



Original Contribution

Association of Genetic Polymorphisms in Cell-Cycle Control Genes and Susceptibility to Endometrial Cancer Among Chinese Women

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Although genetic variations in cell-cycle control genes have been previously linked to cancer risk, no study has specifically evaluated the role of these gene variants in endometrial carcinogenesis. Using data from the Shanghai Endometrial Cancer Study, a population-based case-control study with 1,199 cases and 1,212 age-matched controls (1997–2003), the authors carried out a systematic evaluation of the association of cell-cycle control genes with endometrial cancer risk. Sixty-five tagging or potentially functional single nucleotide polymorphisms in the *CCNB1*, *CCND1*, *CCNE1*, *CDK2*, *CDK4*, *CDK6*, *CDKN1A*, *CDKN1B*, and *CDKN2A* genes were genotyped and evaluated. Three single nucleotide polymorphisms in the *CDKN1B* gene (rs11055027, rs3759216, and rs34330) were related to endometrial cancer risk, although only the association with rs34330 remained statistically significant after adjustment for multiple comparisons. The odds ratios for rs34330 were 1.33 (95% confidence interval (CI): 1.06, 1.66) and 1.51 (95% CI: 1.16, 1.94) for the CT and TT genotypes, respectively, compared with the CC genotype. In vitro luciferase reporter assays showed that the minor allele (A) in rs3759216, which was associated with decreased endometrial cancer risk (odds ratio = 0.73, 95% CI: 0.56, 0.94) without adjustment for multiple comparisons, significantly increased promoter activity. These findings suggest that polymorphisms of the *CDKN1B* gene may play a role in endometrial carcinogenesis.

cell cycle; China; endometrial neoplasms; polymorphism, genetic; polymorphism, single nucleotide; women

Abbreviations: CDK, cyclin-dependent kinase; GRS, genetic risk score; SNP, single nucleotide polymorphism.

Carcinogenesis in humans is believed to result from uncontrolled cell proliferation. Cell-cycle checkpoints are one of the primary defense mechanisms against mutagenic exposure. These checkpoints are regulatory pathways that control the order and timing of cell-cycle transitions to ensure DNA replication and chromosome segregation (1). The key point in cell-cycle regulation is the transition of the restriction point late in the G1 phase, which is crucial in determining the cell's destiny—division, differentiation, senescence, or apoptosis. It is believed that once the restriction point has been overcome, cell-cycle progression occurs almost automatically. Cells may be arrested at the restriction point, temporarily halting the cell cycle and allowing DNA repair to be completed. The loss of this checkpoint and the perturbation of cell-cycle control not only may disrupt the

balance between normal growth and terminal differentiation but may also be accompanied by genomic instability, which may facilitate the development of cancer, as evidenced by the frequent inactivation of cell-cycle control genes, including those for p53, p16, p27, and retinoblastoma protein, in various cancers (2). In mammalian cells, cell division is controlled by the activity of cyclin-dependent kinases (CDKs) and their essential activating coenzymes, the CDK inhibitors (3–5), which may be influenced by genetic variations in the corresponding genes.

Cyclin D1, encoded by the *CCND1* gene, plays an important role in the progress of the cell cycle. It associates with CDKs to phosphorylate the retinoblastoma protein during the G1 phase (6). Cyclin E, dissimilar to the mitogen-dependent cyclin D, is a mitogen-independent activator of

CDK and a critical regulator of G1/S transition (7). The CDK inhibitors are key regulators of the G1/S checkpoint; their concerted action prevents cells from undergoing subsequent division in response to oncogenic signaling or DNA damage (8). Among them, *CDKN1B* belongs to the Cip/Kip family and functions as a cell-cycle checkpoint at G1/S by encoding the p27 protein. The p27 protein is a putative tumor suppressor that inhibits phosphorylation of retinoblastoma protein to regulate G1 cyclin-CDK complexes (9, 10). In knockout mouse models, p27 deficiency has led to gigantism (11, 12), indicating the importance of this gene in tumorigenesis.

Anomalies in cell-cycle control genes have frequently been observed in many human malignancies. *CCNB1*, *CCND1*, and *CCNE1* have distinct expressions in different breast cancer subtypes and invasive ovarian cancer (13, 14). *CDK2*, *CDK4*, *CDK6*, *CDKN1A*, *CDKN1B*, and *CDKN2A* are found to be associated with breast cancer (15, 16), lung cancer (17, 18), and ovarian, pancreatic, and prostate cancers (19–22). Together, these data suggest an important role for CDKs, cyclins, and CDK inhibitors in cancer development. However, to the best of our knowledge, no study has evaluated the effect of genetic variants in those cell-cycle control genes on susceptibility to endometrial cancer. In this study, we examined associations of polymorphisms in 9 cell-cycle control genes with endometrial cancer risk using data from the Shanghai Endometrial Cancer Study, a large population-based case-control study conducted in urban Shanghai, China.

MATERIALS AND METHODS

Study participants

Details on the Shanghai Endometrial Cancer Study have been published elsewhere (23). Briefly, of 1,449 newly diagnosed endometrial cancer patients aged 30–69 years who were identified between January 1997 and December 2003 through the population-based Shanghai Cancer Registry, 1,199 (82.7%) participated in the study. Controls were randomly selected from permanent female residents of urban Shanghai and were frequency-matched to cancer cases by age (5-year interval) at a 1:1 ratio. The random selection was performed by staff of the Shanghai Resident Registry. Women with a prior history of any cancer or hysterectomy were ineligible for the study. Of the 1,629 eligible controls contacted, 1,212 (74.4%) participated in the study. The study protocols were approved by the institutional review boards of all institutions involved in the study, and all participants provided written informed consent before participating in the study.

Detailed information on demographic factors, menstrual and reproductive history, hormone use, prior disease history, physical activity, tobacco and alcohol use, diet, weight history, and family history of cancer was collected for all participants via an in-person interview. Body weight, height, and waist and hip circumferences were measured according to a standardized protocol at the time of interview. Menopause was defined as cessation of menstruation for at least 12 months before the reference date (diagnosis date for

cases and interview date for controls), excluding lapses caused by pregnancy or breastfeeding. Body mass index (weight in kilograms/squared height in meters) and waist-to-hip circumference ratio were calculated using measured anthropometric data.

Of the study participants who completed an in-person interview, 850 cases and 853 controls donated a blood sample and 280 cases and 274 controls provided a buccal-cell sample (187 cases and 186 controls provided samples using a mouthwash method, and 93 cases and 88 controls provided samples using a buccal swab method). Because of the very low DNA yield of the buccal swab method, we did not include buccal swab DNA samples in the genotyping. In addition, DNA samples from 19 controls who donated a blood sample were used up in other studies. Thus, DNA samples from 1,037 cases (86.5%; 850 blood and 187 buccal-cell) and 1,020 controls (84.2%; 834 blood and 186 buccal-cell) were included in this genotyping study. Genotyping data for cell-cycle control genes were obtained from 1,028 cases and 1,003 controls, with success rates of 99.1% and 99.6%, respectively.

SNP selection, identification, and genotyping

We selected haplotype-tagging single nucleotide polymorphisms (SNPs) by searching Han Chinese data from the International HapMap Project (<http://hapmap.ncbi.nlm.nih.gov/>) using the Tagger program (24). The following criteria were used to identify tagging SNPs: 1) the SNPs were located in the cell-cycle control gene or within the 5-kilobase region flanking the gene, 2) the SNPs had a minor allele frequency greater than or equal to 0.05, and 3) the other known unselected SNPs could be captured by one of the tagging SNPs with a linkage disequilibrium of $r^2 \geq 0.90$. Known functional or potentially functional SNPs were forced into the haplotype-tagging SNP selection process. SNP selection was completed in December 2005. Of the 67 selected SNPs, the design of the assay for 2 SNPs (rs2282992 in the *CDK6* gene and rs1801270 in the *CDKN1A* gene) failed, which resulted in 65 SNPs being genotyped for this study (genotyped SNPs are listed in Web Table 1, which is posted on the *Journal's* Web site (<http://aje.oxfordjournals.org/>)).

Genomic DNA was extracted from buffy coat fractions or buccal cells using the QIAamp DNA minikit (Qiagen Inc., Valencia, California) following the manufacturer's protocol. The SNPs were genotyped using the Affymetrix MegAllele Targeted Genotyping System (Affymetrix, Inc., Santa Clara, California) with the molecular inversion probe method (25) as part of a large-scale genotyping effort that included 1,737 SNPs. Genotyping was conducted at the Vanderbilt Microarray Shared Resource (Vanderbilt University, Nashville, Tennessee) following the Affymetrix protocol. As a quality control procedure, we included 39 blinded duplicated quality control samples and 12 HapMap DNA samples in the genotyping. The average consistency rate for these samples was 99.6%. The genotyping of SNPs in cell-cycle control genes was highly successful, with call rates of 99.5%–100% (median, 99.95%). Finally, the laboratory staff was blind to the case-control status and identity of all samples.

In vitro functional experiments

To evaluate the function of SNPs located in the 5' flanking region of the *CDKN1B* gene, we conducted a series of experiments with *CDKN1B* luciferase reporter constructs. First, a 3.6-kilobase fragment, spanning the 3,600 base pairs upstream of the ATG translation start codon and including rs11055027, rs3759216, and rs34330, was generated by polymerase chain reaction with the forward primer 5'-GTGAGCTTAGAAGAATGGTGGAGTTGAGTG-3' and the backward primer 5'-CTATAAGCTTCTGCACGACC-GCCTCTCTC-3'. Template DNA from persons known to have either a major allele or a minor allele for rs11055027, rs3759216, and rs34330 was used. The resulting fragments were cloned into a promoter-less luciferase reporter vector, pGL4.20 (Promega Corporation, Madison, Wisconsin). All DNA constructs were verified through sequencing. Transfection was performed with the use of FuGene 6 Transfection Reagent (Roche Diagnostics GmbH, Mannheim, Germany) in triplicate for each construct. Human 293 cells (2×10^5 each) were seeded in 24-well plates and cotransfected with pGL4.73 (a *Renilla* expressing vector serving as a reference for transfection efficiency) and the luciferase reporter constructs with different combinations of alleles. Thirty-six to 48 hours later, cells were lysed with passive lysis buffer, and luminescence (relative light units) was measured using the Dual-Luciferase Assay System (Promega Corporation). Relative luciferase activity was measured as the ratio of firefly luciferase activity to *Renilla* luciferase activity, and results from 5 independent experiments were averaged.

Statistical analyses

Chi-squared and *t* tests were used to evaluate case-control differences in the distributions and mean values of risk factors and genotypes of the cell-cycle control genes and deviations of genotype frequencies in controls from those expected under Hardy-Weinberg equilibrium. Unconditional multivariable logistic regression models were applied to estimate odds ratios and 95% confidence intervals for measurement of the association between endometrial cancer risk and cell-cycle control genes, adjusting for matching variables and known risk factors for endometrial cancer throughout. Known risk factors included age at interview, educational level, income level, menopausal status, age at menarche, number of livebirths, alcohol consumption (ever), and body mass index. Categorized variables were treated as dummy variables in the model. All tests for trend were performed by entering categorical variables as continuous parameters in the models. *P* values for trend tests on 65 SNPs in additive models were adjusted for multiple comparisons using an approach proposed by Conneely et al. (26) to reduce false-positive rates. SNPs with *P* < 0.05 in the additive model with no adjustment for multiple comparisons are presented here.

To evaluate whether multiple SNPs in the same gene may have an additive effect on endometrial cancer risk, we estimated the combined effect of these SNPs. We treated alleles with an odds ratio greater than or equal to 1 at each locus as

the risk allele (the reference allele is the risk allele if the odds ratio is less than 1) and tallied the total number of risk alleles for each participant to calculate a genetic risk score (GRS). Linkage disequilibrium between polymorphisms was assessed using HaploView software, version 4.1 (27), and haplotype blocks were defined using a method reported by Gabriel et al. (28). The repeated-measures analysis of variance test was used to evaluate the relative value of luciferase activity for each SNP. SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina) was used to test associations between SNPs and endometrial cancer. All statistical tests were based on 2-tailed probability.

RESULTS

Table 1 presents selected demographic and risk factor characteristics of participants genotyped for polymorphisms of the cell-cycle control genes in this study. The 1,028 cases and 1,003 controls were similar with respect to age, educational status, income level, and cigarette smoking habits. Compared with controls, cases were more likely to have been younger at menarche, were less likely to be postmenopausal, had had fewer livebirths, and had a higher body mass index. There were no appreciable differences in the distribution of demographic or risk factors between the entire study population (data not shown) and persons with genotyping data.

A total of 65 SNPs in 9 cell-cycle control genes—3 cyclins (*CCNB1*, *CCND1*, and *CCNE1*), 3 CDKs (*CDK2*, *CDK4*, and *CDK6*), and 3 CDK inhibitors (*CDKN1A*, *CDKN1B*, and *CDKN2A*)—were included in the study. Their associations with endometrial cancer risk are summarized in Web Table 1. The genotype distributions were consistent with Hardy-Weinberg equilibrium (*P* > 0.05) among controls for most SNPs, with the exception of rs649392, rs2237572, rs2282981, rs3829963, rs12528248, and rs12191972.

Of the 65 SNPs under study, 7 SNPs were associated with endometrial cancer risk at *P* < 0.05 (Table 2) before adjustment for multiple comparisons. These included 1 (rs2069433) of 3 SNPs in the *CCNB1* gene, 2 (rs2282978 and rs2282979) of 32 SNPs in the *CDK6* gene, and 4 (rs11055027, rs3759216, rs34330, and rs34322) of 9 SNPs in the *CDKN1B* gene. After multiple comparison adjustment, only the minor allele of rs34330 in the *CDKN1B* gene was significantly associated with endometrial cancer risk compared with its major homozygote, and the other 3 SNPs (rs2069433, rs11055027, and rs3759216) had associations of marginal statistical significance. There was no evidence for any association at a significance level of *P* ≤ 0.05 for the remaining 6 genes studied (*CCND1*, *CCNE1*, *CDK2*, *CDK4*, *CDKN1A*, and *CDKN2A*).

Figure 1 depicts the linkage disequilibrium structure of the *CDKN1B* gene with 3 blocks. The linkage disequilibrium plot of the 9 SNPs revealed low/moderate pairwise *r*² values, and the highest *r*² value (*r*² = 0.60) was seen for rs3759216 and rs34330, indicating that linkage disequilibrium among these SNPs is not strong. Additive effects of multiple SNPs in the same gene were evaluated (Table 3).

Table 1. Demographic Characteristics and Selected Risk Factors for Endometrial Cancer Cases and Controls, Shanghai Endometrial Cancer Study, Shanghai, China, 1997–2003

Participant Characteristic	Cases (n = 1,028)		Controls (n = 1,003)		P Value
	Mean (SD)	%	Mean (SD)	%	
Age, years	54.78 (8.51)		54.88 (8.49)		0.80
Age at menarche, years	14.47 (1.73)		14.77 (1.81)		<0.001
No. of livebirths	1.67 (1.18)		1.76 (1.14)		0.07
Body mass index ^a	25.75 (4.13)		23.78 (3.48)		<0.001
Education					
Primary school or below		21.7		22.0	
Middle school		38.2		37.9	
High school or above		40.1		40.1	0.98
Family income, RMB/year					
<10,000		12.2		10.8	
10,001–19,999		41.6		39.7	
≥20,000		46.2		49.5	0.30
Occupation, %					
Professional		29.1		25.4	
Clerical		22.4		22.6	
Manual laborer		48.5		51.9	0.15
Ever smoking (yes)		3.1		3.6	0.55
Ever consuming alcohol (yes)		3.1		5.3	0.01
Postmenopausal		56.8		61.5	0.03

Abbreviations: RMB, renminbi; SD, standard deviation.

^a Weight (kg)/height (m)².

For the 5 *CDKN1B* SNPs with $P < 0.10$ (Web Table 1), the GRS for each participant ranged from 0 to 10. We categorized participants into 6 groups and used participants with a GRS of 0 as the reference group. The odds ratios for participants with $GRS < 5$ did not differ significantly from the reference group. However, the odds ratios for participants with $GRS \geq 5$ exhibited a significantly increased risk of

endometrial cancer. A woman with a GRS of 9–10 had a 2.5-fold increased risk of endometrial cancer ($P = 0.02$). We found no significant associations for either *CCNB1* or *CDK6*.

To evaluate whether SNPs in the *CDKN1B* gene (rs11055027, rs3759216, and rs34330) affect gene expression, we conducted an in vitro *CDKN1B* promoter/luciferase

Table 2. Genotype-Specific Risks of Endometrial Cancer for Selected Single Nucleotide Polymorphisms^a, Shanghai Endometrial Cancer Study, Shanghai, China, 1997–2003

Gene	Single Nucleotide Polymorphism	Minor/Major Alleles	Region	Minor Allele Frequency	P_{HWE}	Endometrial Cancer Risk					
						Heterozygosity (AB)		Minor-Allele Homozygosity (BB)		Additive P_{trend}	Adjusted P Value ^b
						OR ^c	95% CI	OR ^c	95% CI		
<i>CCNB1</i>	rs2069433	C/T	Intron	7.28	1.00	0.79	0.60, 1.02	0.35	0.08, 1.46	0.030	0.081
<i>CDK6</i>	rs2282978	C/T	Intron	11.30	1.00	0.79	0.63, 1.00	0.75	0.34, 1.68	0.040	0.413
<i>CDK6</i>	rs2282979	C/T	Intron	10.81	0.89	0.80	0.64, 1.01	0.67	0.28, 1.57	0.042	0.424
<i>CDKN1B</i>	rs11055027	C/G	Promoter	18.24	0.73	1.21	0.99, 1.48	1.89	1.12, 3.19	0.006	0.068
<i>CDKN1B</i>	rs3759216	A/G	Promoter	43.66	0.38	0.76	0.62, 0.93	0.73	0.56, 0.94	0.008	0.084
<i>CDKN1B</i>	rs34330	T/C	5' untranslated	49.75	0.18	1.33	1.06, 1.66	1.51	1.16, 1.94	0.002	0.025
<i>CDKN1B</i>	rs34322	C/T	3' flanking	48.35	0.49	0.84	0.67, 1.04	0.73	0.57, 0.94	0.015	0.142

Abbreviations: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratio.

^a Single nucleotide polymorphisms were selected on the basis of $P_{trend} < 0.05$ and $P_{HWE} > 0.05$.

^b Adjusted P values are presented because of multiple correlated tests (26).

^c Adjusted for age at interview, body mass index, menopausal status, education, income, alcohol consumption, age at menarche, number of livebirths, and occupation. Major-allele homozygosity (AA) was the reference category.

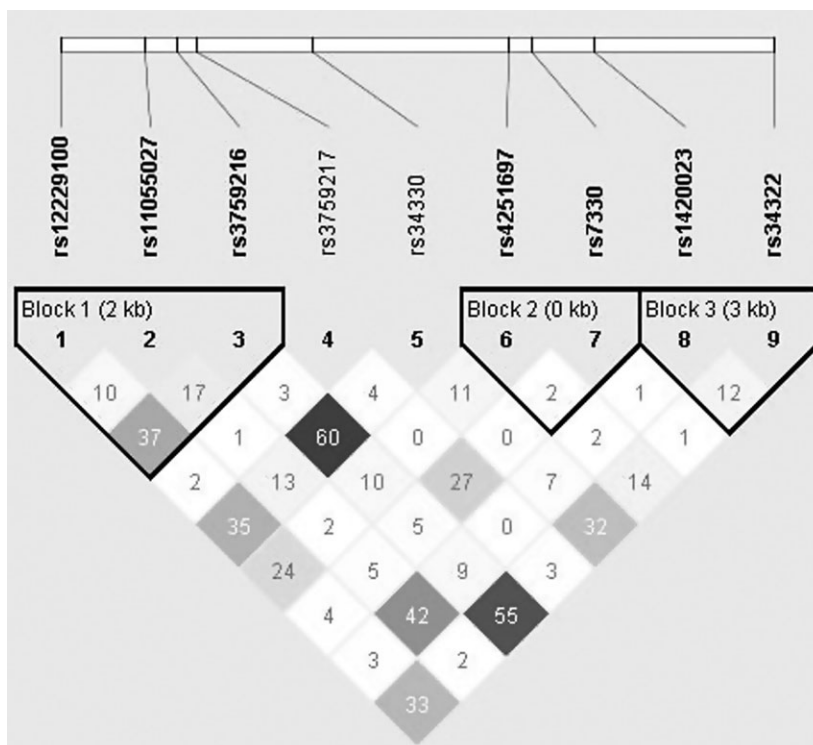


Figure 1. Pairwise r^2 values for the relations between single nucleotide polymorphisms (SNPs) in the cyclin-dependent kinase inhibitor 1B gene (*CDKN1B*). Numbers shown in diamonds are the r^2 values $\times 100$ (10 means 0.10, 1 means 0.01). The block was defined using the method of confidence intervals (28), and the boldface SNPs are selected in blocks (blocks 1, 2, and 3). kb, kilobases.

assay in human 293 cells. We generated several luciferase reporter constructs containing either the major allele or corresponding minor alleles in the promoter region of the *CDKN1B* gene. Human 293 cells were transiently transfected with these constructs. The luciferase assay results showed that the minor allele (A) of rs3759216 increased *CDKN1B* promoter activity by approximately 27% in comparison with the major allele (G) in the 293 cells (0.4906 vs. 0.3849; $P < 0.05$), while no variant of rs34330 or rs11055027 had a significant effect on *CDKN1B* promoter activity in comparison with the major allele and minor constructs (Figure 2).

Since body mass index, menopausal status, years of menstruation, and oral contraceptive use are predominant risk factors for endometrial cancer and because these factors may influence the sex hormone milieu, we also evaluated the possible modifying effect of these factors. The inverse association between GRS for *CDK6* and cancer risk appeared to be more evident among postmenopausal women and women who had not used oral contraceptives. In addition, the positive association between GRS for *CDKN1B* and cancer risk was stronger among postmenopausal women, women with more years of menstruation, women with a lower body mass index, and women who had not taken oral contraceptives. However, none of the tests for multiplicative interaction were statistically significant (data not shown).

DISCUSSION

In this population-based case-control study, we evaluated the association of 65 SNPs in 9 cell-cycle control genes with the risk of endometrial cancer. We found that 4 SNPs in the *CDKN1B* gene, 2 SNPs in the *CDK6* gene, and 1 SNP in the *CCNB1* gene were associated with the risk of endometrial cancer before adjustment for multiple comparisons. The association between the *CDKN1B* SNP rs34330 and endometrial cancer remained statistically significant even after adjustment for multiple comparisons. Our study found no evidence for an association between endometrial cancer and polymorphisms in the *CCND1*, *CCNE1*, *CDK2*, *CDK4*, *CDKN1A*, and *CDKN2A* genes.

Dysregulation of the cell cycle is a hallmark of many cancers, since control and timing of the cell cycle involves checkpoints and regulatory pathways that ensure the fidelity of DNA replication and chromosome segregation (1, 2, 29). These processes involve a large collection of key molecules, including the cyclins, CDKs, and CDK inhibitors. Therefore, genetic variations of those genes are excellent candidates for cancer susceptibility. Several studies have investigated the associations of genetic polymorphisms of the *CDKN1B* gene with cancer risk. The SNP rs34330 in *CDKN1B* has been previously related to risk of prostate cancer, particularly in men affected at ages less than 65 years ($P = 0.0015$) (20).

Table 3. Association Between Number of Risk Alleles in the *CCNB1*, *CDK6*, and *CDKN1B* Genes and Risk of Endometrial Cancer, Shanghai Endometrial Cancer Study, Shanghai, China, 1997–2003

Gene (SNP ^a) and No. of Risk Alleles	No. of Cases	No. of Controls	Odds Ratio ^b	95% Confidence Interval	P Value
<i>CCNB1</i> (rs350104 and rs2069433)					
0–2	381	392	1.0		
3	419	359	1.25	1.03, 1.52	0.02
4	97	98	1.17	0.85, 1.61	0.34
<i>P</i> for trend ^c			0.1603		
<i>CDK6</i> (rs4272, rs2282978, and rs2282983)					
0	547	500	1.0		
1	239	238	0.93	0.75, 1.16	0.54
>1	240	260	1.09	0.73, 1.63	0.66
<i>P</i> for trend			0.0570		
<i>CDKN1B</i> (rs11055027, rs3759216, rs34330, rs1420023, and rs34322)					
0	135	160	1.0		
1–2	121	121	1.24	0.87, 1.76	0.23
3–4	332	353	1.17	0.88, 1.55	0.27
5–6	283	245	1.51	1.12, 2.02	<0.01
7–8	131	100	1.61	1.13, 2.30	<0.01
9–10	20	12	2.49	1.14, 5.46	0.02
<i>P</i> for trend			0.0008		

Abbreviation: SNP, single nucleotide polymorphism.

^a Selection of SNPs for the analysis was based on a low P_{trend} value ($P < 0.10$) for that SNP in Web Table 1 (<http://aje.oxfordjournals.org>).

^b Adjusted for age at interview, body mass index, menopausal status, education, income, alcohol consumption, age at menarche, number of livebirths, and occupation.

^c Tests for trend were performed by entering categorical scores (0–2 for *CCNB1* and *CDK6* and 0–5 for *CDKN1B*) into the models as continuous parameters.

The T allele of rs34330 has also been associated with increased risk of breast cancer in Chinese women (30) and United Kingdom women (31), but this association was not found among women in western Ireland (32). The variant-

containing genotype of rs34330 (CT/TT) was significantly associated with increased risk of lung cancer (odds ratio = 1.27, 95% confidence interval: 1.01, 1.60) and was not associated with bladder cancer risk, compared with carriers of the

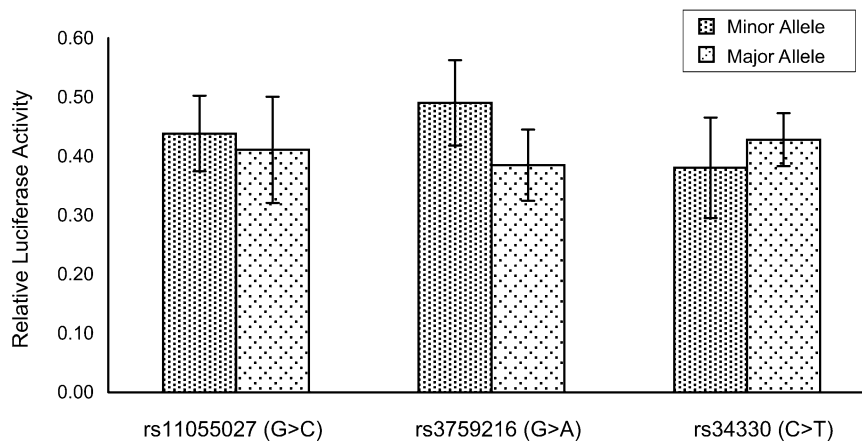


Figure 2. Effects (allele-specific difference) of the single nucleotide polymorphisms rs11055027, rs3759216, and rs34330 on cyclin-dependent kinase inhibitor 1B gene (*CDKN1B*) promoter activity. The difference in relative luciferase activity between the A and G alleles was statistically significant ($P < 0.05$) for rs3759216 (G > A). Bars, standard deviation.

homozygous wild type (18, 33). Few studies have investigated the association between rs3759216 in the *CDKN1B* gene and cancer. We found that rs3759216 was associated with reduced endometrial cancer risk. This was consistent with the finding in Spurdle et al.'s (34) study, in which this SNP was found to have a marginally significant association with breast cancer risk in carriers of *BRCA1* and *BRCA2* mutations.

The *CCNB1* gene is a key regulator of the G2/M transition of the cell cycle through formation of a complex with cell division control protein 2 homolog, which may promote progression to mitosis and augment the cellular growth rate. Overexpression of *CCNB1* has been reported in various tumor types and may be related to increased mitotic activity during malignant transformation (13, 35). In our study, we found that rs2069433 was related to a reduction in endometrial cancer risk. However, to our knowledge, there have been no investigations of associations of SNPs in the *CCNB1* gene with endometrial cancer to date. Thus, the role of this SNP in the *CCNB1* gene in endometrial tumorigenesis remains to be further studied. *CDK6*, as well as *CDK4*, has been shown to phosphorylate, and thus regulate, the activity of the tumor suppressor protein retinoblastoma protein. A previous study investigated 13 SNPs in the *CDK6* gene and did not find associations between those SNPs and breast cancer risk in the British population (31). Two groups of investigators have reported that no association was found in their studies between *CDK6* and lung and ovarian cancers, with the exception of rs8, which was associated with nonserous cancer, a histologic subtype of ovarian cancer (18, 36). Similar to the above studies, there were 32 SNPs in the *CDK6* gene reviewed in our study, and none of them was associated with endometrial cancer risk after adjustment for multiple comparisons.

Although SNPs in other cell-cycle control genes, such as *CCND1* (rs649392), *CDK2* (rs2069414), *CCNE1* (rs3218036), and *CDKN2A* (rs3731257), were found to be associated with ovarian cancer risk (21, 37) and although *CDKN2A* (rs3731239) was associated with breast cancer risk (31), we did not find any associations of these SNPs with endometrial cancer risk in our study.

We investigated the potential mechanisms underlying the associations of the *CDKN1B* SNPs with endometrial cancer risk through in vitro biochemical analyses. We found that the minor allele (A) of rs3759216 in the *CDKN1B* promoter region significantly increased luciferase activity, suggesting that rs3759216 may functionally up-regulate p27 protein expression in subpopulations carrying the A allele(s) of this SNP. This may suppress cell growth, thereby reducing risk of endometrial cancer, which is consistent with our finding that the A allele of rs3759216 was associated with reduced risk of endometrial cancer. Unlike the classic tumor suppressors that follow Knudson's "2-hit hypothesis," homozygous loss or silencing of *CDKN1B* is extremely rare in human malignancies (38–39). The p27 protein, coded by *CDKN1B*, is an atypical tumor suppressor that regulates G1/S phase transition by binding to and regulating the activity of CDKs. The p27 protein has been linked to endometrial hyperplasia and/or carcinoma, which is consistent with its role in driving cell proliferation (40). A decrease in

p27 protein level has been observed in colon, breast, lung, and other cancers (41–44). Our in vitro biochemical study data were consistent with the findings of these studies and suggest that rs3759216 may be a causal variant of endometrial cancer. These data need to be validated in other study populations. Studies are also needed to investigate the relation between the rs3759216 polymorphism and p27 expression.

Many association studies have presented inconsistent results for relations between rs34330 in the *CDKN1B* gene and the risks of breast, ovarian, lung, and prostate cancer (30–33). In a recent study, Landa et al. (45) found that rs34330 was a risk factor for developing the follicular variant of papillary thyroid carcinoma, a subtype of thyroid cancer, with an odds ratio of 2.12 ($P = 0.023$) only in a recessive model. In that study, the risk allele (T) led to a lower transcription rate in cells transfected with a luciferase reporter driven by the polymorphic p27 promoter (45). In our study, rs34330 had a highly significant association with endometrial cancer risk. However, our in vitro biochemical study did not find that this SNP had a direct effect on promoter activity and suggested that rs34330 is unlikely to be the causal SNP, but rather a genetic marker associated with endometrial cancer via its linkage disequilibrium with rs3759216 or other unidentified functional variants.

This study had several strengths, including the population-based study design, the relatively high participation rate, the homogenous ethnic background, and the low frequency of hysterectomy in the study population. The application of the haplotype-tagging SNP approach in SNP selection made it possible to systematically evaluate the genetic markers of the above genes. In addition, we applied P value adjustment methods to control for multiple comparisons and in vitro assays to verify SNP-disease associations. Although, to our knowledge, our study was one of the largest epidemiologic studies of endometrial cancer carried out to date, the sample size was still not sufficiently large to investigate interactions or SNPs with a low minor allele frequency.

In conclusion, we found that 3 SNPs in *CDKN1B* were associated with endometrial cancer risk, and these associations remained statistically or marginally significant after adjustment for multiple comparisons. We found that 1 SNP, rs3759216, was related to increasing luciferase activity and may be a causal variant of endometrial cancer. Our study suggests that genetic variations in *CDKN1B* may contribute to endometrial carcinogenesis. Studies with high coverage of genetic variants in *CDKN1B* are needed to further validate our findings.

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