Associations between Maternal Methylenetetrahydrofolate Reductase Polymorphisms and Adverse Outcomes of Pregnancy: The Hordaland Homocysteine Study

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PURPOSE: Methylenetetrahydrofolate reductase (MTHFR) is involved in the metabolism of folate and homocysteine; a polymorphism in the MTHFR gene (677C→T) has been associated with adverse outcomes of pregnancy. We studied whether two polymorphisms in the MTHFR gene (677C→T and 1298A→C) are associated with pregnancy complications, adverse outcomes, and birth defects.

METHODS: MTHFR polymorphisms were determined in blood collected in 1992 and 1993 from 5883 women aged 40 to 42 years, and linked with 14,492 pregnancies in the same women recorded in the Medical Birth Registry of Norway from 1967 to 1996.

RESULTS: The 677TT genotype in mothers was associated with increased risk of placental abruption (odds ratio [OR] = 2.6; 95% confidence interval [CI]: 1.4 to 4.8) compared with the CC variant. The risk of intrauterine growth restriction increased with number of T alleles (*P* for trend = 0.04). Compared with the 1298AA variant, the CC variant was associated with a reduced risk of very low birth weight infants (OR = 0.4; 95% CI: 0.2 to 0.8). No significant associations were found between MTHFR polymorphisms and birth defects.

CONCLUSION: The maternal MTHFR 677C→T polymorphism was a risk factor for placental abruption. The unexpected protective effect of the 1298A→C polymorphism on very low birth weight needs further study. **Am J Med. 2004;117:26–31.**

Homozygosity of a common polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene—a C to T substitution at nucleotide 677—has been associated with an increased risk of complications and adverse outcomes of pregnancy, including preeclampsia, placental abruption and infarction, recurrent spontaneous abortion, and low birth weight (1–5). However, not all studies have confirmed these results (6–9). Several studies have shown that the T allele is a risk factor for birth defects, such as neural tube defects (1) and oral clefts (1,10,11), but others have not (1,12–14). The effects of another common polymorphism in the MTHFR gene, an A to C substitution at nucleotide 1298, on pregnancy outcomes are uncertain (9,13–20).

The aim of the present study was to examine whether the MTHFR 677C→T and 1298A→C polymorphisms are associated with pregnancy outcomes in a large population-based cohort.

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METHODS

The Hordaland Homocysteine Study is a collaborative study among the University of Bergen, local health services, and the National Health Screening Service. The cohort, which was established in 1992 and 1993 in western Norway, included more than 18,000 persons born between 1925 and 1952 (21,22). The Medical Birth Registry of Norway, based on compulsory notification, comprises all births and stillbirths in the country since 1967 from week 16 of gestation and onwards. By using the national identification number, data from 6348 female participants born between 1950 and 1952 were linked with data from the birth registry (23) to yield 5883 women who from 1967 to 1996 experienced a total of 14,492 pregnancies. The study protocol was cleared by the Regional Committee for Medical Research Ethics of western Norway.

In 1992 and 1993, participants underwent the standard cardiovascular examination of the National Health Screening Service (24). The MTHFR 677C→T and 1298A→C polymorphisms were determined in the packed cell fraction from samples of blood obtained at those examinations. A self-administered questionnaire focusing on cardiovascular risk factors and lifestyle factors was used (21,22). Notifications from the birth registry covered the period from 16 weeks of gestation to the first 8 days of life; data included birth weight, length of gestation, medical conditions, complications during

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pregnancy and birth, congenital malformations, and obstetric interventions. Pregnancy complications and adverse pregnancy outcomes include preeclampsia (at any time during or before week 37 of pregnancy), placental abruption, premature delivery (<37 weeks), low birth weight (<2500 g), very low birth weight (<1500 g), and intrauterine growth restriction (birth weight <10th percentile for gestational age). The number of pregnancies complicated by preeclampsia is lower in this report than in a previous report from the same data set (23) because of a change in the definition of preeclampsia to exclude hypertension and edema without proteinuria.

Detection of the 677C→T and 1298A→C polymorphisms in the MTHFR gene was based on real-time polymerase chain reaction (PCR) or mutagenically separated PCR and multiple-injection capillary electrophoresis (25,26).

Data Analysis

Relations of MTHFR genotypes to pregnancy outcomes were studied with multiple logistic regression analyses. Although the vast majority of the births occurred before blood samples were obtained, this should not affect the associations between genotype and outcomes among surviving women. Odds ratios were calculated using the wild-type variant as a reference. Odds ratios for trend (wild-type homozygosity was coded as 0, heterozygosity as 1, and homozygosity as 2) with 95% confidence intervals are presented after adjustment for parity, age of mother at delivery, and smoking habits reported in 1992 and 1993 (never smoker; former smoker; or smoked one to nine, 10 to 19, or ≥20 cigarettes per day). Statistical analyses were performed using the Statistical Package for the Social Sciences 11.0 for Windows (SPSS Inc, Chicago, Illinois). Two-sided P values <0.05 were considered statistically significant.

RESULTS

Genotype distributions for the MTHFR 677C→T and 1298A→C polymorphisms were consistent with those predicted by Hardy-Weinberg equilibrium (Table 1). About 50% of women had the mutated 677C→T genotype (mainly heterozygotes) and about 55% had the 1298A→C genotype (again mainly heterozygotes). About 15% of women had the wild-type genotype of both polymorphisms (677C and 1298A); there was no double homozygosity for the 677T and 1298C genotypes.

The mean (± SD) age at first birth was 23.3 ± 4.3 years and the number of pregnancies was 2.5 ± 0.9 in all three MTHFR 677C→T genotypes. For the 1298A→C polymorphism, the mean age at first birth did not vary significantly among the different genotypes, but the mean number of pregnancies was marginally smaller in the 1298CC genotype (2.4 ± 0.9) than in the AA and AC genotypes (2.5 ± 0.9, $P = 0.03$).

Table 1. Combined Genotypes of Methylenetetrahydrofolate Reductase 677C→T and 1298A→C Polymorphisms in 5867 Women

MTHFR 1298A→C Genotypes	MTHFR 677C→T Genotypes		
	CC	CT	TT
	Number (%)		
AA	878 (15.0)	1274 (21.7)	504 (8.6)
AC	1388 (23.7)	1176 (20.0)	6 (0.1)
CC	636 (10.8)	5 (0.1)	—

MTHFR = methylenetetrahydrofolate reductase.

Outcomes of Pregnancy

Compared with the MTHFR 677CC genotype, the 677TT genotype was associated with more than a doubling of the risk of placental abruption (Figure 1). There was also a slight increase in the risk of intrauterine growth restriction by the number of T alleles. The maternal 1298CC variant was associated with lower risk of very low birth weight (<1500 g) than the AA variant, and perhaps with a reduced risk of low birth weight (<2500 g) and intrauterine growth restriction. The effects of the 1298A→C polymorphism on pregnancy complications and adverse pregnancy outcomes did not change when women with the 677TT genotype were excluded.

We did not find significant associations between MTHFR polymorphisms and birth defects (Figure 2).

Compound heterozygosity of the 677C→T and 1298A→C polymorphisms was not related to any of the pregnancy outcomes (Table 2).

DISCUSSION

By combining data from a population-based cardiovascular survey of 40- to 42-year-old women and the Medical Birth Registry of Norway, we have shown that the MTHFR 677TT variant was a risk factor for placental abruption, while the 1298A→C polymorphism was associated with reduced risk of very low birth weight infants.

Our findings for placental abruption are consistent with previous reports of a more than threefold risk of placental vasculopathy, including placental abruption, among Dutch women and among Israeli women, in whom a nonsignificant increase was found (27–29).

We found that the risk of intrauterine growth restriction increased slightly with the number of T alleles, consistent with one previous study (28) that reported that the TT genotype was associated with an increased risk of intrauterine growth restriction. Several other studies (6–9), however, have not found such an association. The

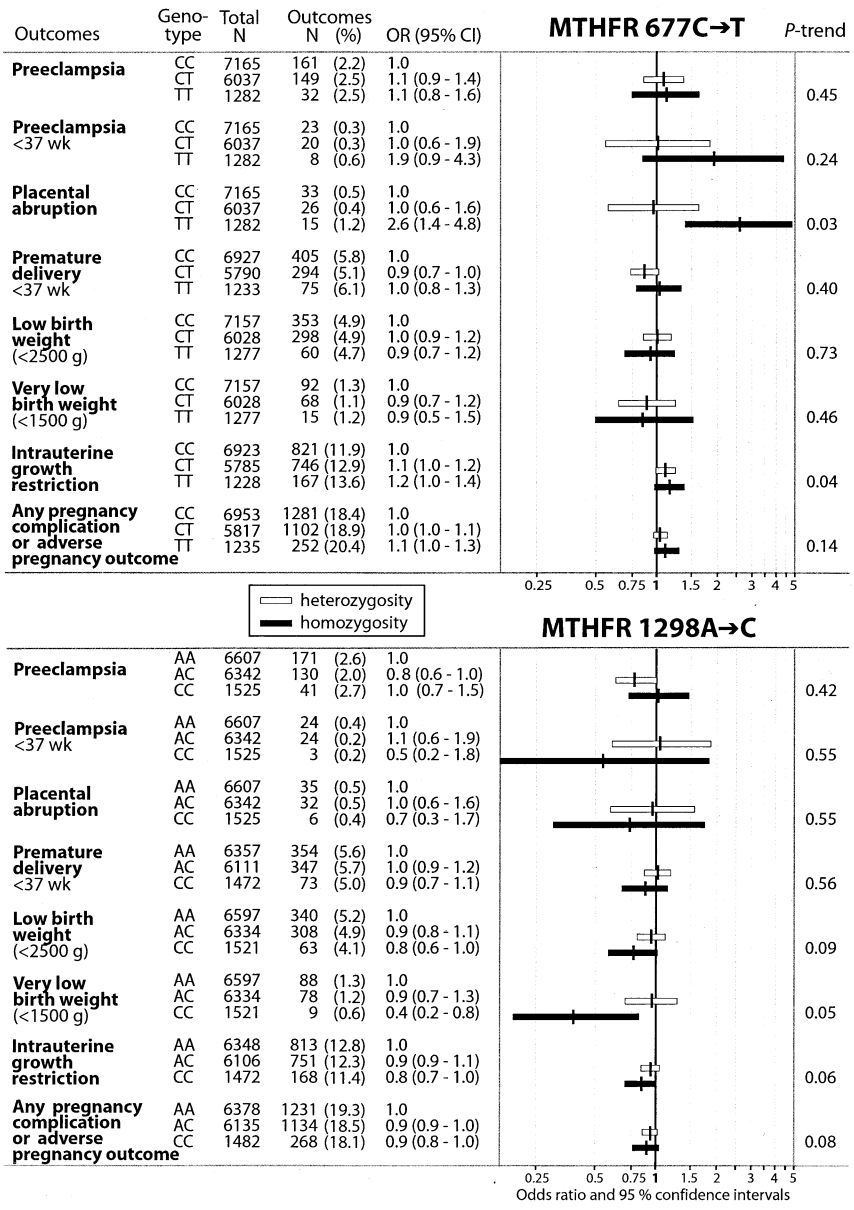

Figure 1. Associations between maternal methylenetetrahydrofolate reductase (MTHFR) 677C→T and 1298A→C polymorphisms and adverse pregnancy outcomes. Multiple logistic regression analysis was carried out in 13,971 pregnancies with complete data. Odds ratios (OR) with 95% confidence intervals (CI) were adjusted for mother's age, parity, and smoking habits reported in 1992 and 1993.

MTHFR 677C→T polymorphism may only be important in women with low folate levels. Intake of folate or multivitamin supplementation was relatively high in three of these negative studies (6,7,9); the fourth study had no information on folate intake (8).

Less is known about the MTHFR 1298A→C polymorphism and pregnancy outcomes. In one study, no association between the 1298A→C polymorphism and a history of preeclampsia was found (20), consistent with our results. We also found that the CC genotype was associated with a reduced risk of very low birth weight infants (<1500 g), and perhaps with intrauterine growth restriction. In a previous study (9), the risk of intrauterine growth restriction was reduced by about 50% in mothers who were homozygous carriers of the 1298A→C genotype.

The MTHFR 677C→T and 1298A→C polymorphisms are in strong linkage disequilibrium, which means that their likelihood of coexisting in the same person is no greater than would occur by chance. Thus, the

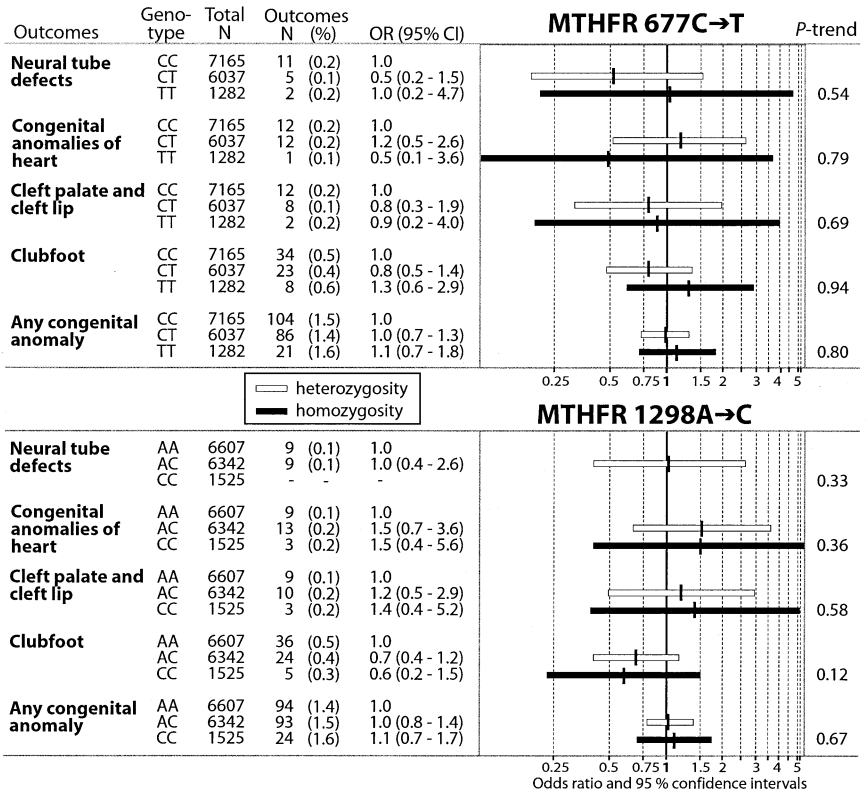

Figure 2. Associations between maternal methylenetetrahydrofolate reductase (MTHFR) 677C→T and 1298A→C polymorphisms and birth defects. Multiple logistic regression analysis was carried out in 14,484 pregnancies with complete data. Odds ratios (OR) with 95% confidence intervals (CI) were adjusted for mother's age, parity, and smoking habits reported in 1992 and 1993.

possibility of confounding the results for the 1298A→C polymorphism by the 677 polymorphism was negligible, as we confirmed.

An increased prevalence of the 677C→T mutation has been observed among children with neural tube defects and their mothers (1,30–32). However, we found no association between a maternal 677C→T polymorphism and neural tube defects, consistent with other reports (12,13,33). It has been suggested that this polymorphism increases the risk of sporadic, but not the hereditary form of, neural tube defects (30). Associations between the 677C→T polymorphism and other birth defects have been less well studied, and the results are conflicting (1). Congenital cardiac malformations, oral clefts, and limb defects have been associated with the polymorphism (1,10,11,34,35), but other studies (1,12,13,36) are consistent with our results showing no association.

The MTHFR 1298A→C polymorphism was not associated with birth defects in this study, as has been seen in studies using different designs (13–16). Neither were combinations of the 677 and 1298 genotypes related to birth defects in our study, although a few other studies have reported that these combinations were associated with birth defects (14,15,17).

Homozygotes for either one of the MTHFR polymorphisms always had a wild type for the other polymorphism (15). Only 11 (0.2%) of the 5867 women had the combinations 677CT + 1298CC or 677TT + 1298AC. Hence, these two MTHFR polymorphisms can occur both in *cis* (together on the same chromosome) and *trans* (on the opposite member of a chromosome pair) configurations (18,19).

Our study has several limitations. Women who gave birth from 1967 to 1993, but who did not survive until the time of enrollment in this study or who did not participate in the Hordaland Homocysteine Study, were not included. Because we enrolled most women well after the time of delivery, we did not have any data on levels of homocysteine, folate, or other B vitamins during pregnancy. Thus, we cannot determine whether the effects of these MTHFR polymorphisms are mediated through, or influenced by, these levels. Finally, our results were not adjusted for multiple comparisons, although we looked at four genotypes (two for each site) and 13 different outcomes.

In conclusion, we found evidence that the MTHFR 677C→T polymorphism was associated with an increased risk of placental abruption and perhaps intra-

Table 2. Pregnancy Complications, Adverse Pregnancy Outcomes, Birth Defects, and Double Heterozygosity of Methylenetetrahydrofolate Reductase 677C→T and 1298A→C polymorphisms

	Combined MTHFR 677C→T and 1298A→C Genotypes		
	CC/AA (n = 2192)	CT/AC (n = 2867)	Odds Ratio (95% Confidence Interval)*
	Number	(%)	
Preeclampsia	56 (2.6)	66 (2.3)	0.9 (0.6–1.3)
Preeclampsia <37 weeks	5 (0.2)	9 (0.3)	1.4 (0.5–4.1)
Placental abruption	8 (0.4)	13 (0.5)	1.4 (0.6–3.6)
Premature delivery	128 (6.0)	143 (5.2)	0.9 (0.7–1.1)
Low birth weight	97 (4.4)	113 (3.9)	0.9 (0.7–1.2)
Very low birth weight	31 (1.4)	26 (0.9)	0.7 (0.4–1.1)
Intrauterine growth restriction	242 (11.4)	333 (12.1)	1.0 (0.9–1.3)
All pregnancy complications and adverse pregnancy outcomes	396 (18.6)	510 (18.4)	1.0 (0.8–1.1)
Neural tube defects	4 (0.2)	2 (0.1)	0.4 (0.1–2.1)
Congenital anomalies of heart	2 (0.1)	6 (0.2)	2.3 (0.5–11.6)
Cleft palate and cleft lip	3 (0.1)	4 (0.1)	1.0 (0.2–4.6)
Clubfoot	15 (0.7)	9 (0.3)	0.4 (0.2–1.0)
All birth defects	30 (1.4)	42 (1.5)	1.1 (0.7–1.7)

* Multiple logistic regression analysis of 14,397 pregnancies with complete data. Odds ratios compare the risks in the CT/AC combined genotype with the CC/AA genotype, and were adjusted for mother's age, parity, and smoking habits in 1992 and 1993.
MTHFR = methylenetetrahydrofolate reductase.

uterine growth restriction, while the 1298CC variant had a weaker association with low birth weight and intrauterine growth restriction. Our data indicate the need for further studies to confirm the unexpected effects of the 1298A→C polymorphism.

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