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Association of Endometriosis-Associated Genetic Polymorphisms From Genome-Wide Association Studies With Ovarian Endometriosis in a Chinese Population

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Abstract

Endometriosis is a common multifactorial disease caused by an interaction between multiple gene loci and environment. Four genome-wide association studies (GWASs) of endometriosis have identified several single-nucleotide polymorphisms (SNPs) associated with endometriosis. However, results from independent replication studies with different populations are inconsistent. The present study aims to evaluate whether the GWAS-derived susceptibility loci are correlated with the risk of the development of ovarian endometriosis in North Chinese women. This case–control study comprised 580 patients with ovarian endometriosis and 606 matched control women. Three SNPs were selected for this association study including rs10965235 in *CDKN2BAS*, rs2235529 located in LINC00339-*WNT4*, and rs12700667 in an intergenic region on 7p15.2. The results show that the G/A genotype of rs12700667 can significantly increase the risk of developing ovarian endometriosis when compared with the G/G genotype (odds ratio [OR] = 1.57, 95% confidence interval [CI] = 1.23-2.00). Similarly, the carriers with A allele showed a higher risk of ovarian endometriosis than those with G allele (OR = 1.23, 95% CI = 1.12-1.68). The study suggests that the endometriosis-associated genetic polymorphisms (rs12700667) from GWAS be associated with the risk of developing ovarian endometriosis in North Chinese women.

Keywords

ovarian endometriosis, genetic polymorphism, replication

Introduction

Endometriosis is a common gynecological disorder characterized by the presence of an endometrium-like glandular epithelium and stroma outside the uterine cavity that includes the ovaries, the uterosacral ligaments, and the pouch of Douglas. Approximately 5% to 10% of reproductive-age women are affected.[1] Women with endometriosis typically present with abdominal pain, dysmenorrhea, dyspareunia, and infertility. Thus, endometriosis has critical implications for the quality of life in women.[2,3]

Although peritoneal endometriosis was first recognized in 1860, the pathogenesis of the disease remains unclear. There is mounting evidence that endometriosis is a complex multifactorial disease in which genetic and environmental factors act together to produce the phenotype. So far, the role of genetic factors in endometriosis susceptibility is supported by a number of different studies,[4-7] including clinical research, epidemiology, cytogenetic, and molecular genetic analyses. In particular, a large number of genetic association studies showed that the genetic polymorphisms may have contributed to the development of endometriosis during the past 20 years.[8-11] However, conflicting results and the absence of replication in independent populations have led to the genetic contribution of endometriosis being questioned.[12] The failure of candidate gene studies is largely attributed to poor study design, insufficient power, and population differences.

Genome-wide association studies (GWASs) analysis using high-density single-nucleotide polymorphism (SNP) arrays have revolutionized gene discovery and our understanding of the genetic contributions to complex diseases. To date, 4 GWASs of endometriosis[13-16] have been published with study populations from Japan, Australia, the United States, and the United Kingdom, and studies have identified several SNPs

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associated with endometriosis. However, the results from following independent replication studies with different populations are not consistent, which may be due to the existence of genetic heterogeneity. Based on the above-mentioned reasons, we hypothesize that 3 SNPs (rs10965235, rs12700667, and rs2235529), which had been reported a strong relation with the susceptibility to endometriosis in the aforementioned GWAS, may affect the risk of ovarian endometriosis in northern Chinese women. In the present study, the association between these 3 SNPs and the risk of developing ovarian endometriosis in a Chinese population was analyzed by a hospital-based case–control study.

Materials and Methods

Study Participants

A case–control study design was used in 580 cases of ovarian endometriosis and 606 matched controls. Part of the samples had been used in our previous studies.[17,18] Patients with ovarian endometriosis underwent laparoscopy or laparotomy and had a histologically confirmed diagnosis at the Fourth Affiliated Hospital, Hebei Medical University, between 2004 and March 2013. According to the revised American Fertility Society classification system (1997), all patients had moderate or severe endometriosis (stage III-IV). General detailed information on each patient was recorded in her medical chart.

The control group consisted of reproductive-age women without any malignant disease, endometriosis, or adenomyosis, as confirmed by surgical exploration (cesarean section), pathological examination after a hysterectomy performed for dysfunctional uterine bleeding, or ultrasound examination. Of these controls, 412 were diagnosed with surgery and 194 were diagnosed with ultrasound. The mean age of the patients and controls was 34.60 $\pm$ 7.30 (range: 19-50) and 34.48 $\pm$ 7.30 (range: 19-50) years, respectively. All of the participants were women of the Han ethnicity in North China. The ethics committee of the Hebei Obstetrics and Gynecology Institute approved this study, and informed consent was obtained from all of the recruited participants.

DNA Extraction

Venous blood (5 mL) from each participant was drawn into Vacutainer tubes containing EDTA, and the tubes were stored at 4°C. Genomic DNA was extracted within 1 week of collection using Proteinase K (Merck, Darmstadt, Germany) digestion, followed by a salting out procedure according to the method of Miller et al.[19]

Genotyping

The genotypes of rs12700667, rs10965235, and rs2235529 were determined by the Shanghai Generay Biotech Co, Ltd (http://www.generay.com.cn) using the polymerase chain reaction/ligase detection reaction (PCR-LDR) method. After the PCR-LDR reactions, the products were analyzed using an ABI 3730XL DNA sequencer (Applied Biosystems, USA). Additionally, the representative PCR products were subjected to direct DNA sequencing to confirm the accuracy of this method, and the results were 100% concordant.

Statistical Analysis

Statistical analysis was performed using the SPSS v.13.0 software package (SPSS, Chicago, Illinois), and $P < .05$ (2 sided) was used as the criterion of statistical significance. Hardy-Weinberg equilibrium (HWE) analysis was performed to compare the observed and expected genotype frequencies in the controls using the χ^2 test. Differences in genotype frequencies in the cases and controls were assessed using the χ^2 test. Bonferroni adjustment was corrected for multiple comparisons, and the threshold for significance was set at a P value of .0167 (=.05/3 variants). The odds ratio (OR) and 95% confidence intervals (CIs) for the genotype-specific risk were calculated using an unconditional logistic regression model and were adjusted accordingly for age.

Results

General Characteristics of the Study Participants

Statistical analysis showed that there was no significant difference in age distribution between the case group and the control group ($t = 0.28$, $P = .78$). The frequency distributions of rs10965235, rs12700667, and rs2235529 genotypes in all control groups did not significantly deviate from that expected for HWE ($P = .08$, .86, .74, respectively).

Association of rs10965235 With the Risk of Ovarian Endometriosis

Genotype frequencies of the rs10965235 C/C, C/A, and A/A were 69.8%, 27.3%, and 2.9% in the cases and 63.9%, 33.5%, and 2.6% in the controls, respectively. The frequencies of the C and A alleles among cases and controls were 80.6% and 19.4% and 83.5% and 16.5%, respectively. There was no significant difference in genotype and allele frequency between the 2 groups ($P = .065$ and $P = .072$; Table 1). However, the C/A genotype may have a significantly decreased risk of developing ovarian endometriosis when compared with the C/C genotype (OR = 0.74, 95% CI = 0.58-0.96; Table 1).

Association of rs12700667 With the Risk of Ovarian Endometriosis

Genotype frequencies of the rs12700667 G/G, A/G, and A/A were 68.3%, 28.6%, and 3.1% in the cases and 58.4%, 38.3%, and 3.3% in the controls, respectively. There was a significant difference between the case and control women ($P = .002$; Table 1). The frequency of the A allele among cases (22.4%) was also significantly higher than in the controls (17.4%; $P = .002$). Especially, the difference in genotype and allele

Table 1. The Association Between 3 SNPs and the Risk of Ovarian Endometriosis.

SNP	Genotype/Allele	Controls, n (%)	Cases, n (%)	P^a	OR (95% CI)
rs10965235	C/C	387 (63.9)	405 (69.8)		1.00
	C/A	203 (33.5)	158 (27.3)	.065	**0.74 (0.58-0.96)**[b]
	A/A	16 (2.6)	17 (2.9)		1.02 (0.54-2.04)[b]
	C	977 (80.6)	968 (83.5)	.072	1.00
	A	235 (19.4)	192 (16.5)		0.83 (0.67-1.02)[c]
rs12700667	G/G	414 (68.3)	339 (58.4)		1.00
	A/G	173 (28.6)	222 (38.3)	**.002**[d]	**1.57 (1.23-2.00)**[e]
	A/A	19 (3.1)	19 (3.3)		1.22 (0.64-2.34)[e]
	G	1001 (82.6)	900 (77.6)	**.002**	1.00
	A	211 (17.4)	260 (22.4)		**1.37 (1.12-1.68)**[f]
rs2235529	A/A	179 (29.5)	171 (29.5)		1.00
	A/G	297 (49.0)	304 (52.4)	.308	0.61 (0.82-1.40)[g]
	G/G	130 (21.5)	105 (18.1)		0.85 (0.61-1.18)[g]
	A	655 (54.0)	646 (55.7)	.420	1.00
	G	557 (46.0)	514 (44.3)		0.94 (0.80-1.10)[h]

[a]Chi-square test.
[b]The odds ratio of the C/A and A/A genotypes against the C/C genotype for rs10965235 polymorphism, respectively.
[c]The odds ratio of the A allele against the C allele for rs10965235 polymorphism.
[d]Bold values indicate that the values have statistical significance.
[e]The odds ratio of the A/G and A/A genotypes against the G/G genotype for rs12700667 polymorphism, respectively.
[f]The odds ratio of the A allele against the G allele for rs12700667 polymorphism.
[g]The odds ratio of the A/G and A/A genotypes against the G/G genotype for rs2235529 polymorphism, respectively.
[h]The odds ratio of the G allele against the A allele for rs2235529 polymorphism.

frequency between cases and controls remained significant after Bonferroni correction (all $P < .0167$). Compared with the G/G genotype, the G/A genotype was associated with a significantly increased risk of developing ovarian endometriosis (OR = 1.57, 95% CI = 1.23-2.00; Table 1). Similarly, the carriers with A allele showed a higher risk of ovarian endometriosis than those with G allele (OR = 1.23, 95% CI = 1.12-1.68).

Association of rs2235529 With the Risk of Ovarian Endometriosis

The genotype frequencies of the rs2235529 A/A, A/G, and G/G were 29.5%, 49.0%, and 21.5% in the cases and 25.9%, 52.4%, 18.1% in the controls, respectively. The frequencies of the A and G alleles among cases and controls were 54.0% and 46.0% and 55.7% and 44.3%, respectively. There was no significant difference in genotype and allele frequency between the 2 groups ($P = .308$ and $P = .420$; Table 1). Compared with the A/A genotype, the A/G and G/G genotypes were not associated with the risk of developing ovarian endometriosis (OR = 0.61, 95% CI = 0.82-1.40; OR = 0.85, 95% CI = 0.61-1.18, respectively).

Discussion

In the present study, we explored the association between the 3 endometriosis-associated SNPs from GWAS[13,15,16] and the risk of ovarian endometriosis in a Chinese population. The results showed that rs12700667 was associated with developing ovarian endometriosis in northern Chinese women. The A/G genotype and G allele of rs12700667 A/G polymorphism significantly increased the risk of ovarian endometriosis. However, the rs10965235 C/A and rs2235529 A/G polymorphisms were not associated with the risk of ovarian endometriosis. To the best of our knowledge, this is the first study to investigate the association between endometriosis-associated SNPs from GWAS and the risk of ovarian endometriosis in a Chinese population.

In 2010, the first endometriosis GWAS was published on a Japanese data set.[13] The study identified a significant association of endometriosis dataset with rs10965235 ($P = 0.00$, OR = 1.44), which is located in *CDKN2BAS* on chromosome 9p21 and encodes the cyclin-dependent kinase inhibitor 2B antisense RNA. Although this SNP was not found, a significant signal in another GWAS data set of a Japanese population was observed.[14] However, Adachi et al found that SNP rs17761446, which is in perfect linkage disequilibrium with rs10965235 ($D'=1$, $r2 = 1$, HapMap JPT population [phase III, Rel no. 2]), was significantly associated with endometriosis ($P = 0.009$; OR = 1.29, 95% CI = 1.10-1.48). Consequently, these data suggest that an endometriosis susceptibility loci may be located at 9p21. Further, a replication study including 673 patients with endometriosis and 500 control patients also confirmed that rs10965235 was significantly associated with endometriosis in a Korean population.[20] In the present study, the genotype frequencies of the rs10965235 C/C, C/A, and A/A were not significantly different between the case group and the control population, but C/A genotype may have significantly decreased risk of developing ovarian endometriosis (OR = 0.74, 95% CI = 0.58-0.96). Therefore, more studies with large number of participants are needed to further assess the association between rs10965235 and endometriosis in Chinese

women. However, it is worth mentioning that rs10965235 is monomorphic in individuals of European descent, which makes comparisons with the Asian population futile. So, rs10965235 may be a potential molecular marker for the risk of endometriosis in Asian populations.

The first GWAS in women of European ancestry was conducted by the International Endogene Consortium involving Australian and UK data sets, with independent replication in a US data set.[15] In this study, the strongest signal was observed for rs12700667 in an intergenic region on chromosome 7p15.2 ($P = 0.00$; OR = 1.22, 95% CI = 1.13-1.32). Subsequently, Nyholt et al[21] conducted a genome-wide association meta-analysis for data from Japanese and European ancestry, and the meta-analysis showed that rs12700667, which was previously found in Europeans, was also found in the Japanese ($P = 0.004$). But, another GWAS in women of European ancestry[16] as well as 2 replication studies[22,23] did not find significant evidence for rs12700667. However, a meta-analysis[24] combined results from 8 GWASs and replication studies, which cross European ancestry populations in Australia, Belgium, Italy, the United Kingdom, and the United States as well as Japanese ancestry populations, showed that rs12700667 was significantly associated with endometriosis ($P = 0.00$). Our study showed that rs12700667 was related to ovarian endometriosis in a northern Chinese population. Compared with the G/G genotype, the carriers with the G/A genotype may have a significantly increased risk of developing endometriosis (OR = 1.57, 95% CI = 1.23-2.00). Thus, these findings suggest that the rs12700667 SNP may be a candidate SNP for the development of endometriosis in Asian and European populations.

In a recent GWAS by Albertsen et al,[16] an association between 1 novel SNP (rs2235529 located in LINC00339-*WNT4* on 1p36.12) and endometriosis was observed in American women ($P = 0.00$, OR = 1.29, 95% CI = 1.18-1.40). WNT4 is a secreted protein that plays a crucial role in the development of the female reproductive tract, human endometrial stromal cell differentiation, and embryonic implantation.[25-28] Several GWASs of endometriosis have detected some endometriosis-related genetic markers in the region close to or within an intron of *WNT4*.[21,15,13] Uno et al[13] noted that rs16826658, which is approximately 16 kb upstream of WNT4, showed a possible association with endometriosis ($P = 0.00$, OR = 1.20) in a Japanese population. Painter et al[15] reported that rs7210902, which is located approximately 22 kb upstream of *WNT4*, also showed evidence for association in a European cohort ($P = 0.00$, OR = 1.16). However, these results were inconsistent. In the present study, we analyzed the association between the rs2235529 SNP and the risk of ovarian endometriosis in a Chinese population; significant differences were not found between rs2235529 and the risk of ovarian endometriosis in a northern Chinese population.

In conclusion, we hypothesize that the endometriosis-associated genetic polymorphisms from GWAS (rs10965235, rs12700667, and rs2235529) may affect the risk of ovarian endometriosis in northern Chinese women. The results showed that rs12700667 polymorphisms, but not rs10965235 and rs2235529, may be associated with the risk of endometriosis in the present study. To further determine the relationship between endometriosis-associated genetic polymorphisms from GWAS and endometriosis, a duplicate study in different ethnic groups and a larger population is necessary.

Declaration of Conflicting Interests

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