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CYP17 PROMOTOR POLYMORPHISM AND OVARIAN CANCER RISK

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The CYP17 gene encodes the cytochrome P450c17α enzyme, which functions at 2 different points in the steroid biosynthesis pathway, and is considered a candidate susceptibility gene for endocrine-related tumors. A *T* to *C* substitution polymorphism exists in the 5′ promoter region of this gene, and creates an additional Sp1-type motif. Several studies have examined this polymorphism as a risk factor for breast cancer, but results have been conflicting. We examined 319 cases of ovarian cancer and 298 unaffected controls for the *T-C* polymorphism. There was no significant difference between cases and controls for the allele frequencies ($p = 0.6$), or for genotype distribution ($p = 0.9$). The odds ratio (95% confidence interval) for ovarian cancer was 1.13 (0.70–1.82) for the putative "cancer susceptibility" *CC* genotype and 1.07 (0.77–1.48) for any *C* allele (*CC* or *CT* genotype). Results were little different after adjustment for age. Stratification of the ovarian cancer cases according to form (benign, low malignant potential or invasive), histology, grade or stage failed to reveal any heterogeneity with respect to *CYP17* genotype. Our data provide no evidence for an association between ovarian cancer risk and the genotype defined by the *CYP17* 5′ promoter region *T-C* polymorphism. *Int. J. Cancer* 86:436–439, 2000.
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Ovarian cancer is the main cause of death among women with gynecological malignancies, and the lifetime risk of ovarian cancer in Australian women is 1 in 99 (AIHW and AACR, 1998). Mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2*, or in the mismatch repair genes *hMSH2* and *hMLH1*, are known to be responsible for most "hereditary" ovarian cancers from multiple-case families (reviewed by Boyd and Rubin, 1997). However, the vast majority (approx. 99%) of Australian patients with ovarian cancer do not present as "high-risk" familial cases. It is likely that "low-risk" genes may account for at least some predisposition to these apparently "sporadic" ovarian cancers, since family history is the highest risk factor for ovarian cancer after age (Parazzini *et al.*, 1991; Purdie *et al.*, 1995), with the life-time risk for ovarian cancer increasing from 1% to 7% if one first degree relative is affected.

Low-risk ovarian cancer genes may include those involved in hormone or carcinogen metabolism in which common allelic variants exist. However, there are only a few reported studies investigating the effects of such polymorphisms on the risk of ovarian cancer, and many of these studies are unconfirmed reports based on relatively small sample sizes. We are using the candidate gene approach to identify low-risk ovarian cancer susceptibility genes in a large cohort of ovarian cancer patients and controls. Genes in the hormone biosynthesis pathway are considered likely candidates, because epidemiological studies indicate that ovarian cancer is an endocrine-related tumor (Parazzini *et al.*, 1991). The CYP17 gene encodes the cytochrome P450c17α enzyme, which functions at 2 different points in the steroid biosynthesis pathway. A 5′ promoter *T* to *C* substitution polymorphism creates an additional Sp1 type (CCACC) promoter site, 34 bp upstream of the initiation of translation but downstream from the transcription start site (Carey *et al.*, 1994). This polymorphism, which alters the recognition site for the *MspA1* restriction site, has been investigated as a risk factor for breast cancer with conflicting results. The initial study by Feigelson *et al.*. (1997) suggested that the *C* allele (otherwise termed *A2* allele) was associated with risk of advanced breast cancer, and a recent study of Swedish breast cancer patients diagnosed before age 36 also reported an association between *C* allele carriers and breast cancer risk (Bergman-Jungestrom *et al.*, 1999). However, all other subsequent association studies have failed to replicate this finding (Dunning *et al.*, 1998; Helzlsouer *et al.*, 1998; Weston *et al.*, 1998; Haiman *et al.*, 1999; Kristensen *et al.*, 1999). Recent *in vitro* evidence suggests that the 5′ Sp1 type site resulting from the *T* to *C* substitution does not actually bind transcription factor Sp1 (Kristensen *et al.*, 1999), but there is still some functional evidence to indicate that this polymorphism may act as a risk factor in hormone-related cancers. Women with a *CC* genotype have been shown to have significantly higher serum estradiol or estrone levels than those with a *TT* genotype (Feigelson *et al.*, 1998; Haiman *et al.*, 1999), and there is a suggestion that the homozygous *CC* genotype may modify the expression of polycystic ovary disease in single gene disorder families (Carey *et al.*, 1994; Gharani *et al.*, 1996; Diamanti-Kandarakis *et al.*, 1999). There are no reports of the association between this polymorphism and epithelial ovarian cancer, and we have therefore undertaken a large case-control comparison to assess the *CYP17* 5′ *T* to *C* transition polymorphism as a risk factor for ovarian cancer.

MATERIAL AND METHODS

Subjects

Unselected subjects with ovarian adenocarcinoma (case subjects) (n = 319) were ascertained as incident cases from the Royal Brisbane Hospital, Queensland, Australia, during the period 1985–1996. Clinical information available from these cases included cancer form [benign, low malignant potential (LMP), or invasive], tumor histology, stage, and grade. The series comprised 34 benign, 44 LMP and 240 invasive tumors, and 1 tumor of unknown form. There were 199 serous, 38 mucinous, 31 endometrioid, 15 clear cell carcinoma, 8 mixed mullerian, 7 mixed, 4 undifferentiated and 1 Brenner tumor(s) as well as 16 of unknown histology. Patients were staged at laparotomy in accordance with the recommendations of the International Federation of Gynaecology and Obstetrics (FIGO). Of the 233 invasive tumors of known stage, there were 25, 19, 162 and 27 at FIGO stages 1, 2, 3, and 4, respectively.

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Grade definitions were drawn directly from pathology reports for 218 invasive tumors, and the sample included 18 grade 1, 5 grade 1/2, 57 grade 2, 34 grade 2/3, 102 grade 3 and 2 grade 4 tumors. The grades 1/2 and 2/3 reflected grades considered indistinguishable by the pathologists themselves. The age at diagnosis of cases ranged from 21–95 years, with an average age at diagnosis of 59.9 years. Case subjects were grouped by age at diagnosis as follows: <40 (n = 28), 40–49 (n = 41), 50–59 (n = 72), 60–69 (n =101) and >70 (n = 77) years. No additional epidemiological information was available on cases.

Control subjects (n = 298) were adult female unrelated monozygotic twins selected from a sample of 3348 twins of almost exclusively European descent, recruited through the volunteer National Australian Twin Registry for the Semi Structured Assessment for the Genetics of Alcoholism (SSAGA) research study (Heath *et al.*, 1998). Subjects had participated in a telephone interview follow-up in 1992–1993, and those providing blood samples for DNA studies between 1993 and 1996 lived in or close to Adelaide, Brisbane, Melbourne, or Sydney. Criteria for selection from the SSAGA sample were that subjects should be monozygotic (DNA from dizygotic twin pairs was in high demand for other projects), female, and that the date-of-birth distribution of control-subjects should match as closely as possible the date of birth distribution observed for ovarian cancer patients, namely, one-third from each of 1900–1925, 1926–1938, and 1939–1970. Age at interview ranged from 30–90 years, with an average age at interview of 50.9 years. Control individuals were grouped by age at interview as follows: <40 (n = 85), 40–49 (n = 61), 50–59 (n = 65), 60–69 (n = 56) and >70 (n = 33) years. Parity data collected at interview was available for 291 of the 298 controls, and the sample included 38 nulliparous women, 169 women with 1–3 liveborn children, and 84 women with 4 or more liveborn children.

Ethics clearance for collection of subject information and blood from cases and controls was given by the Queensland Institute of Medical Research Ethics committee. Germline DNA was extracted from peripheral blood from both cases and controls by a salt-precipitation method, as described by Chenevix-Trench *et al.*. (1997).

Genotype detection

The *CYP17* 5′ *C-T* polymorphism (Carey *et al.*, 1997) was detected using the Perkin Elmer (PE) ABI Prism 7700 Sequence Detection System (SDS) for multi-color real-time or end-point fluorogenic PCR detection (PE Applied Biosystems, Foster City, CA; Catalogue 7700-01-220/240). A 102 bp polymerase chain reaction product was amplified using the forward and reverse primers 5′-GCCTCCTTGTGCCCTAGAGTT-3′ and 5′-AGCAAGAGAGCCACGAGCTC-3′, respectively. MspA1 enzyme digestion and high resolution agarose gel electrophoresis was used to identify *TT* and *CC* homozygote DNA controls required as standards for the SDS allelic discrimination assay. Using the standard protocol for SDS allelic discrimination assay, fluorescently-labeled probes 5′-FAM-TCTACTCCACCGCTGTCTATCTTGCCTAMRA-3′ and 5′-TET-TTCTACTCCACTGCTGTCTATCTTGCCTG-TAMRA-3′ were used to detect the *C* and *T* alleles, respectively. Reaction volumes were initially 25 μl, but were optimized to 15 μl for later experiments. The final concentration of reagents in the PCR mix was 1× TaqMan Universal PCR Master Mix (PE Catalogue no. 4304437), 900 nM each primer, 200 nM FAM-C probe, and 100 nM TET-T probe. Reaction mix was added to 30 ng of genomic sample DNA that had been pre-dried in 96-well plates. PCR reactions were incubated in the ABI 7700 SDS PCR machine for 2min at 50°C, 10 min at 95°C, followed by 40–50 two-step cycles of 15 sec at 95°C and 1min at either 64°C or 66°C. Genotype analysis was performed on amplified samples using the ABI PRISM 7700 software, following standard procedures. Repeatability of the ABI PRISM 7700 SDS genotyping was assessed by re-analysis of a sub-sample of 182 DNA samples, selected on the basis of DNA availability. Successful re-amplification of samples generated confirmatory genotype results in all instances.

Statistical analysis

The Student's *t*-test was used to compare age distribution in cases and controls. The Hardy Weinberg Equilibrium (HWE) assumption was assessed for case groups and control groups, and differences in allele frequency were evaluated by the χ^2 test. The linear-by-linear association test was used to assess differences in the distribution of *CYP17* genotype with increasing age at interview or parity among controls, and with increasing age at onset, grade or stage amongst cases. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to compare case and control subjects with respect to *CYP17* genotype, and age-adjusted ORs were calculated using logistic regression. StatXact, Ottutil, Egret and EpiInfo v 6.0 software were used for statistical analyses.

RESULTS

Genotype data were generated for 319 cases and 298 controls (Table I). There was no evidence for deviation from HWE in control-subjects ($p = 0.9$), or in case-subjects ($p = 0.8$). There was no difference in allele frequency between cases and controls ($p = 0.6$), with a T allele frequency (standard error) of 0.619 (0.020) and 0.615 (0.019) in controls and cases, respectively.

Different approaches were used for comparing the three genotypes defined by the 2 CYP17 alleles (Table I). The *CT* heterozygote genotype and the *CC* genotype were compared to the *TT* genotype in order to assess change in risk with increasing numbers of putative "cancer susceptibility" *C* alleles. The pooled sample of *CT* heterozygotes and *CC* homozygotes was also compared with the *TT* genotype to assess the risk associated with the presence of at least 1 *C* allele, as presented in the original investigation of Feigelson *et al.*. (1997). Both crude and age-adjusted odds ratios (ORs) indicated that there was no significant difference between cases and controls. All ORs for an association between ovarian cancer risk and the putative "cancer susceptibility" *C* allele were marginally greater than 1, with confidence intervals including unity. There was no association between *CYP17* genotype and age at onset in cases ($p = 0.4$) or age at interview in controls ($p = 0.6$), and crude and age-adjusted ORs were little different (Table I), suggesting that the younger average age of the control group (Student's *t*-test, $p < 0.0001$) was unlikely to have masked any effect of *CYP17* genotype on ovarian cancer risk. There was 80% power at a significance level of 5% to detect a 1.9-fold increased risk of ovarian cancer in individuals with the *CC* genotype, and a 1.7-fold increased risk of ovarian cancer in individuals with at least one *C* allele.

TABLE I – *CYP17* GENOTYPE: COMPARISON OF OVARIAN CANCER CASES TO CONTROLS

CYP17 Genotype[1]	Controls		Cases		Crude OR (95% CI)		Age-Adjusted OR (95% CI)	
	n	%	n	%				
TT	115	38	118	37	1.00	Reference	1.00	Reference
CT	139	47	150	47	1.05	(0.74–1.49)	1.03	(0.71–1.48)
CC	44	15	51	16	1.13	(0.70–1.82)	1.11	(0.67–1.84)
CT/CC	183	62	201	63	1.07	(0.77–1.48)	1.05	(0.74–1.48)

[1]*T* and *C* alleles also termed *A1* and *A2*, respectively (Carey *et al.*, 1994).

Epidemiological information such as parity was not available for cases, and it was thus not possible to calculate ORs with adjustment for this and other known ovarian cancer risk factors. However, there was no indication that associations between *CYP17* genotype and ovarian cancer risk could have been masked by differences in parity between cases and controls, since stratification of *CYP17* genotype data by parity showed no difference in the genotype distribution among controls ($p = 0.2$).

Ovarian cancer cases were also stratified by cancer form, histology, grade and stage to investigate the possibility of CYP17 genotype heterogeneity between ovarian cancer subgroups (Table II). There were no statistically significant differences in genotype distribution between any of the subgroups, and thus there was no indication for case subgroup analysis in the comparison of *CYP17* genotype distribution of cases to controls.

DISCUSSION

Our present data on 319 ovarian cases and 298 unaffected controls provide no evidence for an association between ovarian cancer risk and the genotype defined by the *CYP17* 5′ *T* to *C* polymorphism, despite having sufficient power to detect an OR of 1.7 for *CC/CT* genotypes combined, or an OR of 1.9 for the *CC* genotype. There was no reason to suspect that the age difference between cases and controls had generated a false-negative result because age-adjusted ORs were marginally closer to 1.0 than crude ORs. Given the later age at onset of ovarian cancer, there remains a possibility for bias if women with undiagnosed ovarian cancer were included in the younger control group. At the worst extreme, we might assume 3 undiagnosed ovarian cancer cases of "cancer susceptibility" *CC* genotype to be included in the control group of 300 individuals; however, exclusion of 3 *CC* genotypes from the control group to mimic such putative undetected cases did not alter the statistical findings.

Confounding due to differences in ethnicity was also unlikely to have generated a false-negative result. The majority of Australians are Anglo-Celtic in origin, so cases and controls were unlikely to differ appreciably with respect to their ethnic background. In addition, control group genotype frequencies presented in our study were also not significantly different from frequencies reported for control groups from a wide variety of ethnic backgrounds (Feigelson *et al.*, 1997; Dunning *et al.*, 1998; Helzlsouer *et al.*, 1998; Weston *et al.*, 1998; Haiman *et al.*, 1999; Kristensen *et al.*, 1999; Bergman-Jungestrom *et al.*.; 1999), with *p* values for differences in genotype distribution ranging from 0.5 to 1.0. These control group samples included 285 Asian, African-American and Latino women aged 45–75 years (Feigelson *et al.*, 1997), 591 East Anglians aged 45–74 (Dunning *et al.*, 1998), 113 Caucasian Americans of average age 60 years (Helzlsouer *et al.*, 1998), 240 Caucasian, Hispanic and African Americans of unspecified age (Weston *et al.*, 1998), 618 Americans of unspecified ethnicity aged 43–69 years (Haiman *et al.*, 1999), 201 Norwegians aged 20–44 years (Kristensen *et al.*, 1999), and 117 Swedes aged 18–39 years (Bergman-Jungestrom *et al.*, 1999).

The ovarian cancer cases were also stratified according to several criteria to assess whether there was heterogeneity with respect to *CYP17* genotype because there is evidence to suggest that the mutational pathway differs between invasive tumors and benign or low malignant potential ovarian tumors (Berchuck *et al.*, 1994; Chenevix-Trench *et al.*, 1997), and between tumors of different histology (Kvale *et al.*, 1988; Ichikawa *et al.*, 1994; Obata *et al.*, 1998). Stratification of the ovarian cancer sample according to

TABLE II – *CYP17* GENOTYPE HETEROGENEITY WITHIN OVARIAN CANCER CASES[1]

Genotype	Cancer form[2]								*p*
	Benign		LMP		Invasive		Total		
	n	%	n	%	n	%	n	%	
TT	15	44	17	39	85	35	117	37	
TC	14	41	22	50	114	48	150	47	0.8
CC	5	15	5	11	41	17	51	16	
Total	34		44		240		318		

Genotype	Histology[3]										*p*
	END		CCC		MUC		SER		Total		
	n	%	n	%	n	%	n	%	n	%	
TT	15	48	4	27	11	29	73	37	103	36	
TC	12	39	7	47	22	58	97	49	138	49	0.5
CC	4	13	4	27	5	13	29	15	42	15	
Total	31		15		38		199		283		

Genotype	Grade[4]														*p*
	0		1		1/2		2		2/3		3–4		Total		
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
TT	32	41	5	28	2	40	17	30	15	44	38	37	109	37	
TC	36	46	10	56	2	40	26	46	17	50	49	47	140	47	0.6
CC	10	13	3	17	1	20	14	25	2	6	17	16	47	16	
Total	78		18		5		57		34		104		296		

Genotype	Stage								*p*
	1		2		3		4		Total		
	n	%	n	%	n	%	n	%	n	%	
TT	5	20	7	37	62	38	10	37	84	36	
TC	14	56	9	47	72	44	14	52	109	47	0.7
CC	6	24	3	16	28	17	3	11	40	17	
Total	25		19		162		27		233		

[1]Heterogeneity assessed by the chi-squared test (neoplasm form, histology), or the linear-by-linear association test (grade, stage).–[2]LMP = low malignant potential.–[3]END = endometrioid, CCC = clear cell carcinoma, MUC = mucinous, SER = serous.–[4]$p = 0.4$ after exclusion of grades 1/2 and 2/3.

form, histology, grade or stage failed to reveal any significant heterogeneity with respect to *CYP17* genotype, thereby excluding *CYP17* genotype as a significant risk factor for even a substratum of ovarian cancer cases.

In conclusion, the data provide no evidence for an association between ovarian cancer risk and the genotype defined by the *CYP17* 5′ promoter region *T-C* polymorphism. Alternative "low-risk" gene candidates are being investigated, and the possibility of gene-gene interactions between the *CYP17* polymorphism and other candidate genes will be explored as the data become available.

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