

# Association of *FCRL3* Genetic Polymorphisms With Endometriosis-Related Infertility Risk

## *An Independent Study in Han Chinese*

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**Abstract:** The Fc receptor-like 3 (*FCRL3*) gene was reported to be linked to a variety of autoimmune diseases, including endometriosis-related infertility. However, this linkage has not been studied in Chinese population and there has been no meta-analysis on the interrelationship of *FCRL3* gene and endometriosis-related infertility. The aim of the study was to investigate the association between *FCRL3* genetic polymorphisms and the risk of endometriosis-related infertility in Han Chinese, and a further meta-analysis was conducted to confirm our results.

Four single nucleotide polymorphisms (SNPs) (rs7528684 [FCRL3\_3], rs11264799 [FCRL3\_4], rs945635 [FCRL3\_5], and rs3761959 [FCRL3\_6]) on *FCRL3* gene were genotyped in a case-control cohort composed of 217 patients suffering from endometriosis-related infertility and 220 healthy controls using cleaved amplification polymorphism sequence-tagged sites (polymerase chain reaction-restriction fragment length polymorphism, PCR-RFLP). Odds ratio (OR) and its 95% confidence interval (CI) was used to evaluate the association quantitatively. Furthermore, a meta-analysis of previous studies including the present study was implemented through Stata 11.0 (Stata Corporation, College Station, TX).

We found an approximately 1.4-fold significantly increased frequency of the *FCRL3\_3* variant in women with endometriosis-related infertility over the controls (OR = 1.41 [95% CI = 1.08–1.84],  $P = 0.013$ ). However, no significant difference was found between women with endometriosis-related infertility and controls for *FCRL3\_4*, *FCRL3\_5*, and *FCRL3\_6*. Regardless of the symptoms and the revised classification of the American Society of Reproductive Medicine (rASRM) stage of endometriosis, there was a significant association between *FCRL3\_3* variant and an increased risk of endometriosis-related infertility. Meta-analysis of previous studies combined with the present study further confirmed the association between *FCRL3\_3* and the risk of endometriosis-related infertility.

In summary, the present study suggested that *FCRL3\_3* variant was associated with an increased risk of endometriosis-related infertility, regardless of symptoms, and rASRM stage of the patients. Meta-analysis of previous studies combined with the present study further confirmed

our results. Further large-scale studies in the future are warranted to explore the association between *FCRL3* genetic polymorphisms and endometriosis-related infertility, as well as other human diseases, in Asian and other ethnicities.

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**Abbreviations:** CI = confidence interval, *FCRL3* = Fc receptor-like 3, OR = odds ratio, rASRM = the revised classification of the American Society of Reproductive Medicine, SNP = single nucleotide polymorphism.

## INTRODUCTION

Endometriosis, a common and chronically estrogen-dependent gynecological disorder, primarily manifests itself in implantation, growth and development of endometrial tissues in the peritoneal cavity, which are supposed to develop themselves within uterine cavity.<sup>1</sup> Approximately 10% to 15% of women at reproductive age suffer from endometriosis and their clinical symptoms include severe pelvic pain, heavy menstrual pain, irregular menstrual bleeding, pain during intercourse, or exercise.<sup>2,3</sup> Furthermore, nearly 50% of endometriosis patients are persecuted by fertility problems, including infertility.<sup>3</sup> To date, endometriosis could be explained by several etiopathogenesis, including implantation theory, defective immune system, genetic factors, etc. As endometriosis has a relatively high heritability ratio of 51%, some researches regarding genetic risk factors, such as estrogen receptor-1 (*ESR1*), estrogen receptor-2 (*ESR2*), and luteinizing hormone beta-subunit (*LHB*) genes, for endometriosis-related infertility have been conducted.<sup>4,5</sup> Even though several meaningful conclusions have been drawn in previous studies,<sup>6–8</sup> the exact etiology of endometriosis remains unclear and standard treatment for endometriosis has been deficient so far.<sup>9</sup> To sum up, considering the unfavorable outcomes of people who are affected by endometriosis and vague etiology of endometriosis-related infertility, it is of great significance to further explore genetic risk factors associated with endometriosis-related infertility.

Among the possible causes of endometriosis, it has been demonstrated that the deficiency in the immune system might act as an impediment to clear endometrial cells from pelvic cavity.<sup>10</sup> There are also evidences that B lymphocytes produce specific antiendometrial autoantibodies and that Tregs, known as regulatory T-cells, are key regulators to guarantee a specific immune response against ectopic endometrial fractions.<sup>11</sup> Therefore, the genetic factors that regulate the performance of B-cells and T-cells could be closely relevant to endometriosis and even endometriosis-related infertility.<sup>12–14</sup> Recently, Fc receptor-like 3 (*FCRL3*), situated at 1q 21–23, was suggested as a novel risk factor of autoimmune disease, partly because the

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gene involves itself in tyrosine-based activation and inhibition motifs in its cytoplasmic domain.<sup>12,15–17</sup> FCRL3, encoded by this gene, is a member of the immunoglobulin receptor family which has structural homology to the Fc receptor, and it is principally expressed in B lymphocytes of lymph nodes and germinal centers, which have been implied to play a significant role in the etiology of endometriosis via secreting autoantibodies.<sup>18</sup> Furthermore, researches by Swainson et al<sup>19</sup> and Nagata et al<sup>20</sup> showed that the expression of FCRL3 could also be detected in Treg cells, serving to restrict the proliferation and cytokine release of T-cells. It might be hypothesized that FCRL3 is associated with combined effects of B-cells and Tregs within the autoimmune system. Emerging evidence has indicated significant associations of single nucleotide polymorphisms (SNPs) in FCRL3 gene with several autoimmune diseases, including rheumatoid arthritis (RA), thyroid disease,<sup>21</sup> systemic lupus erythematosus,<sup>22</sup> and Graves' disease.<sup>23</sup> Moreover, it is proposed that the same susceptibility loci may lead to diverse autoimmune diseases.<sup>24</sup> Hence, there might be an association between FCRL3 gene and endometriosis, which has gradually attracted increasing attention.

Several studies have suggested that FCRL3 genetic polymorphisms might play a significant role in the pathogenesis of endometriosis-related infertility in Brazilian and Polish population.<sup>25–27</sup> However, the conclusion that FCRL3 gene is associated with endometriosis might not be applied precisely to Chinese population due to diversity in phenotype heterogeneity, ethnic background, and the fact that endometriosis is a multifactorial disease.<sup>25</sup> Therefore, the present study is aimed to investigate the association between FCRL3 gene variations and the risk of endometriosis-related infertility in Han Chinese population firstly, and a meta-analysis was also performed to further confirm our results.

## METHODS

### Study Subjects

A case-control study was performed to confirm the relationship between common mutations of FCRL3 gene and susceptibility of endometriosis-related infertility. Peripheral blood samples were obtained from 217 female endometriosis-associated infertility patients (mean age: 33.26 ± 5.71 years) and 220 fertile women (mean age: 32.79 ± 5.56 years) between January 2013 and December 2014 from Linyi City People's Hospital, China. Recruited subjects are selected among Han ethnicity and all of them have no genetic relationship with each other. They are native to Jiangsu Province. The cases and controls were well matched for age and body mass index

(BMI) (all  $P > 0.05$ ). In the research, patients with endometriosis were diagnosed by laparoscopy and histological examination, and women who did not get pregnancy after at least 12 natural cycles were regarded as infertile.<sup>28</sup> Patients with acute or chronic disorders, especially autoimmune diseases, were excluded from the study. The stage of endometriosis was determined according to the revised classification of the American Society of Reproductive Medicine (rASRM).<sup>29</sup> In this group, minimal/mild (stage I–II) endometriosis was found in 88 cases (40.6%) and moderate/severe (stage III–IV) endometriosis in 129 cases (59.4%). A pretested questionnaire about demographic data and clinical characteristics was compiled by experienced and trained interviewers for all participants. The peripheral blood samples and clinicopathologic information could not be acquired before they were approved by the ethical committee of Linyi City People's Hospital. All volunteers were of their own wills to sign informed consents prior to the commencement of the research.

### Genotyping

Genomic DNA was isolated from the peripheral blood of patients and controls by implementing a standard salting out procedure in strict accordance with a standard protocol.<sup>30</sup> Determination of FCRL3 genetic variants (rs7528684 C/T, rs11264799 A/G, rs945635 C/G, and rs3761959 A/G) was executed by cleaved amplification polymorphism sequence-tagged sites (polymerase chain reaction–restriction fragment length polymorphism, PCR–RFLP). Primers (GenScript, Piscataway, NJ 08854, USA) for multiplex-PCR (m-PCR) and multiplex extension were shown in Table 1, which were designed with usage of computer software MassARRAY™ Assay Design 2.0. The amplification reaction were conducted via a 5 μL reaction compound (QIAGEN, 40724 Hilden, Germany) containing 10 mM dNTP, 15 mM MgCl<sub>2</sub>, 4 μM reverse PCR primers, 4 μM forward PCR primers, and 5 U/μL Hotstar Taq. Then tubes were amplified in light of the next PCR conditions: a single cycle of initial denaturation for 15 min at 95°C; 45 cycles of 20 s denaturation at 95°C; 30 s annealing at 56°C, and 1 min extension at 72°C; and a final extension for 3 min at 72°C. Furthermore, in the primer extension reaction, shrimp alkaline phosphatase enzyme (Sequenom Inc., 3595 John Hopkins Court, San Diego, CA 92121, USA) was firstly employed to dephosphorylate excluded dNTPs from the amplification reaction, after which the MassEXTEND reaction was performed. The reaction mixture (Sequenom, Inc.) was involved with 0.18 μL MassEXTEND primers (50 μM each), 0.2 μL hME EXTEND Mix (50 μM each, containing buffer and d/dNTPs), and 0.04 μL Thermo Sequence™ (32 U/μL). And the cycling

**TABLE 1.** Primers of FCRL3 Genetic Polymorphisms for PCR Amplification

SNP	RS ID	Mutant	Region	PCR Amplification Primer	Extension Primer
FCRL3_3	rs7528684	T > C	5' near gene	F: 5'-ACGTTGGATGTTATGAGGCTTCTGAACAGG-3' R: 3'-ACGTTGGATGCAGATCTGGGTGAGATTACG-5'	5'-GAGATTACGGGAAGTCC-3'
FCRL3_4	rs11264799	G > A	5' near gene	F: 5'-ACGTTGGATGGTGGTGTGAATCACACAGTC-3' R: 3'-ACGTTGGATGTTTTCTGTTCAGAAGCCTC-5'	5'-CCTCATAACAACCTATGTGAAA-3'
FCRL3_5	rs945635	G > C	5' UTR	F: 5'-ACGTTGGATGTTACTACTCACTCAGGATCAGC-3' R: 3'-ACGTTGGATGGTGAGACAGACTTTCCCAAC-5'	5'-TTTCCCAACCCTCTGCCC-3'
FCRL3_6	rs3761959	G > A	Intron 3	F: 5'-ACGTTGGATGCTGCAAGTGTTCCTTCCTG-3' R: 3'-ACGTTGGATGCCTCCGACTTTTTTCAGTCTC-5'	5'-TTTTTCTCCCTACATTACC-3'

F = forward; PCR = polymerase chain reaction; R = reverse; SNP = single-nucleotide polymorphism; UTR = untranslated region.

conditions for obtaining allele-specific extended products were described as follows: 94°C for 2 min, followed by 100 cycles of 94°C for 5 s, 52°C for 5 s, and 72°C for 5 s. Finally, approximately 10 µL desalted PCR products were placed on SpectroCHIP (Sequenom, Inc.) and they were analyzed entirely automatically with MassARRAY system (Bruker-Sequenom, San Diego, CA), which was devised on the basis of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) technology.

**Statistical Analysis**

Chi-squared ( $\chi^2$ ) test was utilized to assess if statistical significance for differences in the frequency of alleles between patients and controls and deviation of the genotype frequency distribution from Hardy–Weinberg expectations existed. The Student *t* test and  $\chi^2$  test were conducted to compare demographic characteristics between patients and controls, such as age of menarche, menstrual cycle length, menstrual period length, smoking status, family history of endometriosis, and sex hormone levels and symptoms. Odds ratios (ORs) adjusted by clinical parameters and their 95% confidence intervals (CIs) were used to evaluate the interconnection between gene variations and the risk of endometriosis-related infertility. For meta-analysis, the  $I^2$  and Cochran’s Q test served to evaluate heterogeneity of between-studies. In the condition of  $I^2 < 50\%$  and  $P \geq 0.05$  for the Q test, the fixed-effects model was applied to

integrate the data; otherwise, the random-effects model was employed instead. Statistic analyses were conducted using the Stata version 11.0 (Stata Corporation, College Station, TX), and a 2-tailed *P* value  $< 0.05$  was considered to be statistically significant.

**RESULTS**

**Sample Characteristics**

A total of 437 women were included in the study (217 endometriosis-related infertility patients and 220 fertile women in the control group). The median infertility duration of patients was 5.28 years. Clinical characteristics and hormonal parameters of the 2 groups were presented in Table 2. Most patients were in advanced stages (stage III and IV, 59.4%). No significant differences were found in some indexes between cases and controls, such as age, BMI, age of menarche, menstrual cycle length, menstrual period length, smoking status, and serum concentration of 6 sex hormones in advanced stages of the disease (all  $P > 0.05$ ). However, higher frequency of infertile family history ( $P < 0.001$ ) was observed for cases compared with controls. In addition, regarding to clinical symptoms, a significant number of cases reported chronic pelvic pain, dysmenorrhea, and dyspareunia. Though endometriosis symptoms were also reported by controls, significant differences were observed between the 2 groups in the prevalence of all

**TABLE 2.** Comparison of Endometriosis-Related Infertility Patients and Controls by Selective Clinical Characteristics

Clinical Characteristics	Infertile Women (n = 217)	Fertile Women (n = 220)	P Value
Age, yr	33.26 ± 5.71	32.79 ± 5.56	0.384
BMI, kg/m <sup>2</sup>	22.91 ± 3.82	23.37 ± 2.74	0.148
Parity	–	1.80 ± 0.75	–
Duration of infertility, yr	5.28 ± 1.14	–	–
Age of menarche, yr	13.39 ± 1.72	13.27 ± 1.33	0.415
Menstrual cycle length, d	28.69 ± 3.77	29.13 ± 4.12	0.245
Menstrual period length, d	5.67 ± 0.97	5.82 ± 1.03	0.118
Smoking status, n (%)			
Yes	67 (30.9)	76 (34.5)	0.414
No	150 (69.1)	144 (65.5)	
Family history, n (%)			
Yes	43 (19.8)	4 (1.8)	<b>&lt;0.001</b>
No	174 (80.2)	216 (98.2)	
Sex hormone levels			
Estrogen, pg/mL	68.92 ± 13.21	66.73 ± 11.80	0.068
Progesterone, mIU/mL	3.07 ± 0.63	2.98 ± 0.58	0.121
Prolactin, ng/mL	13.53 ± 4.71	14.19 ± 6.31	0.217
Testosterone, ng/mL	0.79 ± 0.16	0.81 ± 0.17	0.206
FSH, mIU/mL	7.39 ± 1.28	7.62 ± 1.43	0.077
LH, mIU/mL	5.82 ± 2.07	6.13 ± 2.21	0.131
Symptoms*			
Chronic pelvic pain, n (%)	117 (53.9)	25 (11.4)	<b>&lt;0.001</b>
Dysmenorrhea, n (%)	112 (51.6)	18 (8.2)	<b>&lt;0.001</b>
Dyspareunia, n (%)	101 (46.5)	37 (16.8)	<b>&lt;0.001</b>
rASRM stage			
Minimal/mild (stage I and II), n (%)	88 (40.6)	–	–
Moderate/severe (stage III and IV), n (%)	129 (59.4)	–	–

BMI = body mass index; FSH = follicle stimulating hormone; LH = luteinizing hormone; rASRM = the revised classification of the American Society for Reproductive Medicine. Bold values signifies *P* value less than 0.05.

\* A patient can have more than 1 symptom.



**FIGURE 1.** Genetic location of the 4 tag-single nucleotide polymorphisms in *FCRL3* gene.

symptoms (all  $P < 0.001$ ). Therefore, they were adjusted in the multivariable logistic regression models for analyses of the association between *FCRL3* genetic polymorphisms and the risk of endometriosis-related infertility.

**Association Between *FCRL3* Genetic Polymorphisms and Susceptibility of Endometriosis**

As shown in Figure 1, *FCRL3\_3* and *FCRL3\_4* were located in the 5' near gene region, whereas *FCRL3\_5* and *FCRL3\_6* were, respectively, situated in the 5' untranslated region (UTR) and intron 3.<sup>31</sup> The observed frequencies of each SNP met the assumptions of Hardy–Weinberg equilibrium (HWE) in both the case and control subjects (all  $P > 0.05$ ). The results of the allele frequency analysis are presented in Table 3. We found an approximately 1.4-fold significantly increased frequency of the *FCRL3\_3*C allele in women with endometriosis-related infertility over the controls (allelic model: OR = 1.41 [95% CI = 1.08–1.84],  $P = 0.013$ ). There was also a significantly increased frequency of the C/C and C/T genotypes in patients compared with controls (dominant model: OR = 1.69 [95% CI = 1.12–2.56],  $P = 0.012$ ). With regard to the *FCRL3\_4* (rs11264799) polymorphism, no difference was found between women with endometriosis-related infertility and controls. Similar results were found for the *FCRL3\_5* (rs945635) and *FCRL3\_6* (rs3761959) polymorphisms.

To investigate whether there were other factors that might influence the overall results, further stratified analyses were conducted according to the clinical symptoms and rASRM stage (Table 4). Regardless of the symptoms of the disease, there was a significant association between *FCRL3\_3* variant and an increased risk of endometriosis-related infertility, which was also consistent with the overall analysis. When the case and control groups matched with the rASRM stage, the difference was more evident in the group of stage III–IV (C allele vs T allele: OR = 1.42 [95% CI = 1.04–1.93],  $P = 0.027$ ; CT + CC vs TT: OR = 1.63 [95% CI = 1.01–2.64],  $P = 0.046$ ; CC vs TT: OR = 1.93 [95% CI = 1.04–3.56],  $P = 0.035$ ). For the subgroup of stage I–II, only a moderately significant association between *FCRL3\_3* variant and an increased risk of endometriosis-related infertility was found under dominant model (CT + CC vs TT: OR = 1.79 [95% CI = 1.01–3.14],  $P = 0.041$ ).

**Meta-Analysis of *FCRL3\_3* With the Risk of Endometriosis-Related Infertility**

Meta-analysis of 4 previous studies combined with the present study, including 883 cases and 881 controls, was implemented to confirm the association between *FCRL3\_3*

**TABLE 3.** Allele and Genotype Distributions of *FCRL3* Genetic Polymorphisms in Endometriosis-Related Infertility Patients and Controls

SNP	Mutant	Population	Genotype, n (%)			HWE	Dominant Model		Allele Frequency		Allelic Model	
			11	12	22		P Value	OR (95% CI)	P Value	OR (95% CI)	P Value	
<i>FCRL3_3</i>	T > C	Case (n = 217)	54 (24.9%)	110 (50.7%)	53 (24.4%)	0.838	1.69 (1.12–2.56)	0.012	218	216	1.41 (1.08–1.84)	0.013
		Control (n = 220)	79 (35.9%)	100 (45.5%)	41 (18.6%)							
<i>FCRL3_4</i>	G > A	Case (n = 217)	105 (48.4%)	95 (43.8%)	17 (7.8%)	0.480	1.13 (0.77–1.64)	0.566	129	305	1.12 (0.83–1.50)	0.529
		Control (n = 220)	113 (51.4%)	93 (42.3%)	14 (6.4%)							
<i>FCRL3_5</i>	G > C	Case (n = 217)	64 (29.5%)	110 (50.7%)	43 (19.8%)	0.730	1.24 (0.83–1.85)	0.302	238	196	1.13 (0.86–1.47)	0.389
		Control (n = 220)	75 (34.1%)	104 (47.3%)	41 (18.6%)							
<i>FCRL3_6</i>	G > A	Case (n = 217)	56 (25.8%)	110 (50.7%)	51 (23.5%)	0.832	1.10 (0.72–1.69)	0.650	222	212	1.09 (0.83–1.42)	0.548
		Control (n = 220)	61 (27.7%)	112 (50.9%)	47 (21.4%)							

11 = wild genotype; 12 = heterozygote mutant genotype; 22 = homozygote mutant genotype; CI = confidence interval; HWE = Hardy–Weinberg equilibrium; MAF = minor allele frequency; OR = odds ratio; SNP = single nucleotide polymorphism. Bold values signifies  $P$  value less than 0.05.



**TABLE 4.** Stratified Analyses of FCRL3\_3 (rs7528684) in Endometriosis-Related Infertility Patients and Controls

Subgroup	Case/Control	Allelic Model		Dominant Model		Recessive Model		Homozygous Model	
		OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value
Overall	217/220	1.41 (1.08–1.84)	<b>0.013</b>	1.69 (1.12–2.56)	<b>0.012</b>	1.41 (0.89–2.23)	0.141	1.89 (1.11–3.23)	<b>0.019</b>
Symptoms									
Chronic pelvic pain	117/25	1.90 (1.01–3.62)	<b>0.045</b>	2.56 (1.06–6.21)	<b>0.034</b>	1.81 (0.57–5.70)	0.305	2.90 (0.84–10.10)	0.083
Dysmenorrhea	112/18	2.23 (1.05–4.75)	<b>0.034</b>	3.04 (1.12–8.20)	<b>0.024</b>	2.79 (0.60–12.90)	0.179	4.35 (0.86–21.80)	0.057
Dyspareunia	110/37	2.08 (1.19–3.62)	<b>0.009</b>	2.65 (1.22–5.73)	<b>0.012</b>	1.57 (0.77–3.22)	0.218	3.60 (1.18–11.00)	<b>0.020</b>
rASRM stage									
Stage I–II	88/220	1.39 (0.98–1.97)	0.068	1.79 (1.01–3.14)	<b>0.041</b>	1.28 (0.70–2.35)	0.416	1.84 (0.89–3.77)	0.096
Stage III–IV	129/220	1.42 (1.04–1.93)	<b>0.027</b>	1.63 (1.01–2.64)	<b>0.046</b>	1.50 (0.89–2.53)	0.125	1.93 (1.04–3.56)	<b>0.035</b>

CI = confidence interval; OR = odds ratio; rASRM = the revised classification of the American Society for Reproductive Medicine. Bold values signifies P value less than 0.05.

and the risk of endometriosis-related infertility.<sup>8,25,27,32</sup> Detailed information of included studies was enumerated in Table 5. As shown in Figure 2, the fixed-effects model was used due to low level of between-studies heterogeneity ( $I^2 = 0\%$ ,  $P = 0.929$ ). The overall analysis suggested that FCRL3\_3 variant was associated with a significantly increased risk of endometriosis-related infertility under allelic model (OR = 1.55 [95% CI = 1.36–1.76],  $P < 0.001$ ). Moreover, subgroup analyses were further performed based on the rASRM stage, which classified the patients into stage I–II (minimal/mild endometriosis) and stage III–IV (moderate/severe endometriosis) groups (Table 5). A total of 506 cases and 772 controls as well as 573 cases and 722 controls were, respectively, recruited into subgroup stage I–II and stage III–IV. Regardless of the rASRM stage, there was a significant association between FCRL3\_3 variant and an increased risk of

endometriosis-related infertility, which was consistent with the overall analysis.

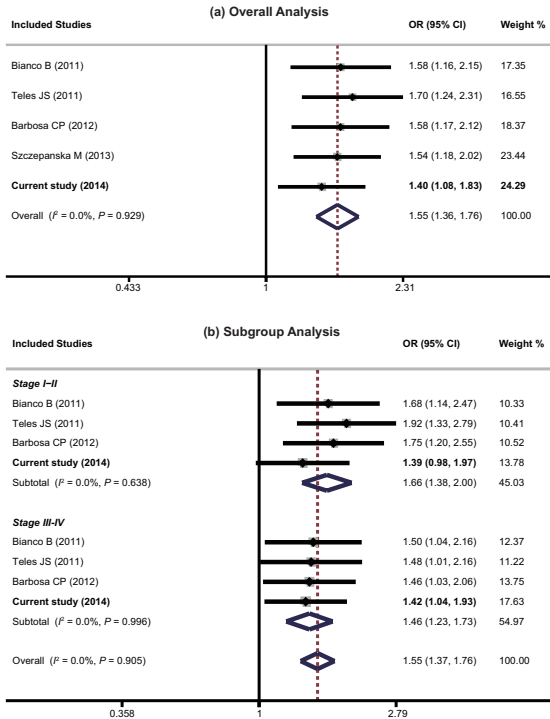
**DISCUSSION**

Endometriosis-related infertility has been reported to co-occur with autoimmune diseases,<sup>33</sup> while FCRL3 genetic polymorphisms discourage regular expression of FCRL3 in B cells, Treg cells, macrophages, etc., during the autoimmune process, indicating that FCRL3 genetic polymorphism might be associated with the presence of endometriosis-related infertility. An association was observed between a short segment composed of 4 SNPs (FCRL3\_3, FCRL3\_4, FCRL3\_5, and FCRL3\_6) in FCRL3 and RA.<sup>21</sup> Simmonds et al<sup>28</sup> also found that mutations of FCRL3\_3 (OR = 1.17 [95% CI: 1.02–1.34]), FCRL3\_5 (OR = 1.18 [95% CI: 1.04–1.35]), and FCRL3\_6 (OR = 1.20 [95% CI: 1.05–1.36]) could heighten susceptibility of Graves'

**TABLE 5.** Meta-Analysis of FCRL3\_3 (rs7528684) in Endometriosis-Related Infertility Patients and Controls

First Author	Year	Country	Population	No. of Case/Control	Case Group				Control Group				Allelic Model	
					CC	CT	TT	MAF	CC	CT	TT	MAF	OR (95% CI)	P Value
Bianco et al	2011	Brazil	All	170/166	40	87	43	0.49	24	78	64	0.38	1.58 (1.16–2.15)	0.004
Teles et al	2011	Brazil	All	167/167	40	86	41	0.49	24	75	68	0.37	1.70 (1.25–2.31)	0.001
Barbosa et al	2012	Brazil	All	188/169	43	97	48	0.48	24	79	66	0.38	1.58 (1.17–2.12)	0.003
Szczepanska et al	2013	Polish	All	141/159	21	73	47	0.59	135	265	119	0.49	1.55 (1.18–2.02)	0.001
Present study	2014	China	All	217/220	54	110	53	0.50	79	100	41	0.41	1.41 (1.08–1.84)	0.013
Overall	–	–	Total	883/881	198	453	232	0.48	286	597	358	0.47	<b>1.55 (1.36–1.76)</b>	<b>&lt;0.001</b>
Bianco et al	2011	Brazil	Stage I–II	170/166	19	40	18	0.51	24	78	64	0.38	1.68 (1.14–2.47)	0.008
Teles et al	2011	Brazil	Stage I–II	167/167	40	86	41	0.50	24	75	68	0.37	1.93 (1.33–2.79)	0.001
Barbosa et al	2012	Brazil	Stage I–II	81/169	21	41	19	0.51	24	79	66	0.38	1.75 (1.20–2.55)	0.004
Present study	2014	China	Stage I–II	88/220	21	47	20	0.49	79	100	41	0.41	1.39 (0.98–1.97)	0.068
Subgroup of stage I–II	–	–	Total	506/722	101	214	98	0.50	151	332	239	0.44	<b>1.66 (1.38–2.00)</b>	<b>&lt;0.001</b>
Bianco et al	2011	Brazil	Stage I–II	170/166	40	87	43	0.49	24	78	64	0.38	1.50 (1.04–2.16)	0.028
Teles et al	2011	Brazil	Stage I–II	167/167	40	86	41	0.50	24	75	68	0.37	1.48 (1.01–2.16)	0.045
Barbosa et al	2012	Brazil	Stage I–II	107/169	22	56	29	0.47	24	79	66	0.38	1.46 (1.03–2.06)	0.033
Present study	2014	China	Stage I–II	129/220	33	63	33	0.50	79	100	41	0.41	1.42 (1.04–1.93)	0.027
Subgroup of stage III–IV	–	–	Total	573/722	135	292	146	0.49	151	332	239	0.44	<b>1.46 (1.23–1.73)</b>	<b>&lt;0.001</b>

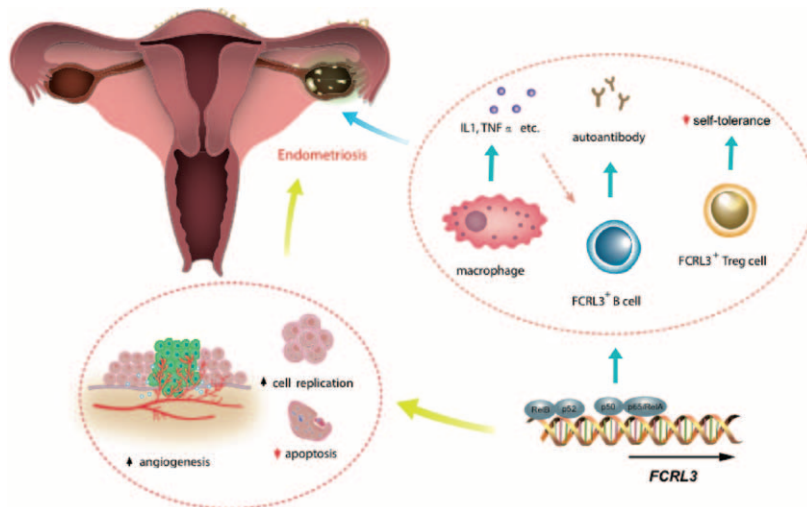
All = endometriosis-related infertility; CI = confidence interval; MAF = minor allele frequency; OR = odds ratio. Stage I–II = minimal/mild endometriosis; Stage I–II and stage III–IV are classified in accordance with ASRM (the revised classification of the American Society for Reproductive Medicine); Stage III–IV = moderate/severe endometriosis. Bold values signifies P value less than 0.05.



**FIGURE 2.** Meta-analysis with the allelic model (C vs T) and fixed effects for the association between FCRL3\_3 and the risk of endometriosis-related infertility. The name of the first author and year of publication of each study are shown. The results of individual and summary odds ratio (OR) estimates, 95% confidence interval (CI), and weight (W) of each study show the association between FCRL3\_3 and the risk of endometriosis-related infertility. Horizontal lines represent 95% CI and vertical-dotted lines represent the value of the summary OR.

disease by means of altering pathways of B cell signaling and activation. Furthermore, it has been identified by 4 whole-genome studies that overlapping cluster of susceptibility loci could bring about clinically distinct diseases, including RA,<sup>34</sup> Graves' disease,<sup>35</sup> multiple sclerosis,<sup>36</sup> and Type 1 diabetes.<sup>37</sup> Thus, the 4 SNPs were selected for the study to investigate the association of FCRL3 genetic polymorphism with risk of endometriosis-related infertility in Han Chinese population and a meta-analysis of previous studies together with the present study was implemented to further confirm our results, concluding that FCRL3\_3 may serve as a susceptible factor for endometriosis-related infertility.

In the present study, FCRL3\_3 was the only genetic polymorphism to be remarkably associated with endometriosis-related infertility among the 4 SNPs. It might be due to the exceptional locus of FCRL3\_3. In fact, FCRL3\_3 was seated in the promoter gene of FCRL3 and it was found to alter nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) binding, which played a determinant role in the expression of FCRL3, both in vitro and in vivo,<sup>38</sup> whereas no study has so far demonstrated how FCRL3\_4, FCRL3\_5, FCRL3\_6 could influence FCRL3 transcription. As shown in Figure 3, abnormal activation of NF-κB, which was a transcriptional factor and was made up of Rel B, p52, p50, and p65/RelA, might lead to expression of genes involved in inhibition of apoptosis, constitutive cell replication, increased angiogenesis, as well as incremental proinflammatory cytokines secreted by macrophages, all of which were indications of a defective immune system.<sup>39</sup> Additionally, the cytokines, such as interleukin 1 (IL1), tumor necrosis factor α (TNFα), etc., also promoted deregulated activation of B cells.<sup>40,41</sup> Furthermore, eccentric NF-κB binding would also contribute to increased FCRL3 expression in B cells and thus signaling pathways were activated or inhibited, disturbing normal B cell functions.<sup>18</sup> The defective immune system as mentioned above, including abnormal B cell activation, would consequently contribute to pelvic pain and subfertility.<sup>42</sup> Besides, it was recently reported that



**FIGURE 3.** Potential mechanism of FCRL3 gene in the pathogenesis of endometriosis-related infertility. Abnormal NF-κB binding, affected by FCRL3\_3, could lead to increased cytokines (IL1, TNFα, etc.) secreted by macrophages and incremental FCRL3 encoded by FCRL3 in B cells, which would altogether abnormally activate B cells and an autoimmune disease onset, and also contribute to increased angiogenesis, constitutive cell replication, and inhibition of apoptosis. Eccentric FCRL3 expression in Treg cells, attributable to abnormal NF-κB binding, also served to disable regulatory activities of the cells. The combined effects of above indications ultimately made pelvic pain and endometriosis-related infertility possible. FCRL3 = Fc receptor-like 3.

FCRL3 could also be expressed on natural killer cells and regulatory T cells (Treg) in addition to B cells,<sup>20,43</sup> especially that FCRL3 positive Tregs have failed to regulate their own activities and could no longer remain self-tolerant.<sup>20</sup> Significant reduction of Tregs in eutopic endometrial tissues was observed in women without endometriosis during secretory phase compared with women with the disease, indicating that the increased FCRL3 positive Tregs discourage novel recruited immune cells from effectually identifying and aiming at endometrial antigens in the process of menstruation, enabling implantation of shed endometrial cells and possible infertility.<sup>11</sup>

Interestingly, the presence of endometriosis-related infertility is accompanied by clinical symptoms as chronic pelvic pain, dysmenorrhea, and dyspareunia, among which dyspareunia is the most significant when associated with FCRL3\_3. The phenomena could possibly be explained by the long-periodic occurrence of chronic pelvic pain and dysmenorrhea (regularly once a month) in comparison with dyspareunia. In addition, patients with moderate/severe endometriosis (stage III–IV) were supposed to be more susceptible to infertility than stage I–II (minimal/mild endometriosis) counterparts. A possible explanation could be that patients with advanced stages of endometriosis revealed more serious clinical symptoms and larger possibility of infertility under the same frequency of FCRL3\_3 variant.

To our best knowledge, this was the foremost case–control study in China about the association of FCRL3 genetic polymorphisms with endometriosis-related infertility risk, in which 2 stratified analyses were also firstly conducted, respectively, in accordance with clinical symptoms (chronic pelvic pain, dysmenorrhea, and dyspareunia) and rASRM stage. Moreover, sample size of case subjects in the present study was relatively bigger than mean sample size of previous studies, partially leading to more convincing results that FCRL3\_3 mutation may promote the occurrence of endometriosis-related infertility, especially for patients with moderate/severe endometriosis. Finally, the first meta-analysis of previous studies coupled with the present study was implemented to further confirm our results, revealing that our conclusion in Chinese population is consistent with antecedent outcomes within Brazilian and Polish population.

However, the excessive consistence in results between our study and previous studies might be attributed to limited sample size in each ethnicity that has been investigated, in which characteristics of the selective subjects may not suffice to be representative of those of the overall population within a certain ethnicity. Furthermore, it was untenable to equate Chinese population with other ethnicities on the association of FCRL3 with endometriosis-related infertility owing to extra possible etiology of endometriosis-related infertility, such as discrepancy in environmental factors, individual genetic, lifestyle, reproductive risk factors, etc.<sup>44,45</sup> Therefore, to further substantiate the effect of FCRL3 mutation on susceptibility of endometriosis-related infertility in Asians and other ethnicities, large-scale and in-depth studies are in huge need.

In summary, the present study suggested that FCRL3\_3 variant was associated with an increased risk of endometriosis-related infertility, regardless of symptoms and rASRM stage of the patients. However, this association was more evident in the group of stage III–IV patients and patients with dyspareunia symptom. Meta-analysis of previous studies combined with the present study further confirmed our results. Further large-scale studies in the future are warranted to explore the association between FCRL3 genetic polymorphisms and endometriosis-

related infertility, as well as other human diseases, in Asian and other ethnicities.

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