FERTILITY AND STERILITY®
VOL. 76, NO. 6, DECEMBER 2001

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Low-penetrance genes are associated with increased susceptibility to endometriosis

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Objective: To investigate whether genetic polymorphisms of *CYP1A1*, *GSTM1*, and *GSTT1* are associated with endometriosis.

Design: Genetic polymorphism analysis.

Setting: University department.

Patient(s): A family with four women in two generations who had endometriosis and one member with suspected endometriosis in the third generation were compared with a group of fertile women.

Intervention(s): Laparoscopic examination.

Main Outcome Measure(s): Blood specimens were obtained from fertile females and available affected female family members. Multiplex polymerase chain reaction (PCR) and restriction fragment length polymorphism PCR was done to determine each participant's genotype.

Result(s): All affected family members had genotype *CYP1A1* wt/m1 and *GSTM1* null deletion. The frequency of this genotype in 54 fertile women was 13%. A 17-year-old family member with suspected endometriosis had the same genotype. One affected member was also a carrier of a *GSTT1* null deletion. This combination was not found in any of the fertile participants. The most frequent genotypes in the sample were *CYP1A1* wt/wt, with *GSTM1* null deletion and at least one functional allele of *GSTT1*, and *CYP1A1* wt/wt, with at least one functional allele of *GSTM1* and *GSTT1* (33% and 31%, respectively).

Conclusion(s): The combination of *CYP1A1* m1 polymorphism and *GSTM1* null deletion is closely associated with penetration of the endometriosis phenotype, whereas *GSTT1* null deletion may add to the penetration of this trait. (Fertil Steril® 2001;76:1202–6. ©2001 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, genetic polymorphism, *CYP1A1*, *GSTM1*, *GSTT1*

Received March 20, 2001; revised and accepted June 5, 2001.

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0015-0282/01/$20.00
PII S0015-0282(01)02865-5

Endometriosis is a common gynecologic disorder that accounts for infertility in 10% to 15% of women of reproductive age. However, its etiology and pathogenesis remain obscure. It seems to result from a complex trait, as does diabetes or asthma, in which multiple gene loci interact with each other and the environment to produce the disease phenotype (1), but thus far, little is known about the candidate genes involved. Genetic polymorphism analysis revealed a correlation of the disorder with glutathione S-transferase class μ1 (*GSTM1*) gene null deletion (2–4), but a recent report associated this polymorphism not with risk for endometriosis but rather with the potential of ectopic endometrium to undergo malignant transformation to endometrioid and clear-cell ovarian cancer (5). The increased interest in this particular gene is due to its contribution to the metabolism of dioxin as a phase II enzyme indicating that *GSTM1* may increase susceptibility to endometriosis, since dioxin is considered a strong contributor to the development of this disease (6).

Cytochrome P450IAI (*CYP1A1*) also plays an important role in dioxin metabolism as a phase I enzyme (7), while glutathione S-transferase θ1 (*GSTT1*) detoxifies smaller hydrocarbons—basically haloalkanes and haloalkenes, such as brominated trihalomethanes, which are by-products frequently present in chlorinated drinking water (8). *CYP1A1* also catalyzes the 2-hydroxylation of 17β-estradiol, and its induction may protect against endometrial or breast tumorigenesis (9–11). *CYP1A1* polymorphism m1, also termed *CYP1A1**2A allele, in the 3′ untranslated region, is an *MspI* restric-

tion fragment length polymorphism (RFLP) that stems from a T→C transition 250 base pairs downstream of the polyadenylation site; these characteristics indicate differences in their regulation and transcript half-life in cytosol (12–14). In contrast, *GSTT1* is characterized by a large deletion of the structural gene, similar to the *GSTM1* polymorphism (15).

We describe a family with four female members affected by endometriosis (mother and three daughters) in two generations and one female member (the oldest granddaughter) in the third generation with severe dysmenorrhea and suspected endometriosis. We examined the possibility that the affected female members carry genomic polymorphisms that alter their metabolizing capability for dioxin and estrogen compared with a group of fertile women.

MATERIALS AND METHODS

Clinical characteristics of the family were examined. Venous blood samples were collected from the family members described below.

Case Patients

The grandmother of the family (case 1), who was 80 years of age, had given birth to four children who had no gynecologic problems. Her daughter (case 2), the 49-year-old mother, gave birth to three children from the age of 16 to 24 years and underwent complete surgical hysterectomy when she was 32 years of age for stage IV bilateral ovarian endometriosis. Her first daughter (case 3) gave birth to two children (one at 14 years of age and the other at 18 years of age) and underwent laparotomy for bilateral ovarian endometriosis at the age of 31 years; this was followed by total surgical hysterectomy after 2 years of conservative treatment because she had stage IV disease and severe clinical symptoms. Her second daughter (case 4) gave birth to two children between 17 and 22 years of age and underwent laparoscopy at 28 years of age, which confirmed stage III endometriosis. Her third daughter (case 5) had severe dysmenorrhea for 4 years and was diagnosed with infertility at 25 years of age; stage II endometriosis was found on laparoscopy.

Finally, the granddaughter of case 2, who is 17 years of age, has severe dysmenorrhea and endometriosis is suspected (case 6); her 13-year-old sister is under observation (case 7). Figure 1 shows the pedigree of the family and affected members.

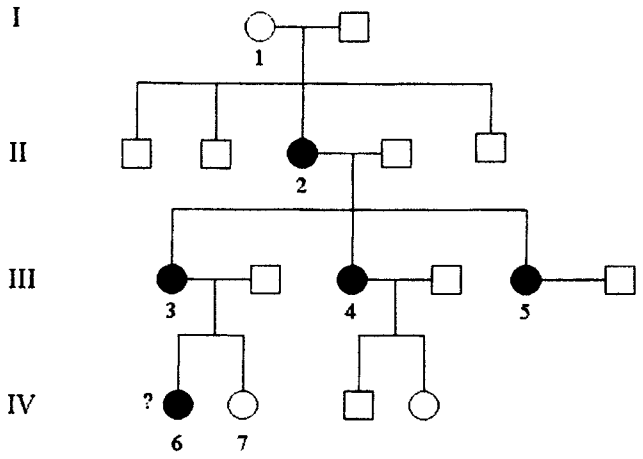
Staging of endometriosis was performed according to the revised American Fertility Society classification system (16). The ethics committee at the University of Crete approved the study, and all the patients gave written informed consent.

Fertile Women

Venous blood samples were collected from 54 confirmed fertile women 18 to 35 years of age (mean [±SD] age, 26.2 ± 6.8 years) who each gave birth to 2 or 3 children at the Department of Obstetrics and Gynecology of the University Hospital of Heraklion, Crete, Greece. All donors gave written informed consent.

FIGURE 1

Pedigree of the case family. Filled circles represent women with endometriosis. The question mark indicates a suspected case of endometriosis. Numbers 1–7 indicate the family members studied.



Arvanitis. Genetic susceptibility to endometriosis. Fertil Steril 2001.

DNA Extraction

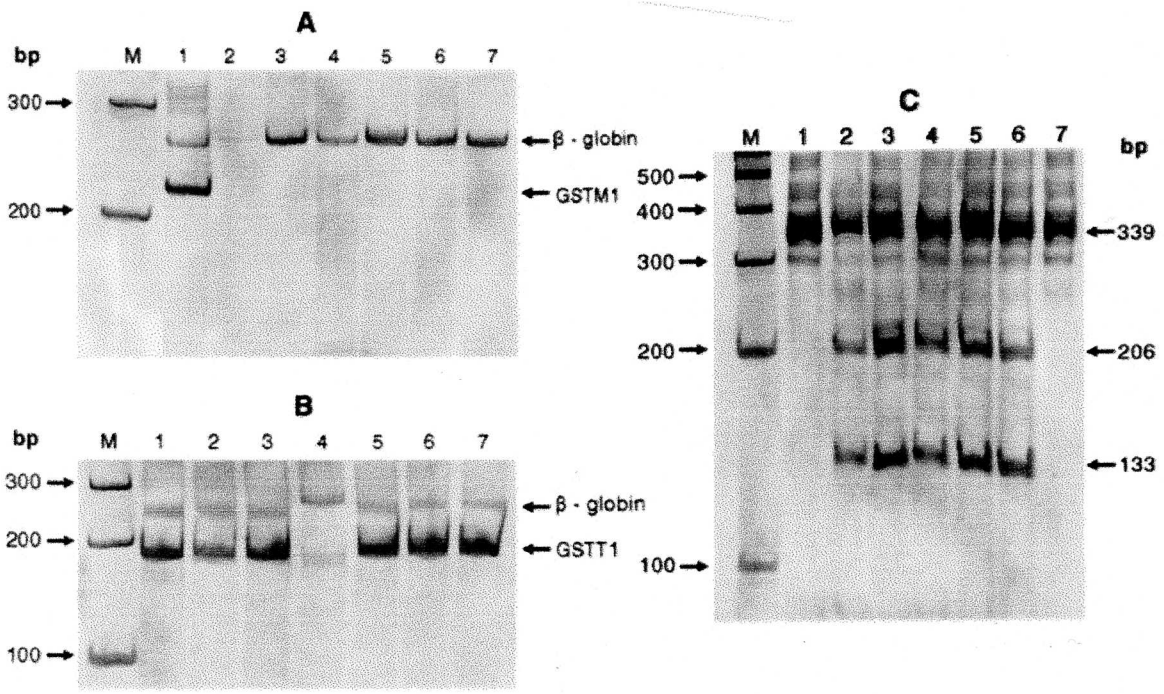
Genomic DNA was extracted from venous blood by using proteinase K, followed by phenol extraction and ethanol precipitation according to standard procedures (17). The DNA was suspended in 50 μL of Tris HCl-EDTA (TE) buffer (10 mM Tris HCl, 1 mM ethylenediamine tetraacetic acid; pH 8.0). Working stocks were prepared by 10-fold dilution in double-distilled H_2O.

Primers and Polymerase Chain Reaction Amplification

Polymerase chain reaction (PCR) assays were performed by introducing 100 ng of genomic DNA in a PCR reaction mixture containing 1× PCR buffer, 200 μM of monodeoxyribonucleoside triphosphates, 2.0 mM of $MgCl_2$, and 0.35 U of Taq DNA polymerase (Life Technologies Ltd., Gaithersburg, Scotland, United Kingdom); the total reaction volume was 15 μL. Amplification was done as follows: 3 minutes of initial denaturation at 94°C; 30 cycles at 94°C for 30 seconds, denaturing, 60°C for 30 seconds, annealing, and 72°C for 30 seconds, elongation. Final extension was done at 72°C for 10 minutes. The oligonucleotide primer sequences for the amplification of *CYP1A1* 3′ untranslated region and for coamplification of *GSTM1* or *GSTT1* with β-globin as an internal positive control were used to a final concentration of 0.3 μM. Their sequences were as follows: *CYP1A1*(forward), 5′-CAG TGA AGA GGT GTA GCC

FIGURE 2

Genotyping of available family members. **(A),** β-globin (267 base pairs), *GSTM1* (218 base pairs). **(B),** β-globin (267 base pairs), *GSTT1* (187 base pairs). **(C),** *CYP1A1* 3′ untranslated region (339 base pairs) product after MspI digestion, resulting in two additional fragments of 206 base pairs and 103 base pairs in wt/m1 heterozygotes. Lanes 1-7 represent the family members studied. M = DNA marker.



Arvanitis. Genetic susceptibility to endometriosis. Fertil Steril 2001.

GCT-3′; *CYP1A1*(reverse), 5′-TAG GAGA TCT TGT CTC ATG CCT-3′, *GSTM1*(forward), 5′-GAA CTC CCT GAA AAG CTA AAG C-3′; *GSTM1*(reverse), 5′-GTT GGG GTC AAA TAT ACG GTG G-3′; *GSTT1*(forward), 5′-ATG ACC CCC ACA CCC ACA GT-3′; *GSTT1*(reverse), 5′-CCA CAT TCC CAG CCT CAC CT-3′; β-globin (forward), 5′-CAA CTT CAT CCA CGT TCA CC-3′; and β-globin (reverse), 5′-GAA GAG CCA AGG ACA GGT AC-3′. The PCR assays were done in a PTC-100 programmable thermal controller (MJ Research, Inc., Watertown, MA).

For analysis of *CYP1A1* m1 polymorphism, the 340–base pair PCR product was digested completely with MspI (New England Biolabs, Inc., Beverly, MA) (18). The sample was characterized as wt/wt (*CYP1A1**1A/*1A) if the product remained uncut; as wt/m1 (*CYP1A1**1A/*2A) if the digestion produced a pattern from two DNA restriction fragments 134 base pairs and 206 base pairs in length, followed by the 340–base pair PCR product; and as m1/m1 (*CYP1A1**2A/*2A) if the only observed bands were the former fragments. Although determinations of *GSTM1* or *GSTT1* null genotypes are absolute, termed *GSTM1* or *GSTT1* [−], the presence of a specific band for *GSTM1* or *GSTT1*, termed *GSTM1* or *GSTT1* [+], was assigned to both homozygotes and hemizygotes for the normal gene.

The PCR products were analyzed by using 8% polyacrylamide gel electrophoresis (29:1 ratio of acrylamide to bisacrylamide) and silver staining. Gels were sealed in plastic transparent bag and scanned on an Agfa SnapScan 1212u (Agfa-Gevaert N.V., Mortsel, Belgium).

RESULTS

Cases 2, 3, and 5, who had endometriosis, and case 6, in whom the disease was suspected, had genotype *CYP1A1* wt/m1, *GSTM1* [−], and *GSTT1* [+]. Case 4 also had genotype *CYP1A1* wt/m1, *GSTM1* [−], and *GSTT1* [−]. Cases 1 and 7 had genotypes *CYP1A1* wt/wt, *GSTM1* [+], and *GSTT1* [+] and *CYP1A1* wt/wt, *GSTM1* [−], and *GSTT1* [+] respectively (Fig. 2). The distribution of combined genotypes of these genes in the control group of 54 fertile women is shown in Table 1. The most frequently found genotypes in the control group were *CYP1A1* wt/wt, *GSTM1* [−], and *GSTT1* [+] (33%) and *CYP1A1* wt/wt, *GSTM1* [+] and *GSTT1* [+] (31%).

TABLE 1

Combined genotypes frequencies in fertile women.

CYP1A1	*GSTM1*	*GSTT1*	No. of cases	Frequency (%)
wt/wt	+	+	17	31
wt/ml	+	+	7	13
ml/ml	+	+	1	2
wt/wt	−	+	18	33
wt/ml	−	+	7	13
ml/ml	−	+	0	0
wt/wt	+	−	1	2
wt/ml	+	−	1	2
ml/ml	+	−	0	0
wt/wt	−	−	2	4
wt/ml	−	−	0	0
ml/ml	−	−	0	0

Arvanitis. Genetic susceptibility to endometriosis. Fertil Steril 2001.

DISCUSSION

Familial aggregation of endometriosis has been well documented (19, 20), but the responsible specific genetic factor has not yet been reported in such families (1). The *GSTM1* and *GALT* genes have been suspected to be genetic susceptibility loci (2–4, 21–23). Environmental factors, such as dioxins (6), and altered estrogen metabolism (24) seem to contribute to development of endometriosis; combinations of relatively common polymorphisms in genes may be responsible for metabolism of these substances. The cytochrome P450-glutathione S-transferase biochemical pathway is predominant in these processes (25, 26).

We describe a family in which four female members (mother and three daughters) have endometriosis and a fifth one (a granddaughter) may have the disease. The three daughters and granddaughter spend their childhood in a different environment from the one in which their mother was raised, and as adults, all live in different places from one another. Investigation of the incidence of genetic polymorphism combinations in *CYP1A1*, *GSTM1*, and *GSTT1* revealed that genotype *CYP1A1* wt/ml and *GSTM1* [−] occurred in all affected family members, including endometriosis case 6; one family member also carried a *GSTT1* null deletion.

Thirteen percent of the 54 controls had the *CYP1A1* wt/ml, *GSTM1* [−], whereas none had the *CYP1A1* wt/ml, *GSTM1* [−], *GSTT1* [−] genotype. The *GSTT1* gene is located on 22q, a region lost in approximately 50% of endometriotic tissues, as determined by comparative genomic hybridization (27).

Our results suggest that the combination of *CYP1A1* polymorphism in the 3′ untranslated region and *GSTM1* null deletion is associated with increased risk for endometriosis, possibly because of their involvement in the dioxin and estrogen metabolism pathway. This is the first report that describes the incidence of common polymorphisms in low-penetrance genes associated with the development of this phenotype.

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