

ABSENCE OF ASSOCIATION OF THROMBOPHILIA POLYMORPHISMS WITH INTRAUTERINE GROWTH RESTRICTION

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ABSTRACT

Background Previous data have demonstrated associations between thrombophilia polymorphisms in pregnant women and an increased risk of intrauterine growth restriction in their offspring, but this finding remains uncertain.

Methods We performed a hospital-based case-control study and a family-based study including 493 newborns with intrauterine growth restriction (defined by birth weight below the 10th percentile for gestational age and sex according to Canadian norms) and 472 controls (with birth weight at or above the 10th percentile). We determined the presence or absence in newborns and their parents of the following polymorphisms: methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C, factor V Leiden G1691A, and prothrombin G20210A. Mothers were interviewed to obtain information on other risk factors for intrauterine growth restriction.

Results The risk of intrauterine growth restriction was not increased among mothers carrying a polymorphism associated with thrombophilia. In the case-control study, the odds ratios associated with two copies of the variant, after adjustment for newborn genotype and other risk factors, were 1.55 for MTHFR C677T (95 percent confidence interval, 0.83 to 2.90) and 0.49 for MTHFR A1298C (95 percent confidence interval, 0.25 to 0.93); heterozygotes for factor V Leiden had an odds ratio of 1.18 (95 percent confidence interval, 0.54 to 2.55), and heterozygotes for prothrombin G20210A had an odds ratio of 0.92 (95 percent confidence interval, 0.36 to 2.35). These polymorphisms in the newborn were not associated with an increased risk. Newborns who were homozygous for the MTHFR C677T variant had a decreased risk of intrauterine growth restriction (odds ratio after adjustment for mother's genotype and other confounders, 0.52 [95 percent confidence interval, 0.29 to 0.94]). The results of the family-based study supported those of the case-control study.

Conclusions Our findings do not indicate that there are associations between maternal or newborn polymorphisms associated with thrombophilia and an increased risk of intrauterine growth restriction. (N Engl J Med 2002;347:19-25.)

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BABIES who are small for their gestational age have increased early morbidity and mortality¹ as well as possible later deficits in neuropsychological development.² Intrauterine growth restriction characterizes a fetus whose weight is less than expected on the basis of gestational age and sex, as determined by population standards. The threshold below which morbidity and mortality increase significantly is still a matter of debate,^{3,4} but a cutoff at less than the 10th percentile is often used.⁵ Causes for intrauterine growth restriction remain unclear, although a number of risk factors, such as cigarette smoking, have been identified.⁵

Polymorphisms in the genes encoding coagulation factor V (G1691A) and prothrombin (G20210A) are associated with thrombophilia (hypercoagulable state).⁶ Two common variants (C677T and A1298C) of the gene encoding 5,10-methylenetetrahydrofolate reductase (MTHFR), resulting from point mutations, have been implicated in the development of hyperhomocysteinemia⁷⁻⁹ and possibly coronary heart disease, although the latter is not a consistent finding.^{10,11}

On the basis of the hypothesis that polymorphisms associated with thrombophilia could affect placental circulation, a large number of case-control studies have been carried out to assess a link between maternal polymorphisms associated with thrombophilia and adverse outcomes of pregnancy.¹² The results have been inconsistent, and few studies have focused on intrauterine growth restriction. One study conducted in Israel included 44 cases of intrauterine growth restriction¹³; the study was later expanded to include 72 such cases.¹⁴ Another study from Italy included 61 cases of intrauterine growth restriction.¹⁵ Both studies found significant associations between polymorphisms associated with thrombophilia in mothers and intrauterine growth restriction in their infants.

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We carried out a large case-control study and a family-based study including both parents and newborns to determine whether polymorphisms associated with thrombophilia in the newborns or mothers were associated with an increased risk of intrauterine growth restriction.

METHODS

Case-Control Study

Selection of Case Subjects and Controls

Case subjects were newborns whose birth weight was below the 10th percentile for gestational age and sex, according to Canadian standards.¹⁶ All case subjects seen at the Centre Hospitalier Universitaire Mère-Enfant de l'Hôpital Sainte-Justine in Montreal between May 1998 and June 2000 who were born alive after the 24th week of gestation and without severe congenital anomalies were eligible for the study if the mother agreed to participate and spoke French or English. During that period, 505 newborns met the criteria for case subjects, of whom 493 participated in the study (97.6 percent).

Controls were born at the same hospital and met the same eligibility criteria, except that their birth weights were at or above the 10th percentile. They were matched to case subjects for gestational week at birth, sex, and race or ethnic group (white, black, Hispanic or Amerindian, and Asian) and were generally born within a week of the matched case subject. The mothers of 480 potential controls were invited to participate, and 472 accepted (98.3 percent). The project was approved by the institutional review board of the hospital. Written informed consent was signed by the mother for the collection of cord and maternal blood.

Interview

A face-to-face interview with all mothers of case subjects and controls was carried out at the hospital within two days after delivery, in French or English. It was based on an interview we had used in a previous study of spontaneous abortions at the same hospital¹⁷ and included questions about potential confounding factors such as demographic characteristics, anthropometric measures before and after pregnancy, complications of pregnancy, maternal chronic diseases, obstetrical history, smoking, and the use of multivitamin supplements.

Family-Based Study

After data were collected on approximately 200 case subjects and 190 controls, we began collecting buccal swabs from fathers of case subjects and controls. The goal was to analyze family trios (mother, father, and newborn) to test for association and linkage.¹⁸ The response rate among fathers of case subjects and controls was 86 percent. We collected 258 case trios and 248 control trios.

Laboratory Investigation

Human genomic DNA was extracted from whole-blood samples (from mothers and newborns) or from buccal swabs (from fathers). Extraction of blood-cell DNA was performed with a DNA extraction kit (Puregene, Gentra Systems). For buccal-swab samples, we used a collection kit containing two sterile cytobrushes in sealed plastic tubes. Fathers were asked to brush the inside of each cheek, using one brush per cheek, to place the brush back in the plastic tube, and to leave it in the mother's room for us to pick up. Brushes were put in microtubes containing 0.5 ml of 50 mM sodium hydroxide and incubated for three hours at room temperature. After centrifugation, brushes were removed, specimens were boiled for five minutes and centrifuged, and supernatant specimens were neutralized with 35 μ l of 1 M TRIS-hydrochloric acid (pH 7.8).

For each polymerase chain reaction (PCR), we used 10 μ l of this solution. PCR was performed with a final reaction volume of 50 μ l with the use of 50 to 100 ng of DNA template per tube under the following conditions: denaturing at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 45 seconds (35 or 40 cycles), and a final extension at 72°C for 10 minutes. The PCR primer sequences were as follows: MTHFR C677T,¹⁹ F-1585 5'GCTGACCTGAAGCACTTGAAGGAGA3' (forward primer) and R-1586 5'AGGACGGTGC GG TGAGAGTG3' (reverse primer); MTHFR A1298C,²⁰ Fp 5'CTTTGGGGAGCTGAAGGACTACTAC3' (forward primer) and Rp 5'CACTTTGTGACCATTCCGGTTTG3' (reverse primer); factor V Leiden G1691A,²¹ F-335 5'TGCCCACTGCTTAACAAGACCA3' (forward primer) and R-336 5'TGTTATCACACTGGTGCTAA3' (reverse primer); and prothrombin G20210A,²² F-1589 5'TCTAGAAACAGTTGCC-TGGC3' (forward primer) and R-1590 5'ATAGCACTGGGAGCATTGAAGC3' (reverse primer).

Allele-specific oligonucleotide hybridization assays were performed as described by others.²³ PCR products were denatured, divided into aliquots, and blotted onto nitrocellulose membranes. Positive and negative controls were included on each membrane. Specimens from family members were assigned randomly to membranes. After hybridization and washing, the membranes were read with the use of a PhosphorImager (Molecular Dynamics) and an automatic scanning program. Membranes were also read visually by two independent observers and again by three observers together. Disagreements after this step were resolved by reamplification, digestion by appropriate restriction enzymes, and gel electrophoresis.

Anticardiolipin antibodies of the IgG and IgM types were measured with an enzyme-linked immunosorbent assay, as previously described.¹⁷ Results are reported in IgG or IgM antiphospholipid units. A specimen containing less than 5 units was considered negative for anticardiolipin antibodies.

All placentas are routinely examined for infarctions by the pathologists on duty, and a report is generally included in the medical record. This information was retrieved by the study nurse coordinator.

Statistical Analysis

Case-Control Study

Of 493 case subjects and 472 controls, 451 were matched for gestational week, sex, and race or ethnic group. Since the matching involved only categorical factors, odds ratios and 95 percent confidence intervals in the case-control study were calculated with the use of unconditional logistic-regression analysis, allowing all study subjects to be included. The first model included only the matching factors and the genotype for either mother or newborn. A second model included the matching factors and genotypes for both mother and newborn. A third model included the matching factors, genotypes for mother and newborn, and the following covariates: weight gain during pregnancy (continuous), prepregnancy body-mass index (the weight in kilograms divided by the square of the height in meters; continuous), parity (primiparous vs. multiparous), history of preeclampsia²⁴ (taken from the medical record and modeled as present or absent in the analysis), history of intrauterine growth restriction (present or absent), and smoking during the third trimester (none, 1 to 20 cigarettes per day, or more than 20 cigarettes per day). Anticardiolipin antibodies and multivitamin intake were not confounders in the data (i.e., they did not materially change the risk estimates for intrauterine growth restriction associated with various genotypes) and so were not included in the final model.

The genotypes were analyzed as variables with three categories (one or two copies of the deleterious allele or no copies) and as single-integer variables (zero, one, or two, corresponding to the number of copies of a given allele). The odds ratios for one copy of the variant were calculated with the use of single-integer variables and are provided in this article; the odds ratios for two copies are

obtained by calculating the square of the odds ratio for one copy. Because folate intake was assumed to modify the effects of MTHFR variants, we tested for interactions between the effects of multivitamin supplementation during pregnancy and MTHFR variants on the risk of intrauterine growth restriction. The Hardy–Weinberg equilibrium (which predicts genotype frequencies in populations) was tested among controls.²⁵

Family-Based Study

In the subsample of available family trios, results were analyzed with the use of the conventional transmission disequilibrium test¹⁸ and the relative risk for case trios (newborn case subjects and parents). Under the null hypothesis, the expected probability of transmission of the variant allele is 50 percent; the transmission disequilibrium test estimates whether the observed probability departs from the expected value. We also used the log-linear likelihood method proposed by Weinberg et al.²⁶ and included control trios (newborn controls and parents) in this analysis.

RESULTS

Case subjects and controls were similar with regard to sex distribution, gestational age, and race or ethnic group, according to the study design (Table 1). The majority of infants were born after the 35th week of pregnancy. Of case and control mothers who were black, 85 percent were born in Haiti. Mothers of case subjects had gained less weight during pregnancy and had a lower body-mass index before pregnancy; they were more likely to be 36 years of age or older, to have smoked during the third trimester of pregnancy, to be primiparous, to have had preeclampsia, and to have had a previous pregnancy with intrauterine growth restriction.

The genotype distributions in controls were consistent with the Hardy–Weinberg equilibrium. The case and control distributions of maternal and newborn genotypes for MTHFR C677T and A1298C, factor V Leiden G1691A, and prothrombin G20210A were generally similar (Table 2). Among white maternal controls, 48 percent were heterozygous carriers of MTHFR C677T, and 11 percent were homozygous carriers; for MTHFR A1298T, these figures were 40 percent and 9.7 percent, respectively. The proportion of carriers of one or two copies among the maternal controls was 5.5 percent for the factor V Leiden mutation and 3.3 percent for the prothrombin G20210A mutation (data not shown).

None of the maternal polymorphisms were associated with an increased risk of intrauterine growth restriction (Table 3). However, mothers homozygous for MTHFR A1298C had a reduced risk of bearing a child with intrauterine growth restriction as compared with mothers with no copy of the variant (adjusted odds ratio, 0.49; 95 percent confidence interval, 0.25 to 0.93). Among the 201 women not taking multivitamin supplements during the third trimester of pregnancy, the odds ratio for intrauterine growth restriction associated with maternal homozygosity for MTHFR C677T was 12.3 (95 percent confidence

TABLE 1. CHARACTERISTICS OF NEWBORNS AND MOTHERS.*

CHARACTERISTIC	CONTROLS (N=472)	CASE SUBJECTS (N=493)
Newborns		
Female sex — no. (%)	253 (53.6)	265 (53.8)
Gestational age — no. (%)		
25–30 wk	20 (4.2)	21 (4.2)
31–35 wk	59 (12.5)	62 (12.5)
36–40 wk	393 (83.3)	410 (83.1)
Birth weight — g	3208.1±734.7	2393.5±606.2
Mothers		
Race or ethnic group — no. (%)		
White	333 (70.6)	330 (66.9)
Black	110 (23.3)	117 (23.7)
Asian	13 (2.8)	24 (4.9)
Hispanic or Amerindian	16 (3.4)	22 (4.5)
Age ≥36 yr — no. (%)	70 (14.8)	86 (17.4)
Schooling ≤12 yr — no. (%)	96 (20.3)	108 (21.9)
Prepregnancy body-mass index	23.2±5.2	22.8±4.3
Weight gain during pregnancy — kg	14.4±5.6	12.7±5.5
Primiparous — no. (%)	234 (49.6)	321 (65.1)
Preeclampsia — no. (%)	12 (2.5)	69 (14.0)
Previous intrauterine growth restriction among parous — no. (%)	23 (9.7)	66 (38.4)
Third-trimester multivitamin use — no. (%)†	374 (80.8)	373 (76.7)
Cigarette smoking during third trimester — no. (%)‡	74 (15.7)	112 (22.8)
Maternal IgG or IgM anticardiolipin antibodies ≥5 U — no. (%)	31 (6.6)	42 (8.5)

*Plus-minus values are means ±SD.

†Data were available for 463 controls and 486 case subjects.

‡Data were available for 470 controls and 492 case subjects.

interval, 1.2 to 126.2). This odds ratio was 1.4 (95 percent confidence interval, 0.7 to 2.8) among the 747 women taking multivitamin supplements. We found no statistical interaction between MTHFR C677T and smoking or between MTHFR A1298C and multivitamin intake or smoking.

The risk of intrauterine growth restriction was not increased in newborns with the polymorphisms MTHFR A1298C, factor V Leiden G1691A, and prothrombin G20210A (Table 4). However, homozygosity for MTHFR C677T was associated with a reduced risk of intrauterine growth restriction (odds ratio, 0.52; 95 percent confidence interval, 0.29 to 0.94).

We also conducted analyses including only case subjects whose weight was below the 5th percentile for gestational age and sex and found similar results. The adjusted odds ratio for intrauterine growth restriction associated with a mother with one copy of MTHFR C677T was 0.85 (95 percent confidence interval, 0.54 to 1.34), and for two copies it was 1.43 (95 percent confidence interval, 0.63 to 1.48). The odds ratio associated with a newborn with one copy

TABLE 2. PREVALENCE IN MOTHERS AND NEWBORNS OF POLYMORPHISMS ASSOCIATED WITH THROMBOPHILIA.

GENOTYPE	MOTHER		NEWBORN	
	CONTROLS (N=472)	CASE SUBJECTS (N=493)	CONTROLS (N=472)	CASE SUBJECTS (N=493)
	no. (%)			
MTHFR C677T				
-/-	233/467 (49.9)	260/490 (53.1)	231/461 (50.1)	255/467 (54.6)
-/+	199/467 (42.6)	185/490 (37.8)	185/461 (40.1)	172/467 (36.8)
+/+	35/467 (7.5)	45/490 (9.2)	45/461 (9.8)	40/467 (8.6)
MTHFR A1298C				
-/-	251/464 (54.1)	276/484 (57.0)	260/458 (56.8)	259/461 (56.2)
-/+	173/464 (37.3)	176/484 (36.4)	167/458 (36.5)	171/461 (37.1)
+/+	40/464 (8.6)	32/484 (6.6)	31/458 (6.8)	31/461 (6.7)
Factor V Leiden				
-/-	452/470 (96.2)	466/488 (95.5)	446/461 (96.7)	446/466 (95.7)
-/+	18/470 (3.8)	22/488 (4.5)	15/461 (3.3)	18/466 (3.9)
+/+	0	0	0	2/466 (0.4)
Prothrombin G20210A				
-/-	460/471 (97.7)	477/489 (97.5)	454/460 (98.7)	457/468 (97.6)
-/+	11/471 (2.3)	12/489 (2.5)	6/460 (1.3)	11/468 (2.4)
+/+	0	0	0	0

TABLE 3. ODDS RATIOS FOR INTRAUTERINE GROWTH RESTRICTION FOR MATERNAL POLYMORPHISMS ASSOCIATED WITH THROMBOPHILIA.

GENOTYPE	Odds Ratio (95% CI)*		
	BASIC MODEL†	ADJUSTED FOR GENOTYPE OF NEWBORN‡	FULLY ADJUSTED‡
MTHFR C677T			
-/+	0.84 (0.63–1.12)	0.91 (0.66–1.24)	0.98 (0.69–1.40)
+/+	1.17 (0.72–1.92)	1.48 (0.84–2.58)	1.55 (0.83–2.90)
MTHFR C677T as a single-integer variable	0.98 (0.79–1.21)	1.07 (0.84–1.38)	1.13 (0.86–1.49)
MTHFR A1298C			
-/+	0.92 (0.70–1.21)	0.81 (0.60–1.10)	0.90 (0.63–1.27)
+/+	0.72 (0.43–1.19)	0.52 (0.29–0.93)	0.49 (0.25–0.93)
MTHFR A1298C as a single-integer variable	0.88 (0.71–1.08)	0.76 (0.59–0.97)	0.78 (0.59–1.02)
Factor V Leiden			
-/+	1.24 (0.65–2.36)	1.00 (0.49–2.05)	1.18 (0.54–2.55)
Prothrombin G20210A			
-/+	1.10 (0.48–2.53)	1.02 (0.43–2.41)	0.92 (0.36–2.35)

*The comparison groups are the -/- genotypes. CI denotes confidence interval.

†The model includes gestational age, sex, and race or ethnic group.

‡The model includes gestational age, sex, and race or ethnic group as well as the genotype of the newborn, weight gain during pregnancy, the prepregnancy body-mass index, smoking during the third trimester, primiparity or multiparity, presence or absence of preeclampsia in the current pregnancy, and presence or absence of previous intrauterine growth restriction.

TABLE 4. ODDS RATIOS FOR INTRAUTERINE GROWTH RESTRICTION FOR NEWBORNS WITH POLYMORPHISMS ASSOCIATED WITH THROMBOPHILIA.

GENOTYPE	Odds Ratio (95% CI)*		
	BASIC MODEL†	ADJUSTED FOR GENOTYPE OF MOTHER‡	FULLY ADJUSTED‡
MTHFR C677T			
-/+	0.84 (0.63–1.12)	0.80 (0.58–1.19)	0.72 (0.51–1.03)
+/+	0.78 (0.49–1.27)	0.71 (0.42–1.22)	0.52 (0.29–0.94)
MTHFR C677T as a single-integer variable	0.87 (0.70–1.05)	0.83 (0.65–1.05)	0.72 (0.55–0.95)
MTHFR A1298C			
-/+	1.05 (0.79–1.39)	1.18 (0.87–1.61)	1.30 (0.91–1.84)
+/+	1.04 (0.61–1.77)	1.41 (0.77–2.56)	1.57 (0.81–3.05)
MTHFR A1298C as a single-integer variable	1.03 (0.83–1.28)	1.18 (0.92–1.52)	1.28 (0.96–1.69)
Factor V Leiden§			
-/+	1.35 (0.76–2.88)	1.36 (0.63–2.94)	1.38 (0.60–3.19)
Factor V Leiden as a single-integer variable	1.58 (0.82–3.06)	1.58 (0.77–3.25)	1.62 (0.67–3.51)
Prothrombin G20210A			
-/+	1.92 (0.70–5.82)	1.93 (0.68–5.45)	1.88 (0.57–6.22)

*The comparison groups are the -/- genotypes. CI denotes confidence interval.

†The model includes gestational age, sex, and race or ethnic group.

‡The model includes gestational age, sex, and race or ethnic group as well as the genotype of the mother, weight gain during pregnancy, the prepregnancy body-mass index, smoking during the third trimester, primiparity or multiparity, presence or absence of preeclampsia in the current pregnancy, and presence or absence of previous intrauterine growth restriction.

§Two case subjects and no controls were homozygous carriers, producing an odds ratio that was infinitely large.

of the gene was 0.63 (95 percent confidence interval, 0.40 to 1.00), and for two copies it was 0.47 (95 percent confidence interval, 0.46 to 0.95). Results for polymorphisms for MTHFR A1298C, factor V Leiden, and prothrombin in mothers and newborns were also not substantially different from the results for all case subjects (data not shown).

Known risk factors for intrauterine growth restriction, such as smoking, a low prepregnancy body-mass index, low weight gain during pregnancy, and primiparity, were all associated with increased risks (data not shown).

At the time of data extraction from the medical records, pathological reports on the presence or absence of placental infarction were available for 77 percent of the case subjects (379 newborns) and for 84 percent of the controls (395 newborns). Of 46 specimens with evidence of placental infarction, 40 were among case subjects. The odds ratio for intrauterine growth restriction associated with placental infarction was 7.6 (95 percent confidence interval, 3.1 to 20.3).

Results of the family-based study of case and control trios (Table 5) were generally similar to the re-

sults of the case-control study (Table 4) and to those of the family-based study of case trios only (Table 5). However, having one copy of the prothrombin variant was associated with a reduced risk of intrauterine growth restriction in the analysis of the case trios and a nonsignificant increase in the risk of intrauterine growth restriction in the analysis of the case and control trios together. These results are explained as follows. Among the 21 case subjects who could have received the deleterious allele, 5 did and 16 did not (relative risk, 0.31). Among controls, 18 could have received the allele but only 2 did (relative risk, 0.12). The point estimate for the relative risk of intrauterine growth restriction associated with one copy of the prothrombin variant is the ratio of those values, or 2.5 (95 percent confidence interval, 0.4 to 14.8).

DISCUSSION

In contrast to previous investigators,¹³⁻¹⁵ we did not find increased risks of intrauterine growth restriction in association with maternal polymorphisms associated with thrombophilia except among the subgroup of women with two copies of the MTHFR C677T variant who were not taking multivitamin supplements

TABLE 5. LOG-LINEAR ANALYSIS WITH RELATIVE RISKS OF INTRAUTERINE GROWTH RESTRICTION FOR POLYMORPHISMS ASSOCIATED WITH THROMBOPHILIA WITH THE USE OF A MAXIMAL NUMBER OF GENOTYPED TRIOS.*

POLYMORPHISM	RELATIVE RISK (95% CI)	
	CASE TRIOS	CASE AND CONTROL TRIOS
MTHFR C677T†		
+/-	0.95 (0.67-1.35)	0.89 (0.53-1.50)
+/+	0.75 (0.39-1.43)	0.78 (0.30-1.98)
MTHFR A1298C‡		
+/-	0.84 (0.58-1.19)	1.02 (0.62-1.70)
+/+	0.78 (0.39-1.52)	1.20 (0.46-3.11)
Factor V Leiden§		
+/-	1.20 (0.52-2.77)	2.16 (0.69-6.76)
Prothrombin G20210A¶		
+/-	0.31 (0.11-0.85)	2.50 (0.42-14.82)
+/+	—	—

*The model using case trios was adjusted for maternal genotype and mating types; the model with case and control trios was adjusted for maternal genotype, mating types, disease status, and the interaction between mating type and disease status. Case trios included newborn case subjects and their parents; control trios included newborn controls and their parents. Mating types were defined by the combinations of genotypes in the trio. CI denotes confidence interval.

†There were 246 case trios and 463 case and control trios. With the use of the Transmission Disequilibrium Test (TDT) among case trios, the relative risk was 0.90 (95 percent confidence interval, 0.68 to 1.19; TDT=0.51; P=0.47).

‡There were 243 case trios and 474 case and control trios. With the use of the TDT among case trios, the relative risk was 0.81 (95 percent confidence interval, 0.60 to 1.07; TDT=2.02; P=0.15).

§There were 240 case trios and 479 case and control trios. With the use of the TDT among case trios, the relative risk was 1.60 (95 percent confidence interval, 0.72 to 3.52; TDT=1.38; P=0.24). Two case subjects and no controls were homozygous carriers, producing an odds ratio that was infinitely large.

¶There were 258 case trios and 501 case and control trios. With the use of the TDT among case trios, the relative risk was 0.31 (95 percent confidence interval, 0.11 to 0.84; TDT=5.7; P=0.01).

during pregnancy. To the extent that folate deficiency is more common in women not taking multivitamin supplements, this is a biologically plausible observation.⁹ We found a reduced risk of intrauterine growth restriction in mothers who were homozygous carriers of the MTHFR A1298C variant and in newborns who were homozygous for the MTHFR C677T variant; however, these observations were based on relatively small numbers of subjects and will require confirmation.

Other studies have not considered the newborn genotypes, nor have they used a family-based approach.¹³⁻¹⁵ Our results differ from those of two recent reports from Israel^{13,14} and Italy.¹⁵ Those studies found substantial increases in the risk of intrauterine growth restriction associated with the maternal polymorphisms MTHFR C677T, factor V Leiden, and

prothrombin G20210A. Our study was considerably larger and had sufficient power to detect odds ratios ranging from 4 to 7, as estimated in the previous studies. Prevalences of polymorphisms in control mothers were generally similar between our study and the two others, but prevalences in case mothers in the other studies were considerably higher than in ours. This suggests that whereas our case subjects and controls came from the same base population,²⁷ there could have been a selection bias for case subjects in the other studies. Although the study from Israel defined intrauterine growth restriction as birth weight below the 5th percentile by American standards,^{13,14} our results did not materially change when we repeated the analyses using only cases below the 5th percentile. The Italian study¹⁵ used criteria similar to ours.

The validity of our study is supported by our finding of the expected associations between intrauterine growth restriction and recognized risk factors, such as cigarette smoking and low weight gain during pregnancy. We adjusted for these factors in the analysis. In addition, the prevalence of polymorphisms in white controls, which was the largest racial group, was consistent with the expected prevalence.^{9,28} In the family-based study of case trios, the nontransmitted alleles from the parents serve as “controls”; in association studies of candidate genes such as ours, this is considered an ideal design, because case subjects (newborns) and controls (their parents) come from the same genetic population.²⁹ Adjusting for race or ethnic group in the case-control study is a reasonable alternative. We did both and found similar results. Control trios in the family-based study provided results that were similar to those from the case-control study of newborns. We are not aware of other studies that used both affected and unaffected trios. Results from the analysis of case as well as control trios for the prothrombin variant were suggestive of a possible effect on fetal survival because there were no homozygous carriers, and the apparent risk in those with one copy of the variant was lower than in those with no copy.²⁶

The meaning of the reduced risks of intrauterine growth restriction observed for mothers homozygous for MTHFR A1298C and for newborns homozygous for MTHFR C677T is not clear. It has been previously reported³⁰ that infant carriers of MTHFR C677T had reduced risks of lymphoblastic or myeloblastic leukemias with mixed-lineage leukemia rearrangements, whereas children who were homozygous carriers of MTHFR A1298C had a reduced risk of lymphoblastic leukemia with hyperdiploid karyotypes. It is possible that the enhanced availability of 5,10-methylenetetrahydrofolate resulting from the MTHFR C677T variant leads to an increased level of extracellular methylenetetrahydrofolate available for thymidylate syn-

thesis and a lesser likelihood that the methylation of uridylylate to thymidylylate for the synthesis of DNA will be deficient. It is conceivable that such a mechanism could protect against intrauterine growth restriction, although this requires further study. Our results do not support previous reports¹³⁻¹⁵ that thrombophilia polymorphisms (including MTHFR C677T, MTHFR A1298C, factor V Leiden G1691A, and prothrombin G20210A) in mothers are associated with an increased risk of intrauterine growth restriction in their offspring.

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