Genetic polymorphisms in *CDH1* are associated with endometrial carcinoma susceptibility among Chinese Han women

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Abstract. The cadherin 1 (CDH1) gene plays critical roles in the epithelial-mesenchymal transition process, potentially offering us a glimpse into the development of endometrial carcinoma (EC). The present study aimed to identify whether genetic variants in CDH1 affect EC susceptibility in Chinese Han women, using a strategy combining haplotype-tagging single nucleotide polymorphisms (htSNPs) association analysis with fine-scale mapping. A total of 9 htSNPs in CDH1 were genotyped among 516 cases and 706 age-matched cancer-free controls. Logistic regression analyses revealed 3 htSNPs (rs17715799, rs6499199 and rs13689) to be associated with increased EC risk and 3 htSNPs (rs12185157, rs10431923 and rs4783689) with decreased EC risk. Furthermore, 14 newly imputed SNPs of CDH1 were identified to be associated with EC risk (P<0.05) using genotype imputation analysis. Notably, multivariate logistic analysis demonstrated that rs13689, rs10431923 and rs10431924 could affect EC susceptibility independently (P≤0.001). Subsequent Generalized Multifactor Dimensionality Reduction analysis revealed several best fitting models for predicting EC risk, including SNP-SNP interactions among rs7100190, rs12185157, rs10431923, rs7186053, rs6499199, rs4783689, rs13689, rs6499197 and rs10431924, and SNP-environment interactions between related SNPs and number of childbirth. Moreover, functional annotations suggest that the majority of these susceptible variants may carry potential biological functions that affect certain gene regulatory elements. In summary, this study suggested that the genetic polymorphisms

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of *CDH1* were indeed associated with EC susceptibility on several levels. If further additional functional studies could verify these findings, these genetic variants may serve as future personalized markers for the early prediction of endometrial cancer in Chinese Han women.

Introduction

As one of the most common gynecologic cancers in China, endometrial carcinoma (EC) has become a major threat to women's health, with an incidence rate of 8.56/100,000 and mortality rate of 1.94/100,000 (1). Endometrial cancer is a complex disease with various risk factors, the most common being unopposed estrogen exposure and obesity (2,3). Genetic factors also play crucial roles in the development of endometrial cancer. While some of the low-frequency, high-penetrance mutations in genes such as MLH1, MSH2, EPCAM, MSH6 or PMS2 contribute to Lynch syndrome, a hereditary cancer syndrome that increases one's risk of developing endometrial cancer and colorectal cancer (4), high-frequency, low-penetrance genetic variants such as single nucleotide polymorphisms (SNPs) are more often associated with sporadic endometrial cancers. There are two common methods to identify disease-related SNPs, genome-wide association studies (GWAS) (5) and candidate genes studies (6). The identification of potentially relevant SNPs could help us further study the occurrence, progression and prognosis of ECs on a populational basis.

Epithelial-mesenchymal transition (EMT) is an important event in tumor cell metastasis, whereby epithelial cells lose their polarity and cell-cell contacts, and shift to mesenchymal cells with a more dispersed morphology as well as an increased motility for migration and invasion (7). Endometrial cancer typically arises from the glandular epithelium, and relies heavily on the EMT process for invasion and metastasis (8). Hallmarks of EMT in EC have been widely reported, such as the levels of E-cadherin, N-cadherin, β -catenin, matrix metalloproteinases and several transcription factors (9). Thus, the cadherin 1 (*CDH1*) gene, encoding E-cadherin, is an important factor regulating the EMT process. We therefore hypothesize that SNPs in *CDH1* are possibly associated with EC susceptibility among the Chinese Han population.

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E-cadherin is a type of cell adhesion molecule within the surface of the epithelium. As a calcium ion-dependent glycoprotein, E-cadherin contributes to maintaining cell-cell and cell-matrix adherent junctions (10). Loss of E-cadherin expression can therefor lead to increased cell motility, thus imparting the EMT process, which accelerates cell invasion during tumor progression. In fact, downregulation of E-cadherin has been found to correlate with EC via CDH1 mutation (11). However, germline mutations are relatively rare and tend to be associated with familiar cancers. SNPs on the other hand, being much more common genetic variants, could serve as better indicators of sporadic cancers in future genetic screening and disease prediction. So far in CDH1, SNPs such as rs13689, rs2059254 and rs12919719 have been found to be associated with breast cancer susceptibility in a Chinese population (12), while other SNPs have been associated with endometriosis susceptibility; could potentially affect the clinical outcome of epithelial ovarian cancer, etc (13,14). However, no studies have yet been reported concerning the association studies of CDH1 SNPs with EC risk among Chinese Han women.

We conducted candidate genes studies, but analyzing all known SNPs in the target gene would be too costly and time-consuming. A more common strategy, based on linkage disequilibrium (LD), is to find haplotype-tagging SNPs (htSNPs), which by definition are a small subset of SNPs capable of capturing the full information of haplotypes (15). This strategy can greatly reduce the expense and scale of the genotyping process, and has been widely used in population-based association studies. In this study, we picked out the htSNPs in CDH1 and comprehensively analyzed the associations between their genetic polymorphisms and EC susceptibility in a Chinese Han population, followed by genotype imputation to fine-map more SNPs that may also be relevant. Functional annotation was also conducted using various bioinformatic tools to predict the functional characteristics of potential causal variants. In the end, we demonstrated that several SNPs in CDH1 may modulate endometrial cancer susceptibility.

Materials and methods

Study population. This case-control study included 516 cases of endometrial adenocarcinoma from Peking University Third Hospital, Beijing Cancer Hospital and Beijing Hospital between 1999 and 2011, all of whom were Chinese Han women with definite pathological diagnoses. Patients who had previous histories of cancer, metastasized cancer originated from other organs, and those who had been treated with radiotherapy or chemotherapy were excluded from the study. A total of 706 controls were from Chinese Han women who participated in a community-based screening program for non-infectious diseases in Beijing, with no history of cancer. The case and control groups were age-matched, and epidemiological information was collected for both groups from clinical records or questionnaires, including: Age, body mass index (BMI), age at menarche, age at menopause, age at first full-term pregnancy (FFTP), number of child birth, smoking history, and family history of cancer in first-degree relatives. The study was approved by the Ethics Committee of Peking University Health Science Center.

SNP selection. Haplotype-tagging SNPs were selected according to the HapMap database (2009-02-06: HapMap Data Release no. 27; CHB (Chinese Beijing) population) using Haploview v.4.2 software. Specific methods and selection criteria have been described in previous research (15-19). For *CDH1*, we identified 10 htSNPs (rs7200690, rs12185157, rs7198799, rs17715799, rs2011779, rs10431923, rs7186053, rs6499199, rs4783689 and rs13689) in the *CDH1* locus (2 kb upstream to 2 kb downstream). As rs2011779 was failed to be directly genotyped in our lab, only the other 9 htSNPs remained to be analyzed in the following study.

DNA isolation and genotyping assay. For the EC cases, genomic DNAs were extracted from archived formalin-fixed paraffin-embedded (FFPE) blocks of non-tumor tissues. For the control group, genomic DNAs were extracted from blood leukocytes. Conventional proteinase K digestion, phenol-chloroform extraction and ethanol precipitation were performed to prepare genomic DNA. Genotyping of all htSNPs were conducted using the ABI 7900HT® Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). TaqMan[®] assay was performed in compliance with the manufacturer's instructions. Primer and probes were supplied by Applied Biosystems and PCR conditions were the same as described by Ruan et al (20). For quality control, positive and negative controls were included in each genotyping plate, and 3% of the samples were repeatedly genotyped, with a concordance rate of more than 99% between the duplicates.

LD block determination and haplotype construction. Lewontin coefficient (D') and squared correlation coefficient (r^2) between the genotyped SNPs in *CDH1* (in cases and controls respectively) were calculated using the Haploview v4.2 software, along with the construction of LD plots and haplotype blocks. The most probable haplotypes for each individual were estimated according to expectation-maximization (EM) algorithm using the SAS 9.1 PROC HAPLOTYPE procedure (21).

Genotype imputation. Genotype imputation serves as an in-silico method to predict missing genotypes of variants that are not directly assayed in existing case-control studies (22,23). Thus, to find out more variants potentially related to EC risk, we performed genotype imputation using the MACH software (24), with reference haplotypes in *CDH1* (spanning from 5 kb upstream to 5 kb downstream) obtained from the CHB population in the 1000 Genomes Project. The imputation helper module in GenGen software tools (http://gengen.openbioinformatics.org/en/latest/) was later applied to convert MACH output files to appropriate formats that could be directly used in subsequent association analyses. Finally, the well-imputed variants were analyzed for their allelic associations with EC risk using the PLINK v1.07 software (25).

Statistical analysis. The epidemiological characteristics between cases and controls were compared using Pearson's χ^2 test or Student's t-test. For each htSNP, Hardy-Weinberg equilibrium was assessed using one-degree of freedom goodness-of-fit test based on genotypes of the controls. Two-sided χ^2 test was conducted to compare the distributions of alleles and genotypes between two groups, with each genotype categorized according to three models: Codominant, dominant and recessive. Cochran-Armitage trend test was also conducted in order to predict the effect of allele dose in each SNP on its association with EC risk. Univariate and multivariate logistic regression models were carried out to estimate the effect of genotypes on EC susceptibility through calculating odd ratios (ORs) and 95% confidence intervals (95% CIs), including unadjusted models as well as models adjusted for BMI, age at menarche, menopause status, age at FFTP, number of birth, and family history of cancer in first-degree relatives.

In order to identify higher-order interactions associated with EC risk, generalized multifactor dimensionality reduction (GMDR) method (GMDR software Beta 0.9) was applied to analyze SNP-SNP and SNP-environment interactions. Originated from the MDR method, GMDR has several advantages: permitting adjustment for covariates, being able to handle both dichotomous and quantitative phenotypes, and applicable to multiple types of population-based study designs (26). During the analysis, cross-validation and/or permutation testing could be applied to evaluate the significance of the models, and the best candidate model with the maximum testing accuracy and/or cross-validation consistency (CVC) could be selected (27). In our study, SNP-SNP interactions were analyzed for 1 to 8-factor models, including 9 htSNPs and 14 newly imputed SNPs that were found to be associated with EC risk during genotype imputation analysis. SNP-environment interactions were also examined for 1-to-8-factor models, including the above 23 SNPs along with age, BMI, family history of cancer in first-degree relatives, number of birth. Both analyses were adjusted for covariates including age, BMI, family history of cancer in first-degree relatives, age at menarche, menopause status, and number of birth.

Functional annotation. In order to further predict the potential functional characteristics of the susceptible SNPs, and to explore the roles they might play in the development of EC, each variant was being functionally annotated via publicly available bioinformatic databases or annotation software. To analyze the correlation between selected variants and mRNA expression levels of corresponding genes, expression Quantitative Trait Loci (eQTL) information was extracted from GTEx Portal (28) (https://gtexportal.org/home/), which could present eQTL results of certain SNPs in various tissues or cell lines. By using HaploReg v4.1 (29) (http://www.broadinstitute. org/mammals/haploreg/haploreg.php) and rSNPBase (30) (http://rsnp.psych.ac.cn/), SNPs were comprehensively and reliably annotated, focusing on DNase I hypersensitivity, histone modification, transcriptional factor binding and motif alteration, as well as eQTL results. UCSC Genome Browser (31) (http://genome.ucsc.edu) was applied to analyze the potential functions of SNPs (especially transcriptional factor ChIP-seq data) in mixed EC cells (ECC-1) (32), which may indicate the particular roles of these variants in endometrial cancer.

Results

Characteristics of the study population. The demographic and epidemiological characteristics of the 516 EC cases and 706 cancer-free controls are presented in Table I. The two groups

were adequately matched in age (P=0.7810). There was no significant difference in smoking history (P=0.6177) between cases and controls, but cases had higher BMIs (P<0.0001) than controls. Moreover, cases more likely had an earlier menarche (P<0.0001), later menopause (P=0.0002), with a lower proportion of individuals in postmenopausal status (P<0.0001), and a younger age at first full-term pregnancy (FFTP) (P=0.0493). In addition, there were also statistical significant differences in the number of childbirth (P<0.0001). The above variables with significant difference were taken into account in the subsequent multivariate logistic regression models to adjust for any possible confounding bias.

Associations between htSNP genotypes and BC susceptibility. All 9 htSNPs were in conformation with Hardy-Weinburg Equilibrium (P>0.05) in the control group (data not shown). Allele and genotype distributions of the 9 htSNPs are shown in Table II. Two-sided χ^2 test revealed significant differences in allele frequencies between cases and controls for rs17715799, rs10431923, rs6499199, rs4783689 and rs13689. Both univariate and multivariate unconditional logistic regression analyses indicated that the genotypes of 6 htSNPs were related with endometrial cancer susceptibility. The genotypes of SNPs rs17715799 (A>T), rs6499199 (C>T) and rs13689 (T>C) were significantly associated with increased endometrial cancer susceptibility (Table I). On the other hand, the genotypes of SNPs rs12185157 (A>G), rs10431923 (T>G) and rs4783689 (C>T) were significantly associated with decreased EC susceptibility (Table II).

Fine-scale genetic mapping of CDH1 by genotype imputation. Based on the 1000 Genomes dataset (Chinese Han Beijing population), there are 381 SNPs with a minor allele frequency (MAF) >1% in CDH1 gene. To identify more SNPs potentially related to EC risk, we performed genotype imputation using the MACH software. By using our directly genotyped data of the 9 htSNPs as well as reference haplotypes of *CDH1* obtained from the 1000 Genomes Project (CHB population), the genotypes of 96 SNPs were well-imputed in cases and controls. Using allelic association tests in PLINK software, the minor alleles of 19 SNPs in *CDH1* were identified to be significantly associated with endometrial cancer susceptibility (P<0.05; Table III), including htSNPs rs17715799, rs10431923, rs6499199, rs4783689 and rs13689, which are consistent with our above htSNP analysis (Table II).

Multivariate logistic analysis to identify independently associated SNPs. In order to seek out the SNPs independently associated with EC susceptibility, we performed multiple logistic regression analysis, which included the 6 susceptible htSNPs and 14 newly imputed SNPs that affected EC risk during genotype imputation analysis. However, only 9 SNPs remained in the multiple logistic regression model, including the 6 susceptible htSNPs (rs12185157, rs17715799, rs10431923, 6499199, rs4783689, rs13689) and 3 susceptible imputed-SNPs (rs6499197, rs10431924, rs1801026), while the other 11 susceptible imputed-SNPs dropped out of the model due to their linear relationships with the other SNPs. We then narrowed our analysis down on the 9 SNPs to further look for SNPs that affected EC susceptibility independently.

Variables	Case, n=516	Control, n=706	P-value
Age, years (mean ± SD)	56.09±10.47	55.95±6.56	0.7810
Age, years, n (%)			0.1327
<55	229 (44.38)	344 (48.73)	
≥55	287 (55.62)	362 (51.27)	
BMI (mean ± SD)	25.85±4.11	24.92±3.17	<0.0001ª
Age at menarche, years (mean \pm SD)	14.76±1.84	15.58±1.94	<0.0001ª
Age at menopause, years (mean \pm SD)	50.51±3.52	49.61±3.53	0.0002^{a}
Age at FFTP, years (mean \pm SD)	25.17±3.36	25.54±2.95	0.0493ª
Menopause status, n (%)			<0.0001 ^a
Premenopause	185 (35.85)	122 (17.28)	
Postmenopause	331 (64.15)	584 (82.72)	
No. of childbirth, n (%)			<0.0001 ^a
0	60 (11.63)	8 (1.13)	
1	186 (36.05)	364 (51.56)	
≥2	270 (52.33)	334 (47.31)	
Family history of cancer in first-degree relatives, n (%)			0.0451ª
Yes	82 (15.89)	144 (20.40)	
No	434 (84.11)	562 (79.60)	
Smoking history, n (%)			0.6177
Yes	19 (3.68)	30 (4.25)	
No	497 (96.32)	676 (95.75)	
^a P<0.05 BMI Body mass index: SD standard deviation: FFTP firs	t full-term pregnancy		

After adjusting for the other SNPs and confounding factors, rs13689 became much more significant in increasing EC risk (aOR=2.87, 95% CI=1.53-5.37, P=0.0010), and rs10431923 as well as rs10431924 became much more significant in reducing EC risk (rs10431923: aOR=0.04, 95% CI=0.02-0.10, P<0.0001; rs10431924: aOR=0.14, 95% CI=0.07-0.26, P<0.0001), whereas the statistical significance for the other SNPs disappeared.

Association between high-order interactions and endometrial cancer risk by GMDR analysis. GMDR is a nonparametric and genetic model-free substitute for linear or logistic regression, serving to capture and depict nonlinear interactions among genetic and environmental factors (33). The interactions between SNP-SNP and SNP-environment are analyzed by GMDR and presented in Table IV. For SNP-SNP interaction, we analyzed all 9 htSNPs and 14 imputed susceptible SNPs for 1-to-8-factors models. After adjusting for covariates, it was indicated that the best one-factor model for predicting EC risk was rs10431923. It carried a testing balanced accuracy of 0.6088 and CVC of 10/10, suggesting that this htSNP may be the primary factor contributing to EC risk among the total 23 SNPs. Among multi-factor models, the one with the highest cross-validation consistency (CVC) was a 7-factor model harboring rs7200690, rs12185157, rs10431923, rs7186053, rs13689, rs6499197 and rs10431924 (testing balanced accuracy=0.8372, CVC=10/10), showing strong synergetic interactions among the 7 SNPs.

As for SNP-environment interaction, after adjusting for covariates, the best six-factor model (including rs7200690, rs12185157, rs10431923, rs13689, rs10431924 and number of childbirth) came to our notice as the model with the highest CVC (10/10) and highest testing balanced accuracy (0.8299; Table IV). Together with two other multi-factor models which also included number of childbirth as a factor, it suggested that in accordance with our knowledge that endometrial cancer is the comprehensive result of both genetic and environmental factors. Genetic variants aside, an individual's number of birth also serves as an important epidemiological factor affecting her chance of developing endometrial cancer.

Functional annotation. According to our aforementioned results, 5 htSNPs, namely rs12185157, rs10431923, rs6499199, rs4783689 and rs13689, were found to be associated with EC susceptibility both in single SNP association analysis and GMDR models. Among the 14 newly imputed SNPs associated with EC risk, rs6499197 and rs10431924 were considered to be relatively important since they were included in some GMDR best models. To identify the possible effects of these 7 SNPs on relevant gene expression, expression Quantitative Trait Loci (eQTL) information of the SNPs was extracted from GTEx Portal and HaploReg, and their summarization can be seen in Table V. Among the 7 SNPs, 6 were found to be associated with the RNA expression level of *CDH1* or other nearby genes in various tissues

Table II. Genotype and allele frequencies of the htSNPs in CDH1 and their associations with EC susceptibility.

SNPs	Genotype	Cases (%)	Controls (%)	P-value ^a	P-value ^b	P _{trend}	OR (95% CI)	P-value	OR (95% CI) ^c	P-value ^c
rs7200690	CC	344	435	0.1924		0.0862	Reference		Reference	
	СТ	(38.137) 147 (28.49)	231 (32 72)				0.81	0.0891	0.83	0.1707
	TT	25	(52:72) 40 (5.67)				0.79	0.3745	0.79	0.4322
	T 11.1.	(4.04)	(3.07)		0.0770		(0.47-1.55)		(0.44 - 1.42)	
		197	311		0.0772					
	frequency	(19.09)	(22.03)				0.80	0.0608	0.82	0 1373
	CI/II vs.						(0.62, 1.02)	0.0098	0.02	0.1373
	CC (D)						(0.63-1.02)	0.5001	(0.64-1.06)	0.5546
	11 vs. C1/						0.85	0.5281	0.84	0.5546
	CC (R)						(0.51-1.42)		(0.47-1.50)	
rs12185157	AA	150 (29.07)	185 (26.20)	0.1495		0.0433ª	Reference		Reference	
	AG	257	339				0.92	0.5444	0.91	0.5267
		(49.81)	(48.02)				(0.70-1.21)		(0.68-1.22)	
	GG	109	182				0.72	0.0401 ^d	0.67	0.0209 ^d
		(21.12)	(25.78)				(0.52-0.99)		(0.47-0.94)	
	G allele	475	703		0.0661					
	frequency AG/GG vs.	(46.03)	(49.79)				0.85	0.2017	0.82	0.162
	AA(D)						(0.66-1.09)		(0.63-1.08)	
	GG vs.						0.75	0.0405^{d}	0.71	0.0192 ^d
	AG/AA(R)						(0.58-0.99)		(0.53-0.95)	
rs7198799	CC	368	508	0.9659		0.8385	Reference		Reference	
		(71.32)	(71.95)							
	СГ	135	180				1.04	0.7936	1.13	0.3898
		(26.16)	(25.50)				(0.80-1.34)		(0.85-1.50)	0.0-01
	11	13	18				1.00	0.9935	0.94	0.8791
	T 11 1	(2.52)	(2.55)		0.0275		(0.48-2.06)		(0.42-2.12)	
		161	216		0.8375					
	CT/TT vs.	(15.00)	(15.30)				1.03	0.807	1.11	0.438
	CC (D)						(0.80-1.33)	0.0726	(0.85-1.46)	0.0175
	11 vs. C1/						0.99	0.9736	0.91	0.8175
m 17715700		200	157	0 0074d		0 0021d	(0.48-2.04) Deference		(0.40-2.03)	
181//13/99	AA	(58.14)	(64.72)	0.0074		0.0031	Reference		Kelelelice	
	۸T	(36.14)	(04.75)				1 21	0 1264	1.25	0.0002
	AI	(33, 33)	(30.50)				(0.05, 1.55)	0.1204	(0.96.1.63)	0.0993
	ТТ	(33.33)	33				2.03	0 0034d	2.06	0 005 ^d
	11	(8 53)	(4.67)				(1.26-3.26)	0.0054	$(1\ 24-3\ 41)$	0.005
	T allele	260	282		0.0021 ^d		(1.20 5.20)		(1.2+ 5.+1)	
	frequency	(25.19)	(19.97)		0.0021					
	AT/TT vs	(23.17)	(15157)				1 32	0 0192 ^d	1 36	0 0163 ^d
	AA (D)						(1.05-1.67)	0.01/2	(1.06-1.75)	0.0100
	TT vs.						1.90	0.0069^{d}	1.91	0.0107 ^d
	AT/AA(R)						(1.19-3.03)		(1.16-3.13)	
rs10431923	TT	217 (42.05)	214 (30.31)	0.0001 ^d		<0.0001 ^d	Reference		Reference	

Table II. Continued.

SNPs	Genotype	Cases (%)	Controls (%)	P-value ^a	P-value ^b	P _{trend}	OR (95% CI)	P-value	OR (95% CI) ^c	P-value ^c
	GT	217 (42.05)	348 (49.29)				0.41	<0.0001 ^d	0.42	<0.0001 ^d
	GG	(+2.0 <i>5</i>) 82	(49.29)				0.25	<0.0001 ^d	0.24	<0.0001 ^d
	00	(15.89)	(20.40)				(0.18-0.35)	<0.0001	(0.17_0.35)	<0.0001
	Gallele	381	636		~0.0001 ^d		(0.10 0.55)		(0.17 0.55)	
	frequency	(36.92)	(45.04)		<0.0001					
	GT/GG vs	(30.72)	(+0.0+)				0.35	<0.0001d	0.35	<0.0001d
	TT(D)						(0.27, 0.46)	<0.0001	(0.27, 0.46)	<0.0001
	$\Gamma \Gamma (D)$						(0.27-0.40)	-0 0001d	(0.27-0.40)	-0 0001d
	GG VS.						0.45	<0.0001*	0.41	<0.0001
710(052	GI/II (R)	0(1	220	0.4624		0.000	(0.33-0.58)		(0.30-0.56)	
rs/186053	GG	261	332	0.4634		0.236	Reference		Reference	
		(50.58)	(47.03)							
	AG	210	306				0.87	0.2653	0.90	0.416
		(40.70)	(43.34)				(0.69-1.11)		(0.70-1.16)	
	AA	45	68				0.84	0.4104	0.77	0.255
		(8.72)	(9.63)				(0.56-1.27)		(0.49-1.21)	
	A allele	300	442		0.2356					
	frequency	(29.07)	(31.30)							
	AG/AA vs.						0.87	0.2194	0.88	0.286
	GG (D)						(0.69-1.09)	0.5054	(0.69-1.12)	0.0006
	AA vs.						0.90	0.5874	0.81	0.3396
(100100	AG/GG (R)	267	525	0.0001		0.001	(0.60-1.33)		(0.53-1.25)	
rs6499199	CC	367	535	<0.0001		0.001	Reference		Reference	
	CT	(71.12)	(75.78)				1.02	0.0050	0.00	0 0000
	CI	(21.71)	100				1.02	0.8850	0.90	0.8008
	TT	(21.71)	(22.00)				(0.77-1.34)	-0.0001	(0.72-1.29)	-0.0001
	11	57 (7.17)	(1.56)				(2, 47, 9, 74)	<0.0001	(2, 21, 0, 18)	<0.0001
	T allele	(7.17)	(1.50)		0.0005		(2.47-9.74)		(2.21-9.10)	
	frequency	(18.02)	(12.89)		0.0005					
	CT/TT vs	(10.02)	(12.07)				1 27	0.0678	1.20	0 197
	CC(D)						(0.98-1.64)	0.0070	(0.91-1.58)	0.177
	TT vs						4 88	<0.0001	4 54	<0.0001
	CT/CC(R)						(2.47-9.66)	0.0001	(2.23-9.23)	(010001
rs4783689	CC	283	321	0.0041		0.0012	Reference		Reference	
		(54.84)	(45.47)							
	СТ	189	303				0.71	0.0051	0.72	0.0117
		(36.63)	(42.92)				(0.56-0.90)		(0.55-0.93)	
	TT	44	82				0.61	0.0149	0.63	0.0329
		(8.53)	(11.61)				(0.41-0.91)		(0.41-0.96)	
	T allele	277	467		0.0009					
	frequency	(26.84)	(33.07)							
	CT/TT vs.						0.69	0.0012	0.70	0.0038
	CC (D)						(0.55-0.86)		(0.55-0.89)	
	TT vs.						0.71	0.0807	0.73	0.1292
	CT/CC (R)						(0.48-1.04)		(0.48-1.10)	
rs13689	TT	247	467	< 0.0001		<0.0001	Reference		Reference	
	_	(47.87)	(66.15)							
	CT	207	218				1.80	< 0.0001	1.71	< 0.0001
		(40.12)	(30.88)				(1.41-2.29)		(1.32-2.27)	

SNPs	Genotype	Cases (%)	Controls (%)	P-value ^a	P-value ^b	$\mathbf{P}_{\mathrm{trend}}$	OR (95% CI)	P-value	OR (95% CI) ^c	P-value ^c
	CC	62 (12.02)	21 (2.97)				5.58 (3.32-9.37)	<0.0001	5.16 (2.97-8.98)	<0.0001
	C allele frequency	331 (32.07)	260 (18.41)		<0.0001		. ,			
	CT/CC vs. TT (D)						2.13 (1.69-2.69)	<0.0001	2.01 (1.57-2.58)	<0.0001
	CC vs. CT/TT (R)						4.46 (2.68-7.41)	<0.0001	4.20 (2.44-7.23)	<0.0001

Table II. Continued.

^aTwo-sided χ^2 test for difference in frequency distribution of genotypes between cases and controls. ^bTwo-sided χ^2 test for difference in frequency distribution of alleles between cases and controls. ^cAdjusted for BMI, age at menarche, age of first birth, number of childbirth, menopause status and family history of cancer in first-degree relatives. ^dP<0.05. BMI, body mass index; OR, odds ratio; CI, confidence interval; htSNPs, haplotype-tagging single nucleotide polymorphisms; *CDH1*, cadherin 1; EC, endometrial carcinoma; D, dominant; R, recessive.

Table III. Well-imputed SNPs associated with E	C susceptibility in CDH.	<i>l</i> (P<0.05) by genot	type imputation
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SNP	Position	P-value ^a	OR (95% CI)	Rsq ^b
rs12599393	Chr16: 68829021	0.02208	1.26 (1.03-1.54)	0.9573
rs6499197	Chr16: 68830473	0.01723	1.28 (1.05-1.58)	0.8524
rs17715799°	Chr16: 68830511	0.002255	1.35 (1.11-1.64)	0.9853
rs8063605	Chr16: 68836665	0.03434	0.64 (0.43-0.97)	0.7150
rs10431923°	Chr16: 68839263	0.003279	0.78 (0.66-0.92)	0.9816
rs10431924	Chr16: 68839302	0.006005	0.78 (0.66-0.93)	0.9296
rs6499199°	Chr16: 68849837	0.0005845	1.48 (1.18-1.85)	0.9903
rs8057342	Chr16: 68849904	0.0007468	1.47 (1.17-1.84)	0.9753
rs34022452	Chr16: 68850384	0.0007224	1.48 (1.18-1.85)	0.9606
rs36029373	Chr16: 68850406	0.004227	1.40 (1.11-1.76)	0.9114
rs8050039	Chr16: 68852074	0.03827	1.42 (1.02-1.97)	0.7898
rs138957735	Chr16: 68852748	0.02279	1.48 (1.05-2.07)	0.7578
rs4783689°	Chr16: 68853671	0.0009422	0.74 (0.62-0.89)	0.9961
rs76685922	Chr16: 68854135	0.009579	1.95 (1.17-3.26)	0.7033
rs34635465	Chr16: 68854703	0.001848	2.33 (1.35-4.01)	0.6781
rs10500544	Chr16: 68855064	0.0102	2.63 (1.22-5.66)	0.6625
rs1801026	Chr16: 68867456	6.71x10 ⁻¹⁵	2.09 (1.73-2.52)	0.9622
rs13689°	Chr16: 68868522	6.71x10 ⁻¹⁵	2.09 (1.73-2.52)	0.9945
rs17690554	Chr16: 68869510	6.71x10 ⁻¹⁵	2.09 (1.73-2.52)	0.9425

^aP-values are calculated using allelic association tests in PLINK v1.07 software to examine the associations of SNPs with EC susceptibility. ^bRsq is a common parameter for measuring imputation quality. In MACH software, we chose Rsq>0.3 as the threshold to drop poorly imputed SNPs. ^chtSNPs in our case-control study. OR, odds ratio; CI, confidence interval; SNPs, single nucleotide polymorphisms; *CDH1*, cadherin 1; EC, endometrial carcinoma; Rsq, r-square; Chr, chromosome.

or cell lines (P<0.05). Notably, individuals with minor allele homozygotes of one important protective locus, rs10431923 (T>G), presented a higher mRNA level of *CDH1* than other genotype carriers (P=1.73x10⁻⁶, Fig. 1). Likewise, people carrying minor allele homozygotes of one newly imputed risk locus, rs6499197 (A>G), showed a lower mRNA level of *CDH1* than other genotype carriers (P=1.49x10⁻⁸; Fig. 1). These results indicate that the above 6 SNP may influence endometrial cancer susceptibility through regulating gene expression, and thus may carry potential biological functions.

To further predict the potential functions of these SNPs, multiple publicly available bioinformatic databases or annotation software were used. The majority of the SNPs could affect

Best models ^a Training balan accuracy ^b	iced Testing balanced accuracy ^b	Sign test (P) ^b	Cross-validation consistency ^b
SNP-SNP			
X5 0.6083	0.6088	0.0107	10/10
X5 X13 0.7688	0.7698	0.0010	10/10
X5 X9 X13 0.7959	0.7887	0.0010	10/10
X2 X5 X9 X13 0.8188	0.8020	0.0010	10/10
X1 X2 X5 X9 X13 0.8412	0.8182	0.0010	10/10
X1 X2 X5 X9 X11 X13 0.8599	0.8147	0.0010	6/10
X1 X2 X5 X6 X9 X11 X13 ^c 0.8788	0.8372	0.0010	10/10
X1 X2 X5 X6 X7 X8 X9 X11 0.8927	0.8159	0.0010	10/10
SNP-environment			
X5 0.6083	0.6088	0.0107	10/10
X5 X13 0.7688	0.7698	0.0010	10/10
X5 X9 X13 0.7964	0.7796	0.0010	8/10
X5 X9 X13 Nbirth 0.8230	0.8077	0.0010	9/10
X2 X5 X9 X13 Nbirth 0.8447	0.8152	0.0010	8/10
X1 X2 X5 X9 X13 Nbirth ^c 0.8685	0.8299	0.0010	10/10
X1 X2 X5 X9 X11 X13 Nbirth 0.8851	0.8201	0.0010	5/10
X1 X2 X5 X6 X9 X11 X13 Nbirth 0.9010	0.8061	0.0010	5/10

Table IV. Comparison of the models identified by GMDR for SNP-SNP and SNP-environment interactions.

^aX1, X2, X5, X6, X7, X8, X9, X11 and X13 represents rs7200190, rs12185157, rs10431923, rs7186053, rs6499199, rs4783689, rs13689, rs6499197 and rs10431924 respectively. Nbirth represents number of childbirth. ^bAdjusted for age, BMI, age at menarche, menopause status, number of childbirth and family history of cancer in first-degree relatives. ^cBest models. BMI, body mass index; SNPs, single nucleotide polymorphisms; GMDR, Generalized Multifactor Dimensionality Reduction.

gene regulatory elements like DNase I hypersensitivity, histone modifications including methylation and acetylation, as well as transcriptional factor binding sites. In the EC cell line ECC-1, which was identified as mixed EC cells (32), all selected SNPs were found to locate in transcriptional factor binding regions (Table V). The functional annotations suggest that these SNPs may affect its binding with transcriptional factors, and affect gene's expression.

Discussion

To our knowledge, this is the first gene-wide association study of CDH1 to comprehensively illustrate the relation between SNPs and endometrial cancer susceptibility in Chinese Han women. We first identified 6 single htSNPs (rs12185157, rs10431923, rs4783689, rs17715799, rs6499199 and rs13689) in CDH1 as susceptible SNPs associated with EC risk, followed by genotype imputation analysis with fine-mapping of the high-density SNPs within CDH1's target region, where 14 SNPs were also identified as possibly associated with EC risk. Among all susceptible SNPs, CDH1 rs10431923, rs10431924 and rs13689 remained to affect EC susceptibility after multiple logistic regression analyses. To identify higher-order interactions associated with EC risk, GMDR analyses were conducted, showing that 7 htSNPs and 2 newly imputed SNPs may be involved in SNP-SNP interaction or SNP-environment interaction in endometrial cancer. Subsequent functional annotations for these susceptible SNPs were carried out, suggesting their considerable biological functions underlying the associations that await future studies.

Our study results show that rs10431923 (G>T) was the single most important independent protective factor for endometrial cancer susceptibility in Chinese Han Women. It yielded an aOR of 0.35 (dominant model) in the logistic regression, and still served as a strong protective factor after adjusting for the other susceptible SNPs in the multiple logistic regression. Moreover, in our GMDR analysis, rs10431923 (G>T) was shown to be the best one-factor model for predicting EC susceptibility, and was involved in almost all other best fitting multi-factor models. Consistent to our findings of a protective effect with GG genotype, rs10431923 (T>G) was previously found to be related to Crohn's disease in a North American population, with its TT genotype related to abnormal aggregation of E-cadherin in epithelial cells resulting in its impaired plasma membrane localization (34). Another study found no association between rs10431923 with colorectal cancer risk (35), but due to small sample size and Italian population, their results might not be generalizable to Chinese Han Women. rs10431923 also showed considerable functional potentials, where it may alter the mRNA expression of CDH1, and was located in TF-binding regions (such as Egr-1, Max, p300, YY1) with significant binding signals in the endometrial cancer cells. This result indicates that the variation of rs10431923 may influence the expression of CDH1 through modifications of the transcription process, thus affecting E-cadherin levels and the EMT process in

				eQTL ^c information		
SNP	Type of SNP ^a	MAF	TF binding in EC cells ^b	Tissue	Correlated gene	P-value
rs12185157	htSNP	0.46	CREB1, FOXM1, p300	EBV-transformed lymphocytes	CDH1	6.99x10 ⁻⁶
rs10431923	htSNP	0.41	CEBPG, Egr-1, Max, p300, TAF1, YY1, ZBTB7A	Spleen	CDH1	1.73x10 ⁻⁶
rs4783689	htSNP	0.31	CREB1, Egr-1, Max, RAD21, TAF1	Esophagus Mucosa	FTLP14	2.87x10 ⁻¹⁴
rs6499199	htSNP	0.17	FOXM1, SRF, TEAD4	Testis	CTD-2033A16.2	1.92x10 ⁻⁵
rs13689	htSNP	0.15	CREB1, YY1, SRF, USF-1	-	-	-
rs6499197	Imputed SNP	0.45	CEBPB, Egr-1, FOXM1, Max, TAF1, TCF12, ZBTB7A	Spleen	CDH1	1.49x10 ⁻⁸
rs10431924	Imputed SNP	0.42	Egr-1, Max, NFIC, p300, SRF, TEAD-4, USF-1, YY1, ZBTB7A	Spleen	CDH1	5.3x10 ⁻⁵

Table V. Functional annotation of selected susceptible SNPs.

^aSusceptible htSNPs (presented with statistical significance in multivariate analysis and also involved in one of the GMDR models) and selected susceptible imputed SNPs (those with P-value <0.05 and were also in one of the GMDR models) were included. ^bData extracted from HaploReg and UCSC Genome Browser, showing binding signals of SNPs with transcriptional factors in ECC-1 cell line, mixed EC cells (33). ^cAll data were collected from existing GTEx Portal database or HaploReg v4.1. '-' indicate no existing eQTL data available for the SNP yet. htSNPs, haplotype-tagging single nucleotide polymorphisms; GMDR, Generalized Multifactor Dimensionality Reduction; MAF, minor allele frequency; EC, endometrial carcinoma; TF, transcription factor; EBV, Epstein-Barr virus.



Figure 1. Association of rs10431923 and rs6499197 with the expression of *CDH1* by eQTL information in GTEx Portal. The eQTL box plots were downloaded from GTEx Portal (Data Source: GTEx Analysis Release V6p) with minor modifications on font size. For rs10431923 (T>G), 'Homo Ref' indicates TT genotype, 'Het' indicates TG genotype, and 'Homo Alt' indicates GG genotype. For rs6499197 (A>G), 'Homo Ref' indicates AA genotype, 'Het' indicates AG genotype.

endometrial cancer. Therefore, rs10431923 was considered the most significant protective locus of EC susceptibility, and its biological functions in the occurrence and progression of EC call for intensive future studies.

Other protective htSNPs in *CDH1*, such as rs12185157 (G>A) and rs4783689 (C>T), were also significantly associated with EC risk in various statistical models. In accordance with previous discoveries, rs12185157 was a part of a three-SNPs

diplotype associated with breast cancer susceptibility in Chinese Han women (16). It was also involved in 6 best-fitting gene-gene models and 5 best-fitting gene-environment models in our GMDR analysis, suggesting its interaction with various susceptible SNPs and environmental risk factors for EC. The other protective htSNP, rs4783689, was found to be associated with a higher risk of endometriosis for C allele carriers in a Japanese population (36). No existing studies have yet revealed the associations between these SNPs and endometrial cancer susceptibility. Functional annotations also suggested their functional potentials, with rs12185157 located in histone modifications regions in cervical carcinoma cells, and was able to change the TF binding motif USF2. It was also located in the TF-binding site of ZEB1, a key activator for the EMT process and metastasis (37), suggesting its potential role in regulating *CDH1* expression in endometrial cancer.

Several risk htSNPs were also discovered to be associated with increased EC risk, among which rs13689 (T>C) stood out as the most important risk SNP. With an aOR of 4.20 (recessive model) and an aOR of 2.87 after adjusting for the other susceptible SNPs, rs13689 was considered a strong independent risk locus for EC risk. It was presented in almost all the best fitting multi-factor models in our GMDR analyses, indicating its vital role in the interaction among SNPs and (or) environmental factors. rs13689 was previously identified as a significant risk factor for breast cancer susceptibility in Chinese Han Population (16), echoing our findings in EC. Functional annotation indicated its location in the 3'-UTR of *CDH1*, suggesting potentials in stabilizing mRNA through miRNAs. It also showed functional potential in regulating some TF factors (CREB1, YY1, SRF, USF-1) in the ECC-1 cell line, and could possibly interact with other genes like ZFP90 and TANGO6 via chromatin loops.

To expand our existing findings, fine-mapped genotype imputation analysis within CDH1's target region was conducted, where 14 newly imputed SNPs were identified to be significantly associated with endometrial cancer susceptibility. Among them, 2 imputed SNPs in CDH1 (rs6499197 and rs10431924) were involved in several best fitting models in our GMDR analyses, suggesting their roles in the interaction with other susceptible htSNPs. Functional annotations also revealed the potential biological functions of these SNPs in DNase I hypersensitivity, histone modifications, TF binding (especially with FOXM1, which is involved in inducing EMT and metastasis (38), and 3D interactions. Two other imputed SNPs (rs1801026 and rs17690554) were previously found to be respectively associated with the susceptibility of gastric cardiac adenocarcinoma, non-small-cell lung cancer, cervical cancer, breast cancer prognosis, or gastric cancer (39-41), while none of these studies were focused on EC. The rest of the imputed SNPs aforementioned have not been studied in any publications yet, therefore warranting future studies.

Our study has three main strengths. To begin with, this is the first comprehensive gene-wide association study of *CDH1* with EC risk in Chinese Han population. By using haplotype-tagging SNPs plus fine-mapped genotype imputation, we nearly covered all common SNPs of *CDH1*. Secondly, after identifying independent susceptible SNPs, we further analyzed SNP-SNP and SNP-environment interactions to identify the joint effect of SNPs and environmental risk factors on EC development. Thirdly, functional annotations using various databases revealed the potential biological functions of the causal SNPs, giving possible directions for future research. This study inevitably has limitations. Due to the small sample size of certain subgroups, we had to merge several groups or leave out some rare subgroups to increase efficiency, though we did use various statistical methods to minimalize false positives. Also, to improve statistical power, we extracted existing eQTL results in multiform races from GTEx Portal instead of using the Han Chinese in Beijing (CHB) population, mainly due to small sample size of the available unrelated CHB population from the HapMap project.

In summary, this study suggests that the genetic polymorphisms of *CDH1* were associated with endometrial cancer susceptibility. Our data found susceptible loci that were independently associated with EC risk, as well as conjoint effects among themselves and with environmental factors. Furthermore, several SNPs might carry potential functions regulating *CDH1* expression, and additional studies are needed to verify and identify the truly causal SNPs. If further supportive studies are validated, these findings may serve to improve personalized evaluation and early prediction of EC susceptibility in the general population.

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Availability of data and materials

The datasets used and analyzed for the present study are available from the corresponding author upon reasonable request.

Authors' contributions

YHG, XXT, DGL, XHL and WGF conceived and designed the experiments. YHG, YMJ, ZFW, LYZ and LC performed the experiments. ZFW and YHG analyzed and interpreted the data, and wrote the manuscript. XXT, WGF, ZFW and YHG revised the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Peking University IRB (reference no. IRB00001052-11029). Written informed consents were obtained from all women in the control group. Genomic DNAs of the patients were extracted from archived formalin-fixed paraffin-embedded normal fallopian tube tissues. Since the contact information of patients treated before 2011 was not obtainable, Peking University IRB approved our application to waive informed consent for the archived samples collected before April 2011. This study only used these samples. All the data/samples were used anonymously.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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