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CYP1A1, *CYP19*, and *GSTM1* polymorphisms increase the risk of endometriosis

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Objective: To investigate the possibility of genetic contribution of *CYP1A1, CYP19, GSTM1,* and *GSTT1* polymorphisms to endometriosis.

Design: Genetic polymorphism analysis.

Setting: Case–control study.

Patient(s): A group of 275 women with sporadic endometriosis was compared with a group of 346 fertile, endometriosis-free women.

Intervention(s): Surgical, laparoscopic, and histological examination.

Main Outcome Measure(s): Blood specimens were obtained from endometriosis cases and controls. Polymerase chain reaction–based assays were performed for the determination of individual's genotype.

Result(s): The *CYP19* VNTR, located in intron 4 $(TTTA)_{10}$ allele increases the risk for endometriosis development (odds ratio [OR], 4.99; 95% confidence interval [95% CI], 1.351 to 18.436). The combined genotype *CYP1A1* wt/m1 or m1/m1 and *GSTM1* null deletion adds to this risk (OR, 1.95; 95% CI, 1.266 to 2.995 and OR, 2.23; 95% CI, 0.631 to 7.906, respectively). In contrast, the *CYP1A1* wt/wt genotype exhibits a protective effect, with a 38% reduction in the odds for endometriosis development (OR, 0.62; 95% CI, 0.440 to 0.883).

Conclusion(s): Our data suggest that *CYP19* VNTR $(TTTA)_{10}$ allele as well as the combined genotype *CYP1A1* m1 polymorphism and *GSTM1* null deletion associate with the endometriosis phenotype, whereas the *GSTT1* null deletion does not. (Fertil Steril® 2003;79(Suppl 1):702–9. ©2003 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, *CYP1A1*, *CYP19*, aromatase, *GSTM1*, *GSTT1*

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Endometriosis is defined as the presence of functional endometrial tissue outside the uterine cavity, affecting 1 in 10 women of reproductive age. It often results in an array of gynecological problems including dyspareunia, dysmenorrhea, pelvic pain, and infertility (1). The etiology and molecular mechanism for the development of this disease are still unclear. However, a large body of laboratory and clinical studies suggests that hereditary genetic factors, in association with environment, are responsible for this gynecological disorder (2–4).

Cytochrome P450IAI (*CYP1A1*), a phase I enzyme responsible for the activation of lipophilic compounds in potent electrophilic adducts (5), glutathione *S*-transferase μ1 (GSTM1) and θ1 (*GSTT1*), and phase II enzymes responsible for the catalysis of many reactions between glutathione (GSH) and these adducts (6), have been linked to endometriosis (7–12). Chemical substances such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), substrate of CYP1A1 and GSTM1, adds to the risk for this disease in primates and mice (13, 14), and it is suspected to cause a similar effect in humans (15, 16).

The incidence of endometriosis tends to regress after menopause or ovariectomy, suggesting that it is estrogen dependent. Exogenous

TABLE 1

Distribution of individual genotypes.

Gene	Polymorphism	Endometriosis cases (%)	Healthy donors (%)	Odds ratio (95% confidence interval)
	wt/wt	173 (62.9)	253 (73.1)	0.62 (0.440–0.883)
CYP1A1	wt/m1	93 (33.8)	87 (25.1)	1.52 (1.066–2.171)
	m1/m1	9 (3.3)	6 (1.7)	1.92 (0.660–5.571)
	[+]	114 (41.5)	165 (47.7)	0.78 (0.561–1.079)
GSTM1	[−]	161 (58.5)	181 (52.3)	1.29 (0.929–1.784)
	[+]	251 (91.3)	315 (91.0)	1.03 (0.582–1.819)
GSTT1	[−]	24 (8.7)	31 (9.0)	0.97 (0.550–1.717)
	$(TTTA)_{7-3}$, $(TTTA)_{7-3}$	24 (8.7)	28 (8.1)	1.81 (1.075–3.047)
	$(TTTA)_{7-3}$, $(TTTA)_{7}$	34 (12.4)	44 (12.7)	1.57 (1.009–2.432)
	$(TTTA)_{7-3}$, $(TTTA)_{8}$	13 (4.7)	20 (5.8)	1.37 (0.722–2.595)
	$(TTTA)_{7-3}$, $(TTTA)_{10}$	4 (1.5)	2 (0.6)	5.08 (1.036–24.89)
	$(TTTA)_{7-3}$, $(TTTA)_{11}$	56 (20.4)	74 (21.4)	1.51 (1.051–2.171)
	$(TTTA)_{7-3}$, $(TTTA)_{12}$	3 (1.1)	5 (1.5)	1.50 (0.443–5.106)
	$(TTTA)_{7}$, $(TTTA)_{7}$	12 (4.4)	16 (4.7)	1.57 (0.786–3.130)
	$(TTTA)_{7}$, $(TTTA)_{8}$	8 (2.9)	16 (4.7)	1.08 (5.511–2.290)
	$(TTTA)_{7}$, $(TTTA)_{10}$	10 (3.6)	2 (0.6)	10.38 (2.297–46.96)
	$(TTTA)_{7}$, $(TTTA)_{11}$	41 (14.9)	56 (16.2)	1.50 (1.007–2.248)
CYP19	$(TTTA)_{7}$, $(TTTA)_{12}$	5 (1.8)	2 (0.6)	5.10 (1.040–24.978)
	$(TTTA)_{8}$, $(TTTA)_{8}$	1 (0.4)	3 (0.9)	0.83 (0.133–5.218)
	$(TTTA)_{8}$, $(TTTA)_{10}$	1 (0.4)	0 (0.0)	—
	$(TTTA)_{8}$, $(TTTA)_{11}$	13 (4.7)	24 (6.9)	1.08 (0.578–2.000)
	$(TTTA)_{8}$, $(TTTA)_{12}$	2 (0.7)	2 (0.6)	1.89 (0.302–11.82)
	$(TTTA)_{10}$, $(TTTA)_{10}$	0 (0.0)	0 (0.0)	—
	$(TTTA)_{10}$, $(TTTA)_{11}$	8 (2.9)	2 (0.6)	8.37 (1.817–38.59)
	$(TTTA)_{10}$, $(TTTA)_{12}$	0 (0.0)	0 (0.0)	—
	$(TTTA)_{11}$, $(TTTA)_{11}$	34 (12.4)	44 (12.7)	1.54 (0.989–2.391)
	$(TTTA)_{11}$, $(TTTA)_{12}$	6 (2.2)	6 (1.7)	1.90 (0.652–5.509)
	$(TTTA)_{12}$, $(TTTA)_{12}$	0 (0.0)	0 (0.0)	—
Total		275 (100.0)	346 (100.0)	

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supplemental estrogens as part of hormone replacement therapy seem to increase the risk for endometriosis (17), whereas certain organochlorines mimic the effects of estrogens (18). The *CYP19* gene encodes a steroid aromatase that mediates the rate-limiting step in the metabolism of C19 androgen steroids to estrogens, an activity detected mainly in the placental syncytiotrophoblasts and ovarian granulosa cells. CYP19 mRNA and protein have been detected in endometriotic tissues and eutopic endometrium of endometriosis patients but not in endometrial specimens of normally menstruating women (19, 20).

In this context, we carried out a genetic distribution analysis of *CYP1A1* m1 (*CYP1A1**2A) polymorphism, *GSTM1* and *GSTT1* null deletions, as well as *CYP19* VNTR in intron 4 $(TTTA)_n$ alleles, in two well-defined populations of women with and free from endometriosis. We assessed the risk of individual or combined genotypes that alter metabolizing capabilities for dioxin and estrogen in the development of endometriosis.

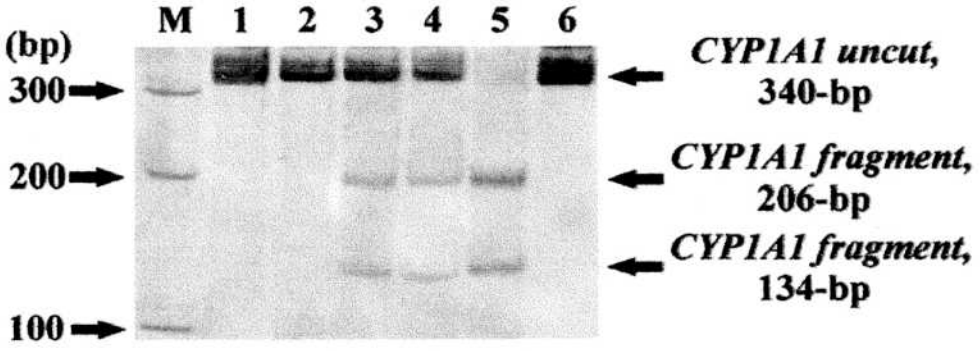
MATERIALS AND METHODS

Specimens

Venous blood samples were collected from 275 sporadic endometriosis patients aged 21–37 (mean±SD; 27.2±3.2) years and 346 fertile, premenopausal endometriosis-free women aged 26–53 (34.5±7.4) years at the Department of Obstetrics and Gynecology of the University Hospital of Heraklion, Crete between 1990 and 2001. Endometriosis patients were diagnosed surgically or by laparoscopy, and the disease was confirmed histologically from biopsies. Staging of the disease was performed according to the revised American Fertility Society classification (21). All members of the control group had given birth to two to five (2.3±0.6) children and had no previous medical record of chronic pelvic pain, dysmenorrhea, or dyspareunia. Both cases and controls were living in the same urban environment and came from the same Greek population with Cretan origin, with no history of endometriosis in their family, at least in two generations. The University of Crete ethics committee

FIGURE 1

Genotyping for *CYP1A1* m1 polymorphism. M = 100-bp DNA ladder. *Lanes 1–5*, PCR products from specimens after *Msp*I digestion. *Lanes 1* and *2*, *CYP1A1* wt/wt, *lanes 3* and *4*, *CYP1A1* wt/m1, *lane 5*, *CYP1A1* m1/m1. *Lane 6*, undigested PCR product.



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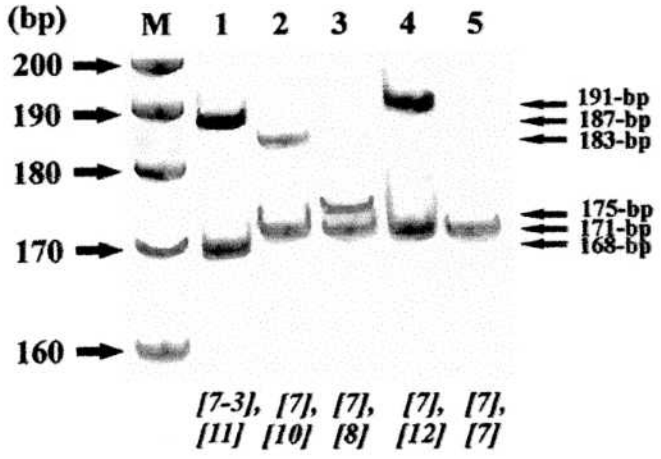
and the National Institute of Public Health in Greece approved this study, and all the patients and healthy donors gave written informed consent.

DNA Extraction

Genomic DNA was extracted from 9 mL of EDTA anticoagulated whole blood using proteinase K, followed by phenol extraction and ethanol precipitation according to standard procedures previously described (22).

FIGURE 2

Genotyping for *CYP19* VNTR in intron 4 $(TTTA)_n$. M = 10-bp DNA ladder. The genotype for each lane is assigned at the bottom of the figure. *Arrows* on the *right* indicate the size of the alleles.



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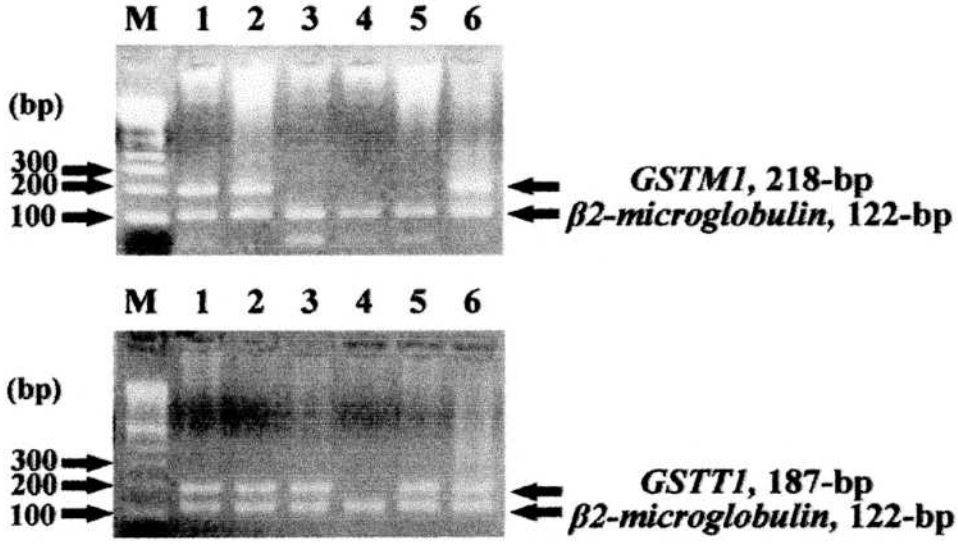
Primers and Polymerase Chain Reaction Amplification

Polymerase chain reaction (PCR) assays and primers for the detection of *CYP1A1**2A polymorphism and *GSTM1* and *GSTT1* null deletions have been described elsewhere (11). Briefly, for the *CYP1A1* m1 polymorphism, the 340-bp PCR product underwent complete digestion with *Msp*I (New England Biolabs Inc., Beverly, MA). If the product remained uncut, then the sample was characterized as wt/wt (*CYP1A1**1A/*1A); as wt/m1 (*CYP1A1**1A/*2A) when the digestion produced a pattern of two DNA restriction fragments sized 134 bp and 206 bp, followed by the 340-bp PCR product; and, when the only observed bands were the two former fragments, as m1/m1 (*CYP1A1**2A/*2A). The *GSTM1* or *GSTT1* null genotypes were determined by multiplex PCR reactions with β2-microglobulin (mp0554 UniSTS primer set 122 bp) product as an internal positive control. Although determinations of *GSTM1* or *GSTT1* null genotypes are absolute, termed as *GSTM1* or *GSTT1* [−], the presence of a specific band for *GSTM1* (218 bp) or *GSTT1* (187 bp), termed as *GSTM1* or *GSTT1* [+], was assigned for both homozygote and hemizygote individuals for the normal gene. The *CYP19* VNTR in intron 4 $(TTTA)_n$ alleles were determined by introducing 100 ng of genomic DNA in a PCR reaction mixture containing 1× PCR buffer, 200 μM dNTPs, 2.0 mM $MgCl_2$, and 0.35 U Taq DNA polymerase (Life Technologies Ltd., Gaithersburg, Scotland, UK) to a 15 μL total reaction volume, using 0.3 μM of the following primers CYP19VNTR(F): 5′-GCA GGT ACT TAG TTA GCT AC-3′ and CYP19VNTR(R): 5′-TTA CAG TGA GCC AAG GTC GT-3′. Amplification parameters were as follows: 3 minutes for initial denaturation at 94°C; 30 seconds at 94°C, 35 seconds at 55°C, 72°C for 30 seconds, these steps repeated for 30 cycles; final extension step at 72°C for

FIGURE 3

Genotyping for *GSTM1* (*upper panel*) and *GSTT1* (*lower panel*) null deletions. In both panels, M = 100-bp DNA ladder. *Upper panel, lanes 3–5, GSTM1* [−]; and *lanes 1, 2,* and *6, GSTM1* [+]. *Lower panel, lane 4, GSTT1* [−]; and *lanes 1–3, 5,* and *6, GSTT1* [+].



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10 minutes. All the reactions were carried out in a PTC-100 programmable thermal controller (MJ Research Inc., Watertown, MA).

Digital Imaging

Polymerase chain reaction products of *GSTM1* or *GSTT1* with β2-microglobulin were analyzed in 2% agarose gels with ethidium bromide stain and visualized on ultraviolet irradiation. Polymerase chain reaction products of *CYP1A1* post digestion and *CYP19* were analyzed in 8% and 10% polyacrylamide gels (29:1 ratio of acrylamide to *bis*-acrylamide), respectively, and silver stained. The gels were scanned on an Agfa SnapScan 1212u (Agfa-Gevaert N.V., Mortsel, Belgium). Integrated density (ID = [mean OD − background OD] × pixels; OD, optical density), of the bands was used as a quantitative parameter, and the PCR product length was calculated by digital imaging using the appropriate DNA ladder (Life Technologies Ltd.).

Statistical Methods

The Pearson χ^2 or Fisher exact test, when applicable, was used to compare genotype distributions between endometriosis and controls. The statistical modeling using logistic regression was used to calculate the relative risk (odds ratio; OR) of genotypes for case–control study. Odds ratios were expressed together with the 95% confidence interval (CI). A P value of $<.05$ was considered statistically significant.

RESULTS

The prevalence of the *CYP1A1, CYP19, GSTM1,* and *GSTT1* polymorphisms in the 275 women with sporadic endometriosis and 346 endometriosis-free women is summarized in Table 1. Characteristic examples of these assays are presented in Figures 1–3. *CYP1A1* wt/wt genotype exhibited a 38% (95% CI, 0.440 to 0.883) reduction of endometriosis risk. *GSTM1* [+] was also protective against the disease phenotype, with a 22% reduction of the risk (95% CI, 0.561 to 1.079). Four infrequent *CYP19* genotypes in the control endometriosis-free group exhibited increased risk for endometriosis development, with OR of 5.08 to 10.38 (Table 1). Moreover, the *CYP19* allele $(TTTA)_{10}$ showed an OR value of 4.99 (95% CI, 1.351 to 18.436). Heterozygosity for this variable number tandom repeat (VNTR) was 0.7418 and 0.7367 in the endometriosis and endometriosis-free groups, respectively. The GSTT1 polymorphism did not show any statistical significant difference between the endometriosis and endometriosis-free women. From the 252 possible combinations of the different *CYP1A1, CYP19, GSTM1,* and *GSTT1* polymorphisms, 100 were detected in both populations tested. The distribution of combined genotypes of these genes was calculated, and OR values with 95% CI are presented in Table 2. We also examined other combinations of these genotypes. In Table 3, *CYP1A1, GSTM1,* and *GSTT1* polymorphisms and their combinations are presented. *CYP1A1* wt/m1 or m1/m1 and *GSTM1* null deletion exhibited OR values of 1.947 and 2.233, respectively. No

TABLE 2

Frequencies and odds ratio of combined genotypes.

Genotype (*GYP1A1, GSTM1, GSTT1, CYP19*)	Endometriosis (%)	Controls (%)	Odds ratio	95% CI (−)	95% CI (+)
wt/wt,[+],[+],[7-3],[7-3]	2.18	2.60	0.835	0.287	2.427
wt/wt,[+],[+],[7-3],[7]	3.64	4.05	0.895	0.385	2.082
wt/wt,[+],[+],[7-3],[8]	1.09	1.73	0.625	0.151	2.595
wt/wt,[+],[+],[7-3],[10]	0.00	0.29	—	—	—
wt/wt,[+],[+],[7-3],[11]	6.18	6.65	0.925	0.478	1.792
wt/wt,[+],[+],[7-3],[12]	0.00	0.29	—	—	—
wt/wt,[+],[+],[7],[7]	0.73	1.45	0.500	0.093	2.684
wt/wt,[+],[+],[7],[8]	0.36	1.45	0.249	0.028	2.239
wt/wt,[+],[+],[7],[10]	1.09	0.29	3.805	0.376	38.530
wt/wt,[+],[+],[7],[11]	4.00	4.91	0.806	0.365	1.779
wt/wt,[+],[+],[7],[12]	0.36	0.29	1.259	0.074	21.402
wt/wt,[+],[+],[8],[8]	0.00	0.29	—	—	—
wt/wt,[+],[+],[8],[11]	1.45	2.31	0.624	0.181	2.145
wt/wt,[+],[+],[8],[12]	0.36	0.29	1.259	0.074	21.402
wt/wt,[+],[+],[10],[11]	0.73	0.29	2.527	0.217	29.431
wt/wt,[+],[+],[11],[11]	3.64	4.05	0.895	0.385	2.082
wt/m,[+],[+],[11],[12]	0.73	0.58	1.260	0.169	9.372
wt/m1,[+],[+],[7-3],[7-3]	0.73	0.87	0.838	0.134	5.237
wt/m1,[+],[+],[7-3],[7]	1.09	1.45	0.752	0.173	3.270
wt/m1,[+],[+],[7-3],[8]	0.36	0.58	0.628	0.054	7.310
wt/m1,[+],[+],[7-3],[11]	1.82	2.31	0.782	0.247	2.475
wt/m1,[+],[+],[7-3],[12]	0.00	0.29	—	—	—
wt/m1,[+],[+],[7],[7]	0.73	0.58	1.260	0.169	9.372
wt/m1,[+],[+],[7],[8]	0.36	0.58	0.628	0.054	7.310
wt/m1,[+],[+],[7],[10]	0.36	0.00	—	—	—
wt/m1,[+],[+],[7],[11]	1.09	1.73	0.625	0.151	2.595
wt/m1,[+],[+],[7],[12]	0.36	0.00	—	—	—
wt/m1,[+],[+],[8],[11]	0.36	0.87	0.417	0.041	4.225
wt/m1,[+],[+],[10],[11]	0.36	0.00	—	—	—
wt/m1,[+],[+],[11],[11]	1.45	1.45	1.007	0.261	3.889
wt/m1,[+],[+],[11],[12]	0.36	0.29	1.259	0.074	21.402
wt/m1,[+],[+],[7-3],[7-3]	0.36	0.00	—	—	—
wt/m1,[+],[+],[7-3],[11]	0.36	0.29	1.259	0.074	21.402
wt/m1,[+],[+],[7],[11]	0.00	0.29	—	—	—
wt/wt,[−],[+],[7-3],[7-3]	2.18	2.89	0.749	0.263	2.132
wt/wt,[−],[+],[7-3],[7]	4.00	4.34	0.919	0.409	2.069
wt/wt,[−],[+],[7-3],[8]	1.82	2.02	0.897	0.275	2.926
wt/wt,[−],[+],[7-3],[10]	0.36	0.29	1.259	0.074	21.402
wt/wt,[−],[+],[7-3],[11]	6.55	7.23	0.899	0.474	1.706
wt/wt,[−],[+],[7-3],[12]	0.36	0.58	0.628	0.054	7.310
wt/wt,[−],[+],[7],[7]	0.73	1.45	0.500	0.093	2.684
wt/wt,[−],[+],[7],[8]	0.73	1.45	0.500	0.093	2.684
wt/wt,[−],[+],[7],[10]	1.09	0.29	3.805	0.376	38.530
wt/wt,[−],[+],[7],[11]	5.09	5.20	0.977	0.470	2.032
wt/wt,[−],[+],[7],[12]	0.73	0.29	2.527	0.217	29.431
wt/wt,[−],[+],[8],[8]	0.00	0.29	—	—	—
wt/wt,[−],[+],[8],[11]	1.45	2.31	0.624	0.181	2.145
wt/wt,[−],[+],[8],[12]	0.00	0.29	—	—	—
wt/wt,[−],[+],[10],[11]	1.09	0.29	3.805	0.376	38.530
wt/wt,[−],[+],[11],[11]	3.27	4.34	0.747	0.316	1.763
wt/wt,[−],[+],[11],[12]	0.73	0.58	1.260	0.169	9.372
wt/m1,[−],[+],[7-3],[7-3]	2.18	1.16	1.907	0.519	7.006
wt/m1,[−],[+],[7-3],[7]	2.18	1.45	1.521	0.448	5.163
wt/m1,[−],[+],[7-3],[8]	1.45	0.87	1.688	0.363	7.842
wt/m1,[−],[+],[7-3],[10]	0.73	0.00	—	—	—
wt/m1,[−],[+],[7-3],[11]	3.27	2.60	1.267	0.487	3.299
wt/m1,[−],[+],[7-3],[12]	0.73	0.29	2.527	0.217	29.431
wt/m1,[−],[+],[7],[7]	1.09	0.58	1.897	0.303	11.861

TABLE 2

Genotype (*GYP1A1, GSTM1, GSTT1, CYP19*)	Endometriosis (%)	Controls (%)	Odds ratio	95% CI (−)	95% CI (+)
wt/m1,[−],[+],[7],[8]	1.09	0.58	1.897	0.303	11.861
wt/m1,[−],[+],[7],[10]	1.09	0.00	—	—	—
wt/m1,[−],[+],[7],[11]	2.55	2.02	1.265	0.429	3.730
wt/m1,[−],[+],[7],[12]	0.36	0.00	—	—	—
wt/m1,[−],[+],[8],[8]	0.36	0.29	1.259	0.074	21.402
wt/m1,[−],[+],[8],[10]	0.36	0.00	—	—	—
wt/m1,[−],[+],[8],[11]	1.09	0.87	1.261	0.244	6.508
wt/m1,[−],[+],[8],[12]	0.36	0.00	—	—	—
wt/m1,[−],[+],[10],[11]	0.73	0.00	—	—	—
wt/m1,[−],[+],[11],[11]	1.82	1.45	1.263	0.353	4.521
wt/m1,[−],[+],[11],[12]	0.36	0.29	1.259	0.074	21.402
m1/m1,[−],[+],[7-3],[7]	0.36	0.29	1.259	0.074	21.402
m1/m1,[−],[+],[7-3],[10]	0.36	0.00	—	—	—
m1/m1,[−],[+],[7-3],[11]	0.36	0.29	1.259	0.074	21.402
m1/m1,[−],[+],[7],[7]	0.36	0.00	—	—	—
m1/m1,[−],[+],[7],[11]	0.36	0.29	1.259	0.074	21.402
m1/m1,[−],[+],[11],[11]	0.73	0.29	2.527	0.217	29.431
wt/wt,[+],[−],[7-3],[7-3]	0.36	0.29	1.259	0.074	21.402
wt/wt,[+],[−],[7-3],[7]	0.36	0.29	1.259	0.074	21.402
wt/wt,[+],[−],[7-3],[8]	0.00	0.29	—	—	—
wt/wt,[+],[−],[7-3],[11]	0.36	0.58	0.628	0.054	7.310
wt/wt,[+],[−],[7],[7]	0.36	0.29	1.259	0.074	21.402
wt/wt,[+],[−],[7],[8]	0.00	0.29	—	—	—
wt/wt,[+],[−],[7],[11]	0.73	0.58	1.260	0.169	9.372
wt/wt,[+],[−],[8],[11]	0.36	0.29	1.259	0.074	21.402
wt/wt,[+],[−],[11],[11]	0.36	0.29	1.259	0.074	21.402
wt/m1,[+],[−],[7-3],[7-3]	0.36	0.00	—	—	—
wt/m1,[+],[−],[7-3],[7]	0.36	0.29	1.259	0.074	21.402
wt/m1,[+],[−],[7-3],[11]	0.36	0.29	1.259	0.074	21.402
wt/m1,[+],[−],[7],[11]	0.36	0.29	1.259	0.074	21.402
wt/m1,[+],[−],[11],[11]	0.36	0.29	1.259	0.074	21.402
wt/wt,[−],[−],[7-3],[7-3]	0.36	0.29	1.259	0.074	21.402
wt/wt,[−],[−],[7-3],[7]	0.36	0.58	0.628	0.054	7.310
wt/wt,[−],[−],[7-3],[8]	0.00	0.29	—	—	—
wt/wt,[−],[−],[7-3],[11]	0.73	0.87	0.838	0.134	5.237
wt/wt,[−],[−],[7],[7]	0.36	0.29	1.259	0.074	21.402
wt/wt,[−],[−],[7],[8]	0.36	0.29	1.259	0.074	21.402
wt/wt,[−],[−],[7],[11]	0.36	0.58	0.628	0.054	7.310
wt/wt,[−],[−],[8],[11]	0.00	0.29	—	—	—
wt/wt,[−],[−],[11],[11]	0.73	0.58	1.260	0.169	9.372
wt/wt,[−],[−],[7-3],[11]	0.36	0.29	1.259	0.074	21.402
wt/wt,[−],[−],[7],[11]	0.36	0.29	1.259	0.074	21.402

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significant correlation was observed between any of the studied polymorphisms and age or severity of the disease.

DISCUSSION

To identify low-penetrance genetic loci correlated with increased endometriosis risk, we studied *CYP1A1, GSTM1, GSTT1,* and *CYP19* common polymorphisms with respect to their functional significance in dioxin detoxification and estrogen production. The selection of both case and control populations is critical in a case–control study. By studying unrelated cases, with no familial disease, in comparison with endometriosis-free women of higher average age and two or more births, the bias was diminished. Moreover all cases and controls were confirmed surgically, by laparoscopic examination, and histologically, to reduce any diagnostic errors.

Previous reports on *GSTM1* null deletion are controversial as some studies found direct correlation of this polymorphism with the disease (7–9), whereas others did not (10, 12). Noteworthily, these studies were performed in different ethnic populations, and different population sizes and control selections were used. Our data did not support a direct interaction between *GSTM1* null deletion and endometriosis. However, the *CYP1A1* m1 allele was found at a frequency of 0.143 in our control group and at 0.202 in cases; thus, this

TABLE 3

Combinations of polymorphisms for *CYP1A1*, *GSTM1*, and *GSTT1*.

Genotype (*CYP1A1, GSTM1, GSTT1*)	Endometriosis (%)	Controls (%)	Odds ratio	95% CI (−)	95% CI (22>)
wt/wt,[+],[+]	26.55	31.79	0.775	0.542	1.109
wt/m1,[+],[+]	9.45	10.98	0.846	0.495	1.448
m1/m1,[+],[+]	0.73	0.58	1.260	0.169	9.372
wt/wt,[−],[+]	30.18	34.10	0.835	0.590	1.182
wt/m1,[−],[+]	21.82	12.43	1.966	1.270	3.046
m1/m1,[−],[+]	2.55	1.16	2.233	0.631	7.906
wt/wt,[+],[−]	2.91	3.18	0.912	0.355	2.345
wt/m1,[+],[−]	1.82	1.16	1.583	0.410	6.117
m1/m1,[+],[−]	0.00	0.00	—	—	—
wt/wt,[−],[−]	3.27	4.05	0.802	0.336	1.916
wt/m1,[−],[−]	0.73	0.58	1.260	0.169	9.372
m1/m1,[−],[−]	0.00	0.00	—	—	—
Genotype *(CYP1A1,GSTM1)*					
wt/wt,[+]	29.45	34.97	0.776	0.548	1.099
wt/m1,[+]	11.27	12.14	0.920	0.556	1.522
m1/m1,[+]	0.73	0.58	1.260	0.169	9.372
wt/wt,[−]	33.45	38.15	0.815	0.581	1.143
wt/m1,[−]	22.55	13.01	1.947	1.266	2.995
m1/m1,[−]	2.55	1.16	2.233	0.631	7.906
Genotype *(GYP1A1,GSTT1)*					
wt/wt,[+]	56.73	65.90	0.678	0.486	0.946
wt/m1,[+]	31.27	23.41	1.489	1.035	2.141
m1/m1,[+]	3.27	1.73	1.917	0.660	5.571
wt/wt,[−]	6.18	7.23	0.846	0.441	1.622
wt/m1,[−]	2.55	1.73	1.480	0.481	4.557
m1/m1,[−]	0.00	0.00	—	—	—
Genotype *(GSTM1,GSTT1)*					
[+],[+]	36.73	43.35	0.758	0.545	1.056
[+],[−]	4.73	4.34	1.095	0.504	2.378
[−],[+]	54.55	47.69	1.316	0.952	1.820
[−],[−]	4.00	4.62	0.859	0.386	1.914

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polymorphism, together with the *GSTM1* null deletion, appears to have a moderate effect on the endometriosis risk. The *GSTT1* null genotype, as shown in a previous study (12), did not exhibit any impact on endometriosis, alone or in combination with any of the other genes tested.

From the biochemical functional aspect of the enzymes encoding these genes, when both phases I (*CYP1A1*) and II (*GSTM1*) of dioxin detoxification enzymes were affected, the risk of endometriosis increased. GSTT1 is a phase II enzyme responsible for detoxification of smaller hydrocarbons, basically haloalkanes and haloalkenes, such as brominated-trihalomethanes, which are frequent byproducts present in chlorinated drinking water (23).

The *CYP19* VNTR, in intron 4 $(TTTA)_{10}$ allele was found to be associated with endometriosis. This rare allele, with only 0.9% frequency in the control group and 4.2% among endometriosis cases, increased by 4.99 times the risk for disease development. A recent study did not find any association of endometriosis genotype with any of the *CYP19* VNTR alleles, but instead with the 3-bp insertion/deletion polymorphism in intron 4 (24). It has been supposed that VNTR in intron 4 may have a direct functional significance in mRNA splicing and therefore in cellular aromatase activity (25). However, it is most likely, as this repeat polymorphism is not close to the splice sites in intron 4, that linkage disequilibrium with other significant functional polymorphisms or mutations could be involved (26, 27).

Because of ethnic variations, it is possible that different variants may be genetically linked with different mutations. Thus, it is important that data from different population studies be interpreted with caution. In breast cancer, such a perplexity is clear because reports for the *CYP19* VNTR in intron 4 from different research groups in different populations found linkage with such different alleles as $(TTTA)_8$, $(TTTA)_{10}$, and $(TTTA)_{12}$ (25–28). Another rationale is that these three alleles are all rare in the populations studied, adding to the population size bias.

In conclusion, we observed that there is a potent connection between $(TTTA)_{10}$ allele of *CYP19* VNTR in intron 4 and endometriosis and, to a lesser extent, between *CYP1A1* m1 and *GSTM1* null polymorphism with the disease.

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