Methylenetetrahydrofolate reductase (MTHFR) is associated with ovarian follicular activity

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Objective: Polymorphisms in the MTHFR gene have been associated with decreased cell division and apoptosis. This finding led us to evaluate whether MTHFR polymorphisms were associated with follicular growth within the ovary. More specifically, we investigated the effect of the two common polymorphisms C677T and A1298C in our population of women undergoing ovarian stimulation.
Design: Prospective cohort study.
Setting: Academic medical center.
Patient(s): Two hundred twenty-three women undergoing ovarian stimulation.
Intervention(s): The DNA from patients was genotyped at the MTHFR C677T and A1298C polymorphisms.
Main Outcome Measure(s): Day 3 FSH, E_2, antral follicle count, amount of gonadotropin used, the number of follicles >13 mm, E_2 on the day of hCG administration, and oocyte number.
Result(s): Women with the variant MTHFR 1298 C allele had significantly higher basal FSH levels, and after ovarian stimulation, produced fewer follicles >13 mm, had lower E_2 levels on the day of hCG administration, and required more ampules of gonadotropin hormone during treatment. Women with the variant MTHFR 677 T allele demonstrated no significant differences.
Conclusion(s): The MTHFR A1298C polymorphism, but not the C677T polymorphism, is associated with higher basal FSH levels and may be a determinant of response to ovarian stimulation. These findings make a compelling case for the MTHFR A1298C polymorphism to modulate folliculogenesis. (Fertil Steril® 2007;88:632–8. ©2007 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization (IVF), MTHFR, folic acid, ovarian reserve, follicle development, ovarian stimulation

Most patients who undergo assisted reproductive technology (ART) rely on controlled ovarian stimulation to override the physiology of dominant follicle selection to increase the number of oocytes available. Follicle-stimulating hormone (FSH) is the essential hormone needed for follicular growth (1). The amount of FSH administered may depend on several biological factors and clinical characteristics, including age, antral follicle count (AFC), day 3 FSH, day 3 E_2, and stimulation protocol (2–5). Yet, the individual ovarian response to FSH may be unpredictable, and may have a genetic basis.

Recent interest has been directed to polymorphisms to determine whether a genetic basis could explain some of the variation observed with drug therapy (6). However, only a small number of genetic factors have been associated with response to ovarian stimulation, such as the FSH receptor (7, 8), the estrogen (E) receptor (9, 10), and bone morphogenetic protein 15 (11).

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MTHFR is a key regulatory enzyme that nonreversibly partitions methyl groups to DNA synthesis/repair or to the methylation cycle. The C677T polymorphism is located in the amino-terminal catalytic domain, whereas the A1298C polymorphism is located in the carboxy-terminal regulatory region (12–14). Although both polymorphisms result in reduced enzymatic activity in vitro (14–16), their activity in vivo has not been clarified, and they appear to function independently and in opposing directions (17–19).

The fact that MTHFR is involved in reproduction is now an active area of investigation (20–24). Recently, it has been suggested that women carrying one of the two common MTHFR polymorphisms (C677T) have decreased ovarian response to stimulation and may have an earlier onset of menopause (24). An increased incidence of this polymorphism, albeit not significant, has also been reported in women failing at least four cycles of IVF (20). It has also been shown that women homozygous for the other common polymorphism (A1298C) are less likely to achieve pregnancy and deliver a baby after IVF (22). These results are controversial, however, as we have recently demonstrated no differences in short-term outcomes after IVF (embryo quality, ongoing pregnancy rate [PR], and abortion rate) in women or their partners with regard to the polymorphisms at C677T and A1298C (25).

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In rapidly proliferating cells, such as cancer, in which the need for folic acid is increased, numerous studies have demonstrated that polymorphisms in MTHFR are associated with disease acquisition, progression, and response to treatment (26–28). We hypothesized that during folliculogenesis, when granulosa cells (GC) proliferate in response to FSH, polymorphisms in MTHFR could associate with ovarian response, especially during ART, when GC growth is maximized. We therefore performed genotypic analysis of the MTHFR C677T and A1298C polymorphisms and determined their relationship with baseline ovarian reserve and response to ovarian stimulation in women undergoing ART.

MATERIALS AND METHODS

Patients

From July 2005 to January 2006, patients and gamete donors presenting to the UCSF Center for Reproductive Medicine for IVF were offered participation in this study. A total of 223 women were recruited into the study. There were no initial exclusion criteria. No participants actively withdrew from the study. This study was approved by the UCSF Committee on Human Research.

Genotype Determination

Each study participant had a cheek swab performed. All samples were prepared and subjected to Taqman SNP genotyping and data analysis as previously described (25). Briefly, buccal samples were base treated and neutralized to prepare the DNA for processing. Taqman reactions were carried out per manufacturer's instructions in 96-well format in an iCycler (Bio-Rad, Hercules, CA). The following primers and probes were used: C677T forward primer, 5'-GCA CTT GAA GGA GAA GGT GTCT; C677T reverse primer, 5'-CCT CAA AGA AAA GCT GCG TGA TG; C677T reporter "C", 5'-VIC dye-ATG AAA TCG GCT CCC GC−NFQ (nonfluorescent quencher); C677T reporter "T", 5'-FAM dye-ATG AAA TCG ACT CCC GC−NFQ; A1298C forward primer, 5'-GGA GGA GCT GCT GAA GAT GTG; A1298C reverse primer, 5'-TGG TTC TCC CGA GAG GTA AAG A; A1298C reporter "A", 5'-VIC dye-CCA GTG AAG AAA GTG TC−NFQ; and A1298C reporter "C", 5'-FAM dye-CAG TGA AGC AAG TGT C−NFQ. The genotypes were defined after 50 cycles of PCR as wild-type (677CC or 1298AA), heterozygous (677CT or 1298AC), and mutant (677TT or 1298CC). Samples with ambiguous genotypes were repeated once or twice until the results were informative.

Ovarian Stimulation

A total of 223 ovarian stimulations were performed, one per patient. The protocols administered for ovarian stimulation included long luteal (n = 147), microdose flare (n = 43), antagonist (n = 28), and short luteal (n = 5). The type of ovarian stimulation administered and start dose (2–6 ampules; 150–450 IU) were based on physician preference and patient characteristics. All long luteal cycles were potential step-down protocols; after 4 days of ovarian stimulation, the dose of gonadotropins was decreased by one if the serum E_2 level was more than 150 pg/mL, and further decreased by day 6 if the E_2 level was more than 350 pg/mL. All patients were triggered when at least two follicles had a mean diameter of $\geq$18 mm with hCG administration of 5,000 or 10,000 IU, if the serum E_2 levels were more or less than 3,500 pg/mL, respectively.

Statistical Analysis

Initial comparisons between genotypes were performed using an analysis of variance (ANOVA). Baseline follicular activity was determined by day 3 FSH and E_2 levels, and AFC. Multivariate linear regression analyses were performed to determine whether MTHFR polymorphisms were predictors of follicular activity while adjusting for patient age and stimulation protocol.

The ovarian response outcomes tested included [1] the number of ampules of gonadotropin used, [2] the number of follicles more than 13 mm, [3] the E_2 level on the day of hCG administration, and [4] the number of oocytes recovered. A multivariate stepwise approach of analysis was undertaken. Initially, the models were adjusted for only age and stimulation protocol to assess an overall effect of the MTHFR variants to the outcome. Subsequently, AFC, day 3 FSH, and E_2 were included in the model to identify whether the MTHFR variants had effects over and above the effect seen at baseline. All data was analyzed in Stata version 7.0 (Stata Corporation, College Station, TX). Tests were declared statistically significant for a two-sided P value $<.05$.

RESULTS

Genotype Determinations

Of the 223 buccal samples collected, one was not informative at the 1298 locus despite repeat testing, and it was excluded from the analysis. The allele frequencies, p and q, respectively, were 0.713 and 0.287 for C677T and 0.696 and 0.304 for A1298C. They were in Hardy-Weinberg equilibrium, and were in similar proportions to the published literature (28, 29). There were no instances of three mutant alleles in a single woman; all cases in which both the 677 and 1298 mutant alleles occurred simultaneously were compound heterozygotes.

Effect of MTHFR Gene Variants C677T and A1298C on Ovarian Reserve

We first determined whether day 3 FSH, day 3 E_2, and AFC are associated with the MTHFR genotypes. These variables did not associate with any of the C677T variants (Table 1, Fig. 1), even after adjusting for age.

The day 3 FSH did associate with the A1298C variants ($P = .003$; Table 1). Using linear regression, women with a heterozygous genotype had significantly higher day 3 FSH level than those with a wild-type genotype (estimated