Variations in folate pathway genes are associated with unexplained female infertility

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ABSTRACT

Objective: To investigate associations between folate-metabolizing gene variations, folate status, and unexplained female infertility.

Design: An association study.

Setting: Hospital-based IVF unit and university-affiliated reproductive research laboratories.

Patient(s): Seventy-one female patients with unexplained infertility.

Intervention(s): Blood samples for polymorphism genotyping and homocysteine, vitamin B12, and folate mea- surements.

MainOutcomeMeasure(s): Alleleandgenotypefrequenciesofthefollowingpolymorphisms:5,10-methylenetetra- hydrofolate reductase (MTHFR) 677C/T, 1298A/C, and 1793G/A, folate receptor 1 (FOLR1) 1314G/A, 1816delC, 1841G/A, and 1928C/T, transcobalamin II (TCN2) 776C/G, cystathionase (CTH) 1208G/T and solute carrier family 19, member 1 (SLC19A1) 80G/A, and concentrations of plasma homocysteine, vitamin B12, and serum folate.

Result(s): MTHFR genotypes 677CT and 1793GA, as well as 1793 allele A were significantly more frequent among controls than in patients. The common MTHFR wild-type haplotype (677, 1298, 1793) CAG was less prev- alent, whereas the rare haplotype CCA was more frequent in the general population than among infertility patients. The frequency of SLC19A1 80G/A genotypes differed significantly between controls and patients and the A allele was more common in the general population than in infertile women. Plasma homocysteine concentrations were influenced by CTH 1208G/T polymorphism among infertile women.

Conclusion(s): Polymorphisms in folate pathway genes could be one reason for fertility complications in some women with unexplained infertility

Key Words: Female infertility, homocysteine, MTHFR, FOLR1, TCN2, CTH, SLC19A1

INTRODUCTION

Folate is an important B vitamin that is believed to be crucial for reproduction (1). Folate metabolism is involved in a large num- ber of physiological and pathophysiological processes in the body. Folates participate in amino acid metabolism, purine and pyrimidine synthesis, and methylation of nucleic acids, proteins, and lipids. Dietary or genetically determined folate deficiency may impair the function of these metabolic path- ways and lead to homocysteine accumulation (2). Homocys- teine, a thiol-containing amino acid, originates from the one-carbon-donating metabolism of methionine and is reme- thylated to methionine, with folates acting as methyl donors (3).

Possible unfavorable effects of folate deficiency and homocysteine accumulation on female reproductive functions include reduced cell division, inflammatory cytokine produc- tion (4), altered nitric oxide metabolism (5), increased oxidative stress (6), elevated apoptosis (7), and disturbed methylation reactions (8). All of these processes are involved in oocyte development, preparation of the endometrial re- ceptivity, embryo implantation, and also, in the following pregnancy.

Severe maternal folate deficiency before conception and during gestation has been shown to hamper female fertility and fetal viability in several animal models, emphasizing the essentiality of folate during mammalian folliculogenesis and fetal development (9). In humans, preconception folic acid supplementation has been shown to increase folate levels and decrease those of homocysteine in follicular fluid (10). In addition, regular use of multivitamin supplements including folate has recently been reported to decrease the risk of an- ovulatory infertility (11). Furthermore, periconceptional sup- plementation with folate and vitamin B12 has been found to be associated with a lower incidence of miscarriage in women planning pregnancy (12).

Several variations have been identified in genes involved in folate absorption and folate-mediated one-carbon metab- olism. These polymorphisms may alter the beneficial effect of folates and other B vitamins that play a role in the me- tabolism of methyl groups and change the flux of folate co- factors between DNA synthesis and methylation reactions (13). The most important variation in folate metabolism in terms of prevalence and impact seems to be the 5,10- methylenetetrahydrofolate reductase (MTHFR) gene poly- morphism 677C/T (14). The MTHFR gene is involved in the folate methylation cycle, where homocysteine is con- verted to methionine. Methionine is the precursor of the methyl group donor S-adenosylmethionine (SAM), which is used in the methylation of DNA, proteins, and lipids (15). The MTHFR 677C/T variation results in an amino acid change at codon Ala222Val,

giving rise to an unstable enzyme with reduced activity (14). This polymorphism re- sults in the accumulation of homocysteine (16) and im- paired methylation reactions. Methylation of DNA is one of the most common repressor mechanisms of tissue-spe- cific genes. Thus, inefficient methylation caused by this polymorphism may affect gene regulation.

These findings lead us to the question of what influence, if any, do polymorphisms in folate pathway-related genes have on female fertility and on folate status among women with unexplained infertility. More than 10% of infertile couples suffer from infertility of an unexplained nature (17). The women in these couples have normal ovulatory cycles and hormonal profiles, and no organ pathologies. Their partners show no evidence of semen quality problems. Hence, the women may be unable to conceive as a result of disturbances in oocyte quality or in endometrial maturation resulting from impaired folate metabolism. To validate this hypothesis we studied the prevalence of 10 polymorphisms in genes in- volved in the folate pathway (MTHFR 677C/T, MTHFR 1298A/C, MTHFR 1793G/A, folate receptor 1 (FOLR1) 1314G/A, FOLR1 1816delC, FOLR1 1841G/A, FOLR1 1928C/T, transcobalamin II (TCN2) 776C/G, cystathionase (CTH) 1208G/T and solute carrier family 19, member 1 (SLC19A1) 80G/A) among women with unexplained infertil- ity and in controls from the general population. In addition, we evaluated the effects of these 10 polymorphisms on blood folate, vitamin B12, and homocysteine concentrations among infertile women.

MATERIALS AND METHODS

Subjects

The Ethics Committee of Karolinska Institutet approved the study and informed consent was obtained from participating women. The patient group consisted of 71 women with unex- plained infertility who attended the Department of Obstetrics and Gynecology, Karolinska University Hospital Huddinge, from 2000–2007. All of the women were of Swedish or Finn- ish origin. Unexplained infertility was diagnosed after the couple had undergone a standard set of diagnostics proce- dures and tests that also included hormone assays and there had been at least two analyses of the partner's semen, show- ing normal results according to World Health Organization criteria (18). The mean age of the women was 33.1 3.2 (SD) years, mean body mass index (BMI) was 21.7 2.5 kg/m^2, mean cycle length was 28.4 1.8 days, and the mean duration of menses was 4.7 0.8 days. All women had normal ovarian function. Their serum concentration of FSH was not more than 11 IU/L during the early follicular

phase of cycle days 2–5. All women had a serum PRL con- centration <20 mg/L and normal TSH as well as thyroid hor- mone serum levels. In addition, all women showed normal tubal patency in hysterosonosalpingography, and no recog- nizable endometriosis according to symptoms, clinical exam- ination, ultrasonography, or diagnostic laparoscopy. However, according to our internal guidelines, only the women with suspicion of endometriosis underwent diagnos- tic laparoscopic examination. The control group consisted of 1,079 individuals from a cross-sectional population studied in central Sweden, the same area in which our group of infer- tile women was recruited. The data concerning the control individuals have already been published (19–22).

Homocysteine, Folate, and Vitamin B12 Assays

Homocysteine was assayed in acidified citrate plasma using a fluorescence polarization immunoassay and an IMx unit (Abbott Laboratories, Chicago, IL). Concentrations of serum folate were measured by means of a solid-phase time-re- solved fluoroimmunoassay based on a competitive reaction between europium-labeled pteroyl-glutamic acid, the stable form of folate, and sample folate for a limited number of binding sites on folate-binding protein (AutoDelfia Folate; Wallac Oy, Turku, Finland). Plasma vitamin B12 concentra- tions were measured by means of a fluorometric method with an Abbott IMx autoanalyzer (Abbott Laboratories). All coef- ficients of variation were <7.5%.

Genotyping

Genomic DNA for polymorphism analysis was extracted from EDTA-collected peripheral blood using a QIAamp DNA Blood Maxi kit (Qiagen, Venlo, the Netherlands). Pre- viously described Pyrosequencing assays (19–22) (Biotage AB, Uppsala, Sweden) were used to genotype the polymor- phisms MTHFR 677C/T (rs1801133), MTHFR 1298A/C (rs1801131), MTHFR 1793G/A (rs2274976), FOLR1 1314G/A (rs2071010), FOLR1 1816delC (rs3833748), FOLR1 1841G/A (rs1540087), FOLR1 1928C/T (rs9282688), TCN2 776C/G (rs1801198), CTH 1208G/T (rs1021737), and SLC19A1 80G/A (rs1051266).

Statistical Analysis

All analyses were performed using Statistical Package for Social Sciences statistical software (SPSS v. 16.0 for Macin- tosh; SPSS Inc., Chicago, IL), with the exception of haplo- type analyses, which were performed with Haploview software (version 4.1) (23). Data are given

as mean SD, un- less otherwise indicated. Nominal variables were analyzed by c^2 tests. Allele frequencies were calculated to investigate de- viation from Hardy-Weinberg equilibrium. All continuous variables were normally distributed, except for serum folate concentrations, which were logarithmic transformed. We an- alyzed the influence of polymorphisms on folate and vitamin B12 concentrations in infertile women by one-way analysis of variance (ANOVA), whereas mean concentrations of folate and vitamin B12 in genotype subgroups were compared by using Tukey's test. The effects of polymorphisms and MTHFR haplotypes on plasma homocysteine concentrations among infertile women were calculated by using analysis of covariance (ANCOVA) after adjusting for folate and age. Polymorphisms and haplotypes were entered as fixed factors and homocysteine as a dependent variable. In calculations of covariance, Bonferroni correction was used. For all analyses, a P value <.05 was considered statistically significant.

RESULTS

Allele and Genotype Frequencies

The genotype and allele frequencies of polymorphisms MTHFR 677C/T, MTHFR 1298A/C, MTHFR 1793G/A, FOLR1 1314G/A, FOLR1 1816delC, FOLR1 1841G/A, FOLR1 1928C/T, TCN2 776C/G, CTH 1208G/T, and SLC19A1 80G/A are presented in Table 1. Data from women with unexplained infertility were compared with data from cross-sectional population studies conducted in the same re- gion (19–22). All genotype distributions in the study subjects were in Hardy-Weinberg equilibrium. Significant differences in allele frequencies between controls and infertile women were detected in polymorphisms MTHFR 1793G/A, with G allele prevalences of 95.3% and 99.2% (P1⁄4.026) and in SLC19A1 80G/A, with G allele frequencies of 55.8% and 59.7% (P1⁄4.002). A significant difference in genotype distri- bution between the study groups was seen in SLC19A1 80G/ A (P1⁄4.011), where the GG genotype was represented in 32.9% of the controls and 35.7% of the infertile women, the GA genotype in 45.8% and 48.2%, and the AA genotype in 21.3% and 16.1%, respectively. The frequencies of variant heterozygous and homozygous genotypes of the studied poly- morphisms are shown in Figure 1. Significant differences in the frequencies of heterozygous genotypes between controls and infertile women were detected with regard to polymor- phisms MTHFR 677C/T (43.6% and 32.4%, respectively; P1⁄4.043) and MTHFR 1793G/A (9.1% and 1.4%, respec- tively; P1⁄4.012). A significant difference in the frequencies of homozygous variant genotypes between study groups was seen with

polymorphism SLC19A1 80G/A; AA genotype frequencies being 21.3% and 16.1% (P1⁄4.026) in controls and infertile women.

Haplotype Analysis

Haplotype analysis of two (677, 1298) and three (677, 1298, 1793) MTHFR loci revealed three and four haplotypes, re- spectively. The prevalence of MTHFR haplotypes in controls and women with unexplained infertility is presented in Table 2. The MTHFR 677-1298 CA nonmutated haplotype occurred significantly less frequently among control subjects when compared with infertile women (36.4% vs. 45.8%; P1⁄4.028). The same pattern was seen when the data were an- alyzed as a three-locus system—the nonmutated MTHFR 677-1298-1793 haplotype CAG was less frequent among controls than in infertile women (36.4% vs. 45.8%, respec- tively; P1⁄4.028). The MTHFR haplotype CCA was detected more frequently in controls than in infertile women (4.7% vs. 0.7%, respectively; P1⁄4.026).

Blood Homocysteine, Folate, and B12 Concentrations in Infertile Women

Among infertile women, whose serum and plasma samples were stored, the mean concentrations of serum folate, plasma vitamin B12, and homocysteine were well within the refer- ence intervals: 19.2 14.0 nmol/L (n 1⁄4 66), 332.5 106.9 pmol/L (n 1⁄4 28), and 8.2 2.7 mmol/L (n 1⁄4 44), re- spectively. A total of 83.0% of the infertile women were using folate supplements during the study. Analysis of variance did not show any correlation between the studied polymorphisms and serum folate and plasma B12 concentrations (data not shown). Analysis of covariance was used to assess the effects of the studied polymorphisms and haplotypes of the MTHFR gene on homocysteine concentrations among women with unexplained infertility, as folate, vitamin B12, and age are co- factors and biological covariates in the metabolism of homo- cysteine. However, no effect of vitamin B12 on homocysteine levels was detected; therefore B12 was excluded from further covariance analyses. Serum folate concentrations were nega- tively correlated with homocysteine values (P<.05). Homo- cysteine concentrations in relation to all studied polymorphisms are shown in Table 3. The TT genotype of the CTH 1208G/T polymorphism had an increasing effect on homocysteine levels among infertile women (P1⁄4.033), re- gardless of all these women were taking folate supplements.

Analysis of covariance did not reveal any effect of MTHFR haplotypes on homocysteine concentrations in the women with unexplained infertility (data not shown).

DISCUSSION

Our findings indicate that polymorphisms MTHFR 677C/T and 1793G/A, as well as SLC19A1 80G/A, may account for infertility in women with an otherwise unspecified reason for their infertility. To the best of our knowledge this is the first time that an association between multiple polymor- phisms in folate-metabolizing genes and unexplained female infertility has been shown. Keeping in mind the interrelation- ship between low folate status and elevated blood homocys- teine levels, it is important in the context of female infertility to understand the genetic background factors influencing the balance between these two essential compounds. Knowledge of such factors could facilitate prompt identification and treatment of those women trying to achieve pregnancy but who have an unfavorable genetic background and an aug- mented risk of folate metabolism abnormalities.

Both folate deficiency and hyperhomocysteinemia are known risk factors of pregnancy complications (1). In follicu- logenesis, hyperhomocysteinemia may activate apoptosis, thereby leading to follicular atresia (8). Negative correlations between follicular fluid homocysteine concentrations and the degree of maturity of retrieved oocytes (24) and in vitro em- bryo quality on culture day 3 have also been reported (25). However, the results of a recent study have shown a positive correlation between follicular homocysteine concentrations and diameter of the follicle (10). Hyperhomocysteinemia also affects IVF outcome, as pregnancy and implantation rates have been shown to be significantly lower, whereas the abortion rate is higher in women with elevated homocys- teine concentrations (26).

It is commonly known that individuals carrying the MTHFR 677 T allele, particularly TT homozygotes, have in- creased plasma homocysteine concentrations. However, peo- ple with the 677 TT genotype have increased blood homocysteine concentrations when their folate intake is in- sufficient, but normal homocysteine values when folate in- take is adequate (27). In our study group of infertile women, no effect on plasma homocysteine concentrations was detected in connection with any of the MTHFR polymor- phisms. Although a haplotype-based approach has been re- ported to be somewhat superior to a simple genotype-based approach in detecting a genetic influence on homocysteine concentrations (20), no association was found between MTHFR haplotypes and homocysteine concentrations. However, it is of importance that the majority of the infertile women had been taking folate supplements, thus the adverse effects of MTHFR gene variations might have been masked by sufficient folate intake.

Of the other tested polymorphisms, only CTH 1208G/T appeared to influence homocysteine concentrations, irrespec- tive of the folate status and supplement use. Similarly to a pre- vious finding (28), infertile women with the CTH 1208 TT genotype showed higher homocysteine values compared with subjects with wild-type and heterozygous genotypes. The CTH gene encodes the enzyme cystathionase, which converts cystathionine to cysteine in the trans-sulfuration pathway. The CTH 1208G/T polymorphism causes a change in the conserved residue Ser403Ile, which might influence enzyme activity and thereby the folate metabolism (28). However, this result should be interpreted with caution as a re- sult of the limited sample size of infertile women with a TT genotype. Further investigation with a larger study group of women with infertility and early pregnancy complications is warranted.

Our finding that the MTHFR 677 heterozygous CT geno- type was less prevalent among the infertile women than among controls is unexpected, as MTHFR 677 T allele car- riers have previously been shown to have ovulatory distur- bances, diminished responses to ovarian stimulation, and lower serum E_2 concentrations (29, 30). However, in agree- ment with our result, a recent study revealed that the MTHFR 677 CT heterozygous genotype, rather than the ho- mozygous CC genotype, is associated with increased chan- ces of having had a previous IVF pregnancy and a live birth in the current IVF cycle (31). Furthermore, spontaneously aborted embryos have been shown to exhibit a significantly higher frequency of the MTHFR 677 CC wild-type geno- type and a lower frequency of the heterozygous CT geno- type compared with child and adult control groups (32). Correspondingly, the MTHFR 677 T allele has been sug- gested to increase embryo viability in the presence of an ad- equate folate-containing diet, based on the observation that the T allele frequency has risen in the Spanish population over the years (33). Decreased viability of embryos with the MTHFR 677 CC genotype may be caused by increased DNA hypermethylation associated with the more active form of the wild-type MTHFR enzyme, indicating that ele- vated methionine concentration may have more influence on embryo survival than high homocysteine concentrations (32).

In addition to the lower prevalence of the MTHFR 677 CT genotype, we detected the 1793 wild-type G allele more fre- quently and the GA genotype less frequently in infertile women than in the general population. The MTHFR 1793G/A polymorphism results in Arg594Gln amino acid substitution (34). The functional relevance of this variation is not clear, although higher homocysteine concentrations have been reported in association with the wild-type

geno- type among Swedish adolescents (20). We also detected higher homocysteine values among 1793 GG carriers, but not at a statistically significant level.

The MTHFR polymorphisms 677C/T, 1298A/C, and 1793G/A were found to be in linkage disequilibrium among our study group of infertile women, in agreement with previous reports (35, 36). The common wild-type haplotype CAG was more prevalent among infertile women than in the general population. This unexpected result could be ex- plained by the high prevalence of heterozygosity in all MTHFR polymorphisms studied among control individuals. As indicated previously, heterozygosity at the MTHFR gene locus could possibly be beneficial in terms of effective repro- duction. Along this line of thinking, the MTHFR 677C/T polymorphism has been proposed to provide protection against some forms of cancer (37).

Collectively, our findings indicate that variations in the MTHFR gene have a role in female infertility. Besides alter- ing homocysteine concentrations, MTHFR gene variants have been shown to play role in hemostasis (38, 39). Based on these previous studies it could be hypothesized that poly- morphisms in the MTHFR gene affect embryo implantation by altering the hemostatic balance between hemorrhage and thrombosis. The hemostatic balance may prove critical at the time of implantation, when the blastocyst interacts with the endometrium and blastocyst-derived syncytiotro- phoblasts breach endometrial blood vessels, thereby estab- lishing the primordial uteroplacental circulation. Indeed, inherited thrombophilias are associated with implantation failure (38, 40). Furthermore, the genes encoding thrombo- genic proteins are involved, in addition to participating in coagulation processes, in fertilization, embryogenesis, and tissue remodeling (41).

Anotherimportantfindinginourstudyistheassociation between the major allele G of the polymorphism SLC19A1 80G/A, as well as wild-type and heterozygous genotypes, and unexplained female infertility. The SLC19A1 gene en- codes the protein reduced folate carrier, which is considered to be the major folate transporter at physiological conditions in most tissues (42). The variation 80G/A introduces the amino acid change His27Arg (43). If the polymorphism were to interfere with the folate-transporting capacity of the reduced folate carrier, alterations in folate concentrations at the site of embryo implantation could have a negative effect on the rapidly dividing embryonic and maternal cells. How- ever, cellular folate intake has been shown not to be affected in vitro by this variation (44). Nonetheless, GG genotype car- riers present with elevated plasma homocysteine concentra- tions (43). Likewise, we detected higher homocysteine concentrations among infertile women carrying the G allele, although that did not reach a statistically significant level. Thus, the negative

effect of the SLC19A1 80G allele on fertil- ity could be mediated by elevated maternal homocysteine concentrations, which have been associated with defective chorionic villous vascularization in women with recurrent early pregnancy loss (45). In addition, the minor allele A has been proposed to offer a protective effect against throm- bosis (46). Hypercoagulation and microthrombosis at the im- plantation site have been hypothesized to cause implantation failure and miscarriage (40, 47). Hence, an elevated G allele frequency among women with unexplained infertility could be associated with an imbalance in coagulation at the implantation site, hampering trophoblast invasion and embryo implantation.

A major limitation of our study is the relatively low num- ber of patients, which may have reduced the statistical power to detect associations between the studied polymorphisms and unexplained female infertility. Women diagnosed with unexplained infertility are a unique study group; however, it is not considered to be homogeneous (48). In addition, some women with endometriosis and presenting no clinical signs of the disease could have been misdiagnosed as unex- plained infertility in the absence of laparoscopic examination (49). Furthermore, some important associations between the gene variants and blood folate, vitamin B12, and homocys- teine levels could have been overlooked in the situation where the majority of patients used vitamin supplements.

In conclusion, our study indicates that polymorphisms in folate-metabolizing pathway genes may contribute to fertility problems in some women with unexplained infertility. The effect could be explained by the potential of polymorphisms to alter homocysteine status, affecting the hemostatic bal- ance, and shifting more folate cofactors to either nucleotide or methyl donor synthesis. Finally, the influence of a single variation on a phenotype may be weak, but it may become ev- ident when coexisting with other polymorphisms or in case of folate deficiency.

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TABLE 1

Genotype and allele frequencies of polymorphisms in genes of the folate-metabolizing pathway in controls from a Swedish population and women with unexplained infertility.

		Controls	c^2	Infertile women	c^2	*P* value
MTHFR 677C/T[28]	CC	330 (47.7)		40 (56.3)		
	CT	302 (43.6)		23 (32.4)		
	TT	60 (8.7)	0.605	8 (11.3)	2.481	.183
	p(C)	0.685		0.725		
	q(T)	0.305		0.275		.454
MTHFR 1298A/C[28]	AA	302 (43.6)		39 (54.9)		
	AC	322 (46.5)		26 (36.6)		
	CC	68 (9.8)	1.785	6 (8.5)	0.307	.187
	p(A)	0.669		0.740		
	q(C)	0.331		0.291		.125
MTHFR 1793G/A[28]	GG	628 (90.8)		70 (98.6)		
	GA	63 (9.1)		1 (1.4)		
	AA	1 (0.1)	0.200	0	0.003	.079
	p(G)	0.953		0.992		
	q(A)	0.047		0.008		.026
FOLR1 1314G/A[27]	GG	338 (86.9)		57 (87.7)		
	GA	49 (12.6)		8 (12.3)		
	AA	2 (0.5)	0.024	0	0.279	.843
	p(G)	0.932		0.936		
	q(A)	0.068		0.064		1.000
FOLR1 1816delC[27]	CC	387 (99.5)		54 (96.4)		
	Cdel	2 (0.5)		2 (3.6)		
	DelDel	0	0.0026	0	0.018	.079
	p(C)	0.997		0.981		
	q(-)	0.003		0.019		.079
FOLR1 1841G/A[27]	GG	386 (99.5)		54 (96.4)		
	GA	2 (0.5)		2 (3.6)		
	AA	0	0.0026	0	0.018	.079
	p(G)	0.997		0.981		
	q(A)	0.003		0.019		.080
FOLR1 1928C/T[27]	CC	368 (95.1)		54 (96.4)		
	CT	19 (4.9)		2 (3.6)		
	TT	0	0.245	0	0.018	1.000
	p(C)	0.975		0.981		
	q(T)	0.024		0.019		1.000
*TCN2*776C/G[29]	CC	124 (31.9)		20 (32.2)		
	CG	184 (47.3)		29 (46.8)		
	GG	81 (20.8)	0.695	13 (21)	0.170	.997
	p(C)	0.555		0.568		
	q(G)	0.445		0.458		1.000
CTH 1208G/T[a]	GG	203 (52.2)		22 (39.3)		
	GT	156 (40.1)		29 (51.8)		
	TT	30 (7.7)	1.521	5 (8.9)	0.504	.191
	p(G)	0.723		0.627		
	q(T)	0.277		0.298		.146
SLC19A1 80G/A[30]	GG	128 (32.9)		20 (35.7)		
	GA	178 (45.8)		27 (48.2)		
	AA	83 (21.3)	2.005	9 (16.1)	0.0004	.011
	p(G)	0.558		0.597		
	q(A)	0.442		0.401		.002

Note: The numbers of subjects and percentages are shown, and c^2 in Hardy-Weinberg equilibrium testing. Values of *P* indicate the significance of differences in genotype and allele frequencies between the study groups.

[a] Unpublished data, T.K. Nilsson 2008.

TABLE 2

Haplotype prevalences of *MTHFR* 677C/T, 1298A/C, and 1793G/A polymorphisms in controls and in women with unexplained infertility.

Haplotype	Controls	Infertile	*P* value
Two-locus system 677-1298			
CA	0.364	0.458	.028
CC	0.331	0.268	.125
TA	0.305	0.275	.454
Three-locus system 677-1298-1793			
CAG	0.364	0.458	.028
TAG	0.305	0.275	.454
CCG	0.284	0.261	.555
CCA	0.047	0.007	.026

Note: Values of *P* indicate haplotype prevalence differences between the study groups.

TABLE 3

Homocysteine concentrations (mmol/L) in relation to polymorphisms in folate-metabolizing pathway genes among women with unexplained infertility.

Genotype (n)	Mean ± SD	*P* value
MTHFR 677		
CC (22)	8.19±0.45	
CT (12)	7.58±0.61	
TT (8)	9.33±0.74	.192
MTHFR 1298		
AA (24)	8.59±0.43	
AC (17)	7.78±0.52	
CC (1)	7.50±2.21	.471
MTHFR 1793		
GG (41)	8.29±0.33	
GA (1)	5.84±2.11	
AA (0)	—	.257
FOLR1 1314		
GG (35)	8.40±0.36	
GA (7)	7.42±0.86	
AA (0)	—	.312
FOLR1 1816		
CC (32)	8.36±0.39	
Cdel (2)	7.63±1.62	
deldel (0)	—	.666
FOLR1 1841		
GG (32)	8.36±0.39	
GA (2)	7.63±1.62	
AA (0)	—	.666
FOLR1 1928		
CC (33)	8.30±0.39	
CT (1)	9.16±2.30	
TT (0)	—	.713
TCN2 776		
CC (14)	8.15±0.61	
CG (12)	8.56±0.66	
GG (8)	8.26±0.83	.896
CTH 1208		
GG (13)	7.88±0.57	
GT (18)	8.11±0.48	
TT (3)	11.50 ±1.20	.033
SLC19A1 80		
GG (12)	8.92±0.63	
GA (15)	8.45±0.56	
AA (7)	7.02±0.83	.200

Note: Homocysteine concentrations have been adjusted for folate and age.

FIGURE 1

Percentages of subjects heterozygous and homozygous for polymorphisms in folate-metabolizing pathway genes among controls from a Swedish population in comparison with women with unexplained infertility. Heterozygosity was compared with both homozygous genotypes. Minor allele homozygosity was compared with heterozygous and wild-type homozygous genotypes. *Statistically significant difference in genotype frequencies between study groups ($P<.05$).