1298 genotype, unadjusted homozygous-dominant models (*AA* compared with *AC*/*CC*) revealed a modest but unsignificant difference between maternal genotypes and MDI-24 scores (mean ± SE: *AA* = 90.8 ± 1.0 compared with *AC*/*CC* = 94.7 ± 2.0, *P* = 0.09; **Figure 2**). We did not observe any significant associations between child *MTHFR* 677 or *MTFHR* 1298 genotypes and MDI-24 scores.

Multiple regression models of maternal and child *MTHFR* genotypes and MDI-24 scores adjusted for maternal age, IQ, and low folate intake, gestational age, and parity are shown in **Table 4**. We observed a −3.52 point reduction in MDI-24 scores for each additional copy of the maternal *MTHFR* 677T variant allele after controlling for covariates (*P* = 0.004). No significant associations were observed between other *MTHFR* genotypes and MDI-24 scores. Low maternal folate intake was not associated with MDI-24 scores. No significant interactions were shown between *MTHFR* genotype × low folate intake and *MTHFR* genotype × prenatal lead concentrations on MDI-24 scores.

To further illustrate the influence of maternal *MTHFR* 677 genotypes on MDI-24 scores, we plotted the cumulative distributions of MDI-24 scores and compared the 677 *CC*/*CT* and *TT* genotypes in the 255 mother-child pairs (**Figure 3**). We observed a shift in the cumulative distribution of MDI-24 scores such that children born to mothers who were 677 *TT* carriers had poorer MDI-24 scores than did children born to mothers who were carriers of the 677 *CC* or *CT* genotypes (Figure 3B). The extent of shift in the cumulative distribution of MDI-24 scores was less apparent when the child *MTHFR* 677 genotype was plotted (Figure 3A).

Finally, we examined the association of *MTHFR* haplotypes with child neurodevelopment (**Table 5**). The 677T-1298A haplotype was the most frequent allele predicted in our population for both mothers and children (62.4%); whereas the haplotypes 677C-1298A and 677C-1298C were predicted at lower frequencies. The rare 677T-1298C haplotype was not predicted for our population. In covariate-adjusted regression models, children of mothers with the 677T-1298A haplotype had lower MDI-24 scores than did children of mothers with the 677C-1298A haplotype (89.9 ± 0.8 compared with 93.3 ± 1.2; *P* = 0.02). No significant associations were shown between child *MTHFR* haplotypes and MDI-24 scores.

DISCUSSION

In the current study, we observed that increased copies of the maternal *MTHFR* 677T variant allele were associated with lower child neurodevelopment at 24 mo. The maternal *MTHFR* haplotype 677T-1298A was also associated with lower neurodevelopment compared with other predicted haplotypes. However, child *MTHFR* 677 genotypes and haplotypes were not associated with child neurodevelopment, which suggests that the effect, if not because of chance, must be related to maternal-fetal metabolism of folate in pregnancy. Finally, maternal and child

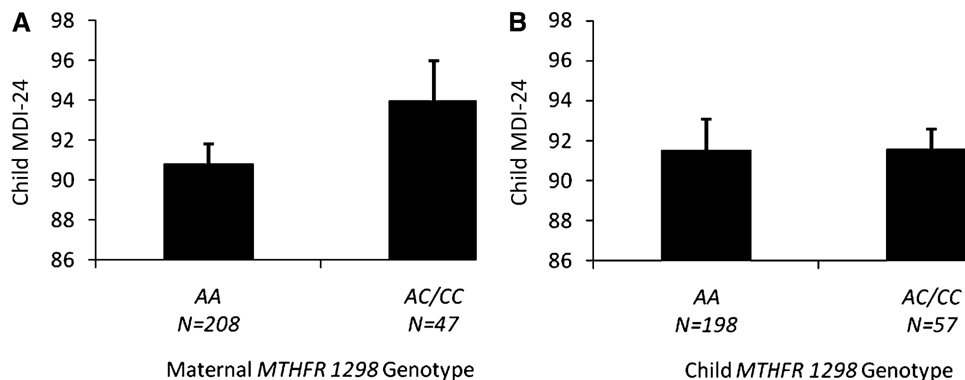

FIGURE 2. A: Unadjusted mean scores of the Bayley Mental Development Index at 24 mo of age (MDI-24) by maternal *MTHFR* 1298 genotype (*P* = 0.09). B: Unadjusted mean MDI-24 scores by child *MTHFR* 1298 genotype (*P* = 0.98).

TABLE 4
Multiple regression models of maternal and child *MTHFR* genotypes and Bayley Mental Development Index at 24 mo of age scores[1]

	Maternal		Child	
Variable	β (95% CI)	*P*	β (95% CI)	*P*
MTHFR 677[2]	−3.52 (−6.12, −0.93)	0.004	−1.73 (−4.31, 0.85)	0.19
Maternal age	−0.23 (−0.62, 0.17)	0.26	−0.22 (−0.62, 0.18)	0.28
Gestational age	0.72 (−0.54, 21.98)	0.26	0.86 (−0.42, 2.13)	0.19
Umbilical cord lead	−0.71 (−1.20, −0.21)	0.005	−0.71 (−1.21, −0.21)	0.006
Maternal IQ	0.12 (0.04, 0.20)	0.002	0.13 (0.05, 0.21)	0.002
Parity	−2.75 (−6.58, 1.08)	0.16	−2.67 (−6.56, 1.23)	0.18
Low folate intake[3]	1.32 (−3.60, 6.23)	0.53	0.79 (−4.17, 5.75)	0.75
Marital status	4.00 (0.11, 7.88)	0.04	4.27 (0.35, 8.20)	0.03
MTHFR 1298[4]	−2.78 (−7.61, 2.04)	0.26	−0.33 (−4.57, 3.90)	0.88
Maternal age	−0.25 (−0.65, 0.15)	0.22	−0.23 (−0.63, 0.17)	0.25
Gestational age	0.86 (−0.41, 2.14)	0.18	0.86 (−0.43, 2.14)	0.19
Umbilical cord lead	−0.70 (−1.20, −0.20)	0.007	−0.73 (−1.22, −0.23)	0.004
Maternal IQ	0.12 (0.04, 0.20)	0.002	0.13 (0.05, 0.21)	0.002
Parity	−2.84 (−6.72, 1.30)	0.15	−2.94 (−6.83, 0.96)	0.14
Low folate intake[3]	0.63 (−4.33, 5.59)	0.80	0.68 (−4.30, 5.67)	0.79
Marital status	4.31 (0.38, 8.23)	0.03	4.46 (0.53, 8.39)	0.03

[1] IQ, intelligence quotient.
[2] *CC* compared with *CT* compared with *TT*.
[3] <520 dietary folate equivalents/d, which is the estimated average requirement for pregnant women.
[4] *AA* compared with *AC*/*CC*.

MTHFR 1298 genotype status was not associated with child neurodevelopment. In this population there is a high prevalence of *MTHFR* 677 *TT* and a low prevalence of *MTHFR* 1298 *CC* genotypes as previously reported for populations of Mexican descent (19).

To our knowledge, this is the first study to find a main effect of maternal *MTHFR* 677 genotype status on child neurodevelopment. The finding that maternal genotypes but not child genotypes are related to child neurodevelopmental outcomes suggests that this effect is due to developmental processes that occur during pregnancy such that maternal genetic variations in folate metabolism may program the trajectories of offspring neurodevelopment.

MTHFR domains/activity

Optimal dietary intake of folate, vitamin B-12, choline, and methionine during pregnancy is necessary for proper fetal

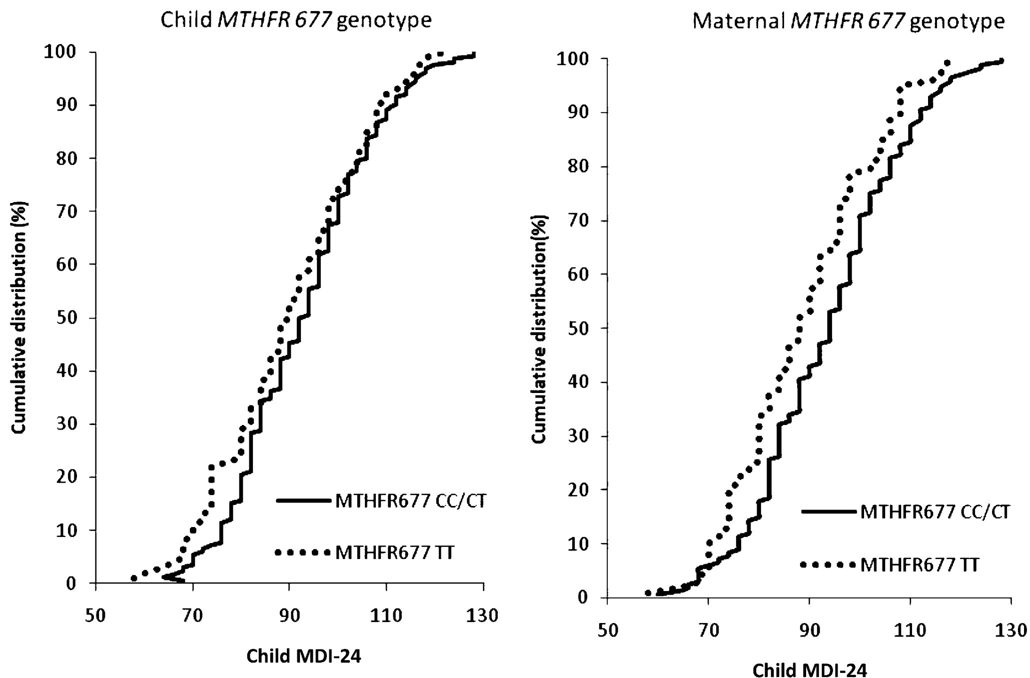

FIGURE 3. Cumulative frequency distributions of scores of the Bayley Mental Development Index at 24 mo of age (MDI-24) comparing *MTHFR* 677 *CC*/*CT* and *TT* genotypes in 256 mother-child pairs.

TABLE 5
MTHFR haplotype frequencies in relation to Bayley Mental Development Index at 24 mo of age (MDI-24) scores

Haplotype	Values	Unadjusted MDI-24 scores	Adjusted MDI-24 scores[1]
Mothers			
677C-1298A	27.8 (142)[2]	93.2 ± 1.2[3]	93.3 ± 1.2
677C-1298C	9.8 (50)	94.6 ± 2.0	93.4 ± 2.1
677T-1298A	62.4 (318)	90.4 ± 0.8[4,5]	89.9 ± 0.8[4]
Children			
677C-1298A	26.1 (133)	92.9 ± 1.3	92.6 ± 1.2
677C-1298C	11.6 (59)	91.7 ± 1.8	91.4 ± 1.8
677T-1298A	62.4 (318)	91.0 ± 0.8	90.5 ± 0.8

[1] Models adjusted for maternal age, intelligence quotient, low folate intake, gestational age, parity, marital status, and umbilical cord blood lead.
[2] Percentage; *n* in parentheses (all such values).
[3] Mean ± SE (all such values).
[4] 677C-1298A compared with 677T-1298A ($P < 0.05$).
[5] 677C-1298 compared with C677T-1298A ($P < 0.05$).

development, especially neurogenesis (38). Together, these nutrients are essential components of one-carbon metabolism-dependent nucleotide synthesis and biomethylation reactions. *MTHFR* is an essential flavoprotein of one-carbon metabolism that catalyzes the NADPH-linked reduction of 5,10-methyleneTHF to 5-methyTHF; the latter provides a methyl group for the de novo generation of methionine from homocysteine for downstream biomethylation reactions (39). The human *MTHFR* is a dimer of ≈70-kDa subunits; each monomer is composed of both an *N*-terminal catalytic domain that binds folate and the FAD cofactor and a C-terminal regulatory domain that binds the allosteric inhibitor *S*-adenosylmethionine (SAM) (13). The 2 monomers are thought to associate in a "head to tail" confirmation to form the catalytically active enzyme dimer (40). The *MTHFR* 677 and 1298 polymorphic sites are harbored within the catalytic and regulatory domains, respectively. The 677 *TT* variants have been reported as thermolabile with reduced enzyme activity (12, 13), whereas the 1298 *CC* variants are associated with only a mild reduction of enzymatic activity in vivo (14). Together, our genotype and haplotype data indicate that the maternal polymorphism located within the catalytic domain (eg, *MTHFR* 677) may play an important role in child neurodevelopment.

MTHFR prevalence and implications

The prevalence of the *MTHFR* 677 and 1298 variants has a wide geographic and ethnic variability. It has been reported that Mexico populations have one of the highest frequency of the 677T allele worldwide, whereas they have the lowest frequency of the 1298C allele (19). Our results are consistent with these reports; we showed that >85% of subjects in our study had at least one 677T allele, and > 38% of subjects were *TT* carriers. In our study, the 1298 *CC* genotype was only present in ≈1% of subjects.

A well-known interaction exists between the *MTHFR* 677 genotype and folate status such that a reduction of MTHFR activity occurs in *TT* carriers under conditions of low but not high folate status. Recombinant studies have shown that lower folate concentrations increase the propensity for *MTHFR* to lose its FAD cofactor and dissociate into monomers (13). The downstream biological consequences of the *MTHFR* 677 *TT* × low folate interaction include elevated plasma homocysteine concentrations (15, 16) and decreased genomic DNA methylation concentrations (17). The high prevalence of the *MTHFR* 677T allele and low folate intake in our study imply that a reduction of MTHFR activity would be likely. del Río Garcia et al (41) reported a significant interaction between low maternal folate intake (<400 μg/d) and the maternal *MTHFR* 677 *TT* genotype on child neurodevelopment. However, in our study we observed no significant interactions between the *MTHFR* 677 *TT* genotype and low folate intake. The discrepancy between studies could be explained by different units of measure and cutoffs for low folate intake. First, we converted all folate values to DFEs to account for the increased bioavailability of folic acid derived from supplements. We also used the EAR for pregnant women (520 DFEs/d) to define low folate intake, which resulted in the majority of mothers (83.4%) being classified as having low folate intake. The high prevalence of low folate intake in our population might have limited the statistical power necessary to detect interactions. Finally, although both studies were conducted in Mexico City, our study included measures of lead biomarkers, which increased the precision in estimating the effect of *MTHFR* genotypes on cognition.

It is important to note that most nutrient components of one-carbon metabolism are actively transported across the placenta against a concentration gradient; for example, folate concentrations are typically 3 times higher in the umbilical cord than in maternal blood (42, 43). Studies have shown that the *MTHFR* 677 *TT* genotype is associated with reduced plasma and red cell folate concentrations (15–17) and an altered distribution of red blood cell folate isoforms, where 5,10-methyleneTHF is more prevalent than 5-methylTHF (18). Although the maternal-to-fetal transfer of folate is mediated by the placental folate receptor, which preferentially binds 5-methylTHF isoforms (44), it is unknown to what extent maternal *MTHFR* 677 genotypes influence the transfer of folate and/or its isoforms across the placental barrier. Shifts in the distribution of folate isoforms in the developing fetus could affect downstream biomethylation reactions or nucleotide synthesis. As in the case of the diomethylation reactions, these could have important implications for the developing nervous system in light of the key role of epigenetic mechanisms, such as DNA and histone methylation, related to neuronal function and memory formation (45).

Lead/cognition

Studies on the effect of nutrition on lead-induced neurocognitive deficits, especially concerning one-carbon metabolism, are underrepresented. SAM, the universal methyl donor for one-carbon metabolism, has been shown to be beneficial in the treatment of lead intoxication by enhancing thiol contents and glutathione concentrations (46). In addition, a recent study revealed that the injection of SAM at postnatal day 22 improved hippocampal long-term potentiation and water-maze performance in rats with lead exposure in early life (47).

In humans, Solon et al (20) showed that folate and/or iron deficiency increased the susceptibility to the negative cognitive effects of lead in children in the central Philippines. Previous work from our group showed that prenatal lead exposure was a significant

predictor of poorer child neurodevelopment (7). In the current study, we extend these findings by reporting that the maternal *MTHFR* 677 *TT* genotype is also an independent predictor of poorer child neurodevelopment. However, we showed no significant interactions between *MTHFR* genotypes and lead measures.

A limitation to this study was the use of the semiquantitative FFQ to assess dietary intake. Maternal dietary intake was assessed 1 mo postpartum and was designed to capture dietary habits of women over the previous year (27). Despite the well-know interaction between *MTHFR* 677 *TT* carriers and folate deficiency, we did not observe a significant interaction between maternal *MTHFR* 677 *TT* carriers and low folate intake on child neurodevelopment. This may be explained, in part, from estimating folate intake from the FFQ rather than assessing folate intake directly through blood measures.

In conclusion, we report that the maternal *MTHFR* 677T allele is an independent predictor of child neurodevelopment at 24 mo. Our results indicate that maternal genes that influence folate metabolism may influence neurodevelopment trajectories, possibly through fetal programming during pregnancy. Maternal MTHFR genotypes may have important implications on prospective child neurodevelopment in populations that carry a high prevalence of the *MTHFR* 677T allele, such as in Mexico and areas of China. Additional studies are warranted to determine whether these results are consistent across geographic and/or ethnic groups and to investigate potential molecular pathways that bridge *MTHFR* genotypes and child neurodevelopment.

We thank the field staff and the American British Cowdray Medical Center in Mexico City and the study participants who made this research possible. We also thank Karen Peterson for critical review of the manuscript and insightful comments.

The authors' responsibilities were as follows—JRP: manuscript preparation, statistical analyses, and interpretation of results; HH, ROW, ASE, AC, MMT-R, KK, and MH-A: study design and assistance in the interpretation of results; BNS and DC: assistance in statistical analyses and in interpretation of results; ALL: haplotype analysis; and LS: cognition measurements. None of the authors declared personal or financial conflicts of interest.

REFERENCES

1. Godfrey KM, Barker DJ. Fetal programming and adult health. Public Health Nutr 2001;4:611–24.
2. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet 1989;2:577–80.
3. Rodier PM. Environmental causes of central nervous system maldevelopment. Pediatrics 2004;113:1076–83.
4. Altmann L, Weinsberg F, Sveinsson K, Lilienthal H, Wiegand H, Winneke G. Impairment of long-term potentiation and learning following chronic lead exposure. Toxicol Lett 1993;66:105–12.
5. Gilbert ME, Kelly ME, Samsam TE, Goodman JH. Chronic developmental lead exposure reduces neurogenesis in adult rat hippocampus but does not impair spatial learning. Toxicol Sci 2005;86:365–74.
6. Nihei MK, Desmond NL, McGlothan JL, Kuhlmann AC, Guilarte TR. N-methyl-D-aspartate receptor subunit changes are associated with lead-induced deficits of long-term potentiation and spatial learning. Neuroscience 2000;99:233–42.
7. Gomaa A, Hu H, Bellinger D, et al. Maternal bone lead as an independent risk factor for fetal neurotoxicity: a prospective study. Pediatrics 2002;110:110–8.
8. Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. Longitudinal analyses of prenatal and postnatal lead exposure and early cognitive development. N Engl J Med 1987;316:1037–43.
9. Hu H, Tellez-Rojo MM, Bellinger D, et al. Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. Environ Health Perspect 2006;114:1730–5.
10. Stover PJ. Physiology of folate and vitamin B12 in health and disease. Nutr Rev 2004;62:S3-12; discussion S13.
11. Stanger O. Physiology of folic acid in health and disease. Curr Drug Metab 2002;3:211–23.
12. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.
13. Yamada K, Chen Z, Rozen R, Matthews RG. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. Proc Natl Acad Sci USA 2001;98:14853–8.
14. van der Put NM, Gabreels F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 1998;62:1044–51.
15. Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 1996;93:7–9.
16. Girelli D, Friso S, Trabetti E, et al. Methylenetetrahydrofolate reductase C677T mutation, plasma homocysteine, and folate in subjects from northern Italy with or without angiographically documented severe coronary atherosclerotic disease: evidence for an important genetic-environmental interaction. Blood 1998;91:4158–63.
17. Friso S, Choi SW, Girelli D, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci USA 2002;99:5606–11.
18. Bagley PJ, Selhub J. A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci USA 1998;95:13217–20.
19. Gueant-Rodriguez RM, Gueant JL, Debard R, et al. Prevalence of methylenetetrahydrofolate reductase 677T and 1298C alleles and folate status: a comparative study in Mexican, West African, and European populations. Am J Clin Nutr 2006;83:701–7.
20. Solon O, Riddell TJ, Quimbo SA, et al. Associations between cognitive function, blood lead concentration, and nutrition among children in the central Philippines. J Pediatr 2008;152:237–43.
21. Andrews KW, Savitz DA, Hertz-Picciotto I. Prenatal lead exposure in relation to gestational age and birth weight: a review of epidemiologic studies. Am J Ind Med 1994;26:13–32.
22. Kim R, Aro A, Rotnitzky A, Amarasiriwardena C, Hu H. K x-ray fluorescence measurements of bone lead concentration: the analysis of low-level data. Phys Med Biol 1995;40:1475–85.
23. Wigg NR, Vimpani GV, McMichael AJ, Baghurst PA, Robertson EF, Roberts RJ. Port Pirie Cohort study: childhood blood lead and neuropsychological development at age two years. J Epidemiol Community Health 1988;42:213–9.
24. Wasserman G, Graziano JH, Factor-Litvak P, et al. Independent effects of lead exposure and iron deficiency anemia on developmental outcome at age 2 years. J Pediatr 1992;121:695–703.
25. Baghurst PA, Robertson EF, Oldfield RK, et al. Lead in the placenta, membranes, and umbilical cord in relation to pregnancy outcome in a lead-smelter community. Environ Health Perspect 1991;90:315–20.
26. Rothenberg SJ, Schnaas L, Cansino-Ortiz S, et al. Neurobehavioral deficits after low level lead exposure in neonates: the Mexico City pilot study. Neurotoxicol Teratol 1989;11:85–93.
27. Kordas K, Ettinger AS, Lamadrid-Figueroa H, et al. Methylenetetrahydrofolate reductase (MTHFR) C677T, A1298C and G1793A genotypes, and the relationship between maternal folate intake, tibia lead and infant size at birth. Br J Nutr 2009;102:907–14.
28. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
29. Willett WC, Reynolds RD, Cottrell-Hoehner S, Sampson L, Browne ML. Validation of a semi-quantitative food frequency questionnaire: comparison with a 1-year diet record. J Am Diet Assoc 1987;87:43–7.
30. Romieu I, Hernandez-Avila M, Rivera JA, Ruel MT, Parra S. Dietary studies in countries experiencing a health transition: Mexico and Central America. Am J Clin Nutr 1997;65:1159S–65S.
31. Hernandez-Avila M, Romieu I, Parra S, Hernandez-Avila J, Madrigal H, Willett W. Validity and reproducibility of a food frequency questionnaire to assess dietary intake of women living in Mexico City. Salud Publica Mex 1998;40:133–40.
32. Bailey LB. New standard for dietary folate intake in pregnant women. Am J Clin Nutr 2000;71:1304S–7S.

33. Suitor CW, Bailey LB. Dietary folate equivalents: interpretation and application. J Am Diet Assoc 2000;100:88–94.
34. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, DC: National Academy Press, 1998.
35. Mejia-Rodriguez F, Sotres-Alvarez D, Neufeld LM, Garcia-Guerra A, Hotz C. Use of nutritional supplements among Mexican women and the estimated impact on dietary intakes below the EAR and above the UL. J Am Coll Nutr 2007;26:16–23.
36. Stephens M, Scheet P. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am J Hum Genet 2005;76:449–62.
37. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.
38. Zeisel SH. Importance of methyl donors during reproduction. Am J Clin Nutr 2009;89:673S–7S.
39. Green JM, Ballou DP, Matthews RG. Examination of the role of methylenetetrahydrofolate reductase in incorporation of methyltetrahydrofolate into cellular metabolism. FASEB J 1988;2:42–7.
40. Ulvik A, Ueland PM, Fredriksen A, et al. Functional inference of the methylenetetrahydrofolate reductase 677C > T and 1298A > C polymorphisms from a large-scale epidemiological study. Hum Genet 2007;121:57–64.
41. del Rio Garcia C, Torres-Sanchez L, Chen J, et al. Maternal MTHFR 677C>T genotype and dietary intake of folate and vitamin B(12): their impact on child neurodevelopment. Nutr Neurosci 2009;12:13–20.
42. Tamura T, Picciano MF. Folate and human reproduction. Am J Clin Nutr 2006;83:993–1016.
43. Hall M, Gamble M, Slavkovich V, et al. Determinants of arsenic metabolism: blood arsenic metabolites, plasma folate, cobalamin, and homocysteine concentrations in maternal-newborn pairs. Environ Health Perspect 2007;115:1503–9.
44. Antony AC. In utero physiology: role of folic acid in nutrient delivery and fetal development. Am J Clin Nutr 2007;85:598S–603S.
45. Levenson JM, Sweatt JD. Epigenetic mechanisms in memory formation. Nat Rev Neurosci 2005;6:108–18.
46. Paredes SR, Fukuda H, Kozicki PA, Rossetti MV, Conti H, Batlle AM. S-adenosyl-L-methionine and lead intoxication: its therapeutic effect varying the route of administration. Ecotoxicol Environ Saf 1986;12:252–60.
47. Cao XJ, Huang SH, Wang M, Chen JT, Ruan DY. S-adenosyl-L-methionine improves impaired hippocampal long-term potentiation and water maze performance induced by developmental lead exposure in rats. Eur J Pharmacol 2008;595:30–4.