ORIGINAL ARTICLE

A nonsynonymous variant of *IL1A* is associated with endometriosis in Japanese population

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Our previous genome-wide association study has demonstrated that single-nucleotide polymorphisms (SNPs) located in intronic and downstream regions of *IL1A* (interleukin 1 α) were associated with the risk of endometriosis. These SNPs on the genome-wide association study platform could be only surrogates for the true causal variant. Thus, we resequenced all the exons of *IL1A* in 377 patients with endometriosis and 457 healthy controls. We detected seven rare variants (minor allele frequency <0.01) and four common variants. All the rare variants were not associated with endometriosis. The four common variants (rs17561, rs1304037, rs2856836 and rs3783553) in *IL1A* were significantly associated with endometriosis ($P=0.0024$, 0.0024, 0.0014 and 0.0061, respectively). All the four SNPs were within a linkage disequilibrium block. Among them, only rs17561 was nonsynonymous (p.A114S), which has been reported to be associated with susceptibility to ovarian cancer. Taken together, we examined association between rs17561 and endometriosis in an independent validation data set (524 patients and 533 healthy controls) replicating significant association ($P=4.0 \times 10^{-5}$; odds ratio (OR), 1.91; 95% confidence interval (CI), 1.41–2.61). Meta-analysis by combining results from the two stages strengthened the evidence of association ($P=2.5 \times 10^{-7}$; OR, 1.90; 95% CI, 1.49–2.43). Our findings demonstrated that the nonsynonymous variant of *IL1A* might confer genetic susceptibility to endometriosis in Japanese population.

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INTRODUCTION

Endometriosis is an estrogen-dependent inflammatory disease and characterized by the presence of uterine endometrial tissue outside of the uterus. The main symptoms are pelvic pain, dysmenorrhea, pain in intercourse and infertility. The prevalence of endometriosis is 6–10% in women of reproductive age.¹ Endometriosis is a common complex disease caused by genetic and environmental factors and their interactions. Several studies demonstrated a familial aggregation of endometriosis, suggesting the influence of genetic factors on the pathogenesis of endometriosis.^{2–6} The incidence in first-degree relatives of women with endometriosis is seven times higher compared with that in women without family histories in USA.² The relative risk of endometriosis in female siblings is estimated to be 5.7 in Japan.⁶ The risk ratio of affected versus population prevalence is 3.58 for monozygotic twins and 2.32 for dizygotic twins in an Australian twins

study.⁴ The heritability of endometriosis was estimated to be 51%.⁴ Furthermore, severe symptoms of endometriosis were found more often in patients with positive family history than among those without.³

Encouraged by several lines of evidence supporting genetic contribution on endometriosis,^{2–6} much effort has been concentrated on identifying susceptibility genes by using candidate gene approaches and whole-genome linkage studies. Based on putative functional relevance to endometriosis, genes involved in estradiol metabolism, steroid-synthesizing pathways, inflammation, immune system, growth factor system, adhesion molecules, apoptosis, cell cycle regulation and tumorigenesis have been intensively studied. However, the systematic searches for published literatures that evaluate the associations between genetic variants and endometriosis based on these methods reveals that robustly replicated genetic associations have not been discovered.^{7,8}

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Recently, genome-wide association (GWA) studies using high-density single-nucleotide polymorphism (SNP) array have identified genetic susceptibility for many complex diseases^{9,10} including endometriosis.^{11–13} Uno *et al.*¹¹ have reported in their GWA study that rs10965325, which is located in *CDKN2BAS* on chromosome 9p21, was significantly associated with endometriosis in the Japanese population. Painter *et al.*¹³ identified the evidence of association with SNP rs12700667 located on intergenic region of chromosome 7p15. Suggestive evidence of association provided by Uno *et al.*, which was closely mapped to *WNT4* on chromosome 1p36, has been confirmed by European study.^{11,13} In our previous study, we confirmed that SNP rs17761446, which is in complete linkage disequilibrium (LD) with rs10965325 ($r^2 = 1$, HapMap JPT population (Phase III, Rel no. 2)), showed P -value = 8.9×10^{-3} with odds ratio (OR), 1.29 (95% confidence interval (CI), 1.10–1.48).^{11,12} On the other hand, we have previously demonstrated in our GWA study that four of the top five SNPs with P -values $< 10^{-5}$ were located in and around interleukin-1 alpha gene (*IL1A*).¹²

The *IL1a* encoded by *IL1A* is a member of the IL-1 cytokine family. This cytokine is involved in immune response, inflammatory process and hematopoiesis, and categorized into proinflammatory cytokines. The increased expression levels of several cytokines such as IL-1, 6, 8 and 10, tumor necrosis factor-alpha and vascular endothelial growth factor in the peritoneal fluid of women with endometriosis have been reported, which may stimulate the activity of the adhesion molecules and then enhance the implantation of fragments of menstrual endometrium on peritoneal surfaces.¹⁴ Therefore, *IL1A* might be involved in genetic susceptibility to endometriosis. The associated SNPs were located in intronic and downstream regions of *IL1A*. These SNPs on the genome-wide association study platform are presumably surrogate for the true causal variant. Therefore, our aim of the current study is to clarify the association between genetic alterations of *IL1A* and endometriosis in Japanese population.

MATERIALS AND METHODS

Subjects

All women with endometriosis were registered at the Niigata University Hospital, the Nagasaki University Hospital, the Kumamoto University Hospital, the Takarazuka City Hospital, the National Hospital Organization Kyoto Medical Center, the Nippon Medical School and several hospitals in Niigata, Toyama and Yamagata Prefecture. The endometriosis patients comprised 901 Japanese women who fulfilled any of the following diagnostic criteria: (i) women who underwent laparotomy or laparoscopic surgery for diseases other than endometriosis, each of which procedure was also used to provide biopsy proven evidence of endometriosis, (ii) women who were verified to have endometriosis in diagnostic laparoscopy and (iii) women who were diagnosed to have ovarian cysts by imaging diagnostics. The records of the revised American Fertility Society (rAFS) classification¹⁵ for 586 patients were available: Stage 1, 11; stage 2, 4; stage 3, 301; stage 4, 270. The majority of the patients with rAFS classification (97.4%) were grouped into moderate-to-severe endometriosis (rAFS stage 3 and 4).

All control samples for the association study were collected from 990 Japanese women from various resources as follows: (i) 621 fertile women or those with benign gynecological tumors, with no history of endometriosis diagnosed at Niigata University Hospital and (ii) 369 healthy women with no history of endometriosis diagnosed at Tokai University. In the control group, none of the women had any history of endometriosis or any of the following endometriosis-related symptoms: infertility, dysmenorrhea, hypermenorrhea or irregular menstruation.

The ethics committees of the participating institutions approved the study protocol, and each participant gave written informed consent.

DNA sample handling

Genomic DNA was extracted from peripheral blood lymphocytes per sample using a QIAamp DNA Blood Maxi Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's protocol.

Mutation screening and genotype analysis

The entire coding regions including seven exons and the intronic boundary regions of *IL1A* (NM_000575.3) were sequenced in both forward and reverse directions to detect unknown variants. Primer sequences and PCR amplification conditions are shown in Supplementary Table 1. The PCR products were sequenced by the dideoxy methods using the ABI BigDye Terminator v3.1 Cycle Sequencing kits on the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

For the replication study using an independent data set, TaqMan SNP genotyping assay was performed on ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). In brief, a PCR was conducted by using TaqMan Universal Master Mix (Applied Biosystems). PCR was conducted in a 5- μ l final reaction volume using 20 ng of genomic DNA. Thermal cycling conditions were 95 °C for 10 min, followed by 40 cycles of 92 °C for 15 s and 60 °C for 1 min.

Statistical analysis

Hardy–Weinberg equilibrium was tested using the χ^2 -test by comparing the expected and actual genotype frequencies by using the R statistical environment version 2.13.0. We conducted a two-stage association study to evaluate the association between genetic variants and endometriosis. We firstly examined association of all the variants detected in the entire region of *IL1A* using 377 cases and 457 controls. In the second stage, we analyzed the significantly associated and likely functioning variants in an independent data set including 524 cases and 533 controls. The association analysis was performed by using a 2×2 contingency table based on allele frequency. The statistical significance was evaluated by means of χ^2 -test, and OR and 95% CI were calculated. Finally, we performed a meta-analysis,¹⁶ where the per-allele ORs from the first and second stages were combined using both fixed-effects model and Dersimonian-Laird random-effects model meta-analyses.¹⁷ Homogeneity across studies was examined by Cochran's Q test at the significance level of 0.1.¹⁸ The extent of between-study heterogeneity was quantified by I^2 .^{19,20} I^2 values over 50% indicate large heterogeneity. The meta-analysis was performed by using STATA version 11.0 (StataCorp, College Station, TX, USA).

Pairwise LD between eight SNPs (four SNPs reported in the previous study and four SNPs detected in this study)¹² was estimated using D' statistics, and the results were visualized using Haploview software v4.2 (<http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview>).

RESULTS

To identify novel genetic alterations of *IL1A* in Japanese population, we resequenced all of the *IL1A* exons using 377 samples from Japanese patients with endometriosis and 457 samples from healthy controls. We identified 10 single-nucleotide and one deletion variants, which were not in our previous genome-wide association study platform. Among seven rare variants (minor allele frequency in controls < 0.01), six variants were novel (not recorded in dbSNP Build 137) but one was known nonsynonymous variant (rs79919093) (Table 1). All the rare variants did not show statistically significant associations with endometriosis (Table 1). It was partly due to the limited sample size of this study to evaluate the associations of these rare variants. For the common variants (rs17561, rs1304037, rs2856836 and rs3783553), we confirmed that the genotypes were in the Hardy–Weinberg equilibrium in controls ($P > 0.05$). The allele frequencies of these common variants (rs17561, rs1304037, rs2856836 and rs3783553) were significantly different between patients with endometriosis and controls ($P = 0.0024$, 0.0024, 0.0014 and 0.0061, respectively) (Table 2).

We investigated LD structure among eight variants, of which four SNPs were reported in Adachi *et al.*¹² located in intronic and downstream regions of *IL1A*. For the analysis of LD structure, we analyzed genotype data of patients with endometriosis that were used in both previous⁸ and current studies. As shown in Supplementary Figure 1, these eight SNPs were located in the same LD block, indicating that these eight SNPs represented the same association signal. We could not statistically distinguish the effect of these SNPs due to high LD. Among them, the SNP rs17561 results in amino acid substitution (p.A114S), which was predicted to be deleterious by PolyPhen-2.²¹ Therefore, it could serve as a plausible candidate for disease susceptibility. Thus, only rs17561 was considered in further analyses.

We examined replication study to validate the association between rs17561 and endometriosis by using an independent data set of 524 endometriosis cases and 533 controls. The frequency of the rs17561 G allele was higher in women with endometriosis than that in controls (4.0×10^{-5} ; OR, 1.91; 95% CI, 1.41–2.61) (Table 3). We did not observe significant heterogeneity between the results from the original and validation studies in terms of Cochran's Q test ($P = 0.95$) and I^2 index ($I^2 = 0.0$) (Table 3), indicating the consistency of the results

from these two studies. By combining the results from these studies with the meta-analysis, the association between *IL1A* rs17561 and endometriosis was reinforced ($P = 2.5 \times 10^{-7}$; OR, 1.90; 95% CI, 1.49–2.43) (Table 3).

It is reported that genetic associations were stronger for patients with severe endometriosis than those with mild endometriosis.¹³ Therefore, we evaluated the effect of SNP rs17561 on disease severity by excluding patients with minimal endometriosis (rAFS stage 1 and 2 disease) and patients without rAFS records. The OR was stronger for patients with moderate-to-severe endometriosis (rAFS stage 3 and 4) ($P = 1.5 \times 10^{-6}$; OR, 2.06; 95% CI, 1.53–2.77).

DISCUSSION

In this study, we sought functionally plausible candidate variant in coding region of *IL1A* for susceptibility to endometriosis. We validated the association of genetic variations of *IL1A* with endometriosis using independent data sets, and demonstrated that the nonsynonymous variant (rs17561, p.A114S) of *IL1A* was significantly involved in endometriosis in the Japanese population.

GWA studies are designed to search the whole-genome for common genetic variations that contribute to common diseases and more than 2000 common variants have been linked to common diseases thus far.²² GWA studies are generally based on the common disease-common variant hypothesis that the variants being sought are relatively high frequencies and associated with the disease. Recently, 'common disease-multiple rare variants' hypothesis that multiple rare variants underlie susceptibility to common diseases, is proposed,²³ and several studies have already shown the relevance of rare variants in complex diseases.^{24,25} In this study, we could not identify rare variant of *IL1A* conferring a causality of endometriosis.

IL1a encoded by *IL1A* is one of representative inflammatory cytokines. As the essence of endometriosis is a pelvic inflammatory process, cytokines including IL1a could have important roles in the development and progression of endometriosis.^{26,27} Interestingly, it is known that IL1a levels in peritoneal fluid and serum are higher in women with endometriosis than those in women without the disease,^{28–30} and, therefore, functional alterations of IL1a might influence the pathogenesis of endometriosis. Our results showed that there were significant differences in the allelic frequencies of three SNPs

Table 1 Allele frequencies of rare variants of *IL1A* in Japanese endometriosis patients and Controls

Location	Nucleotide change	Codon	Rare variant frequency		NCBI database ^a
			Case (n = 377)	Control (n = 457)	
Exon 4	c.134A>T	Glu45Val	0	0.0043	Novel
Exon 5	c.315A>G	Asn102Ser	0.0027	0.0021	rs79919093
3'-UTR	c.816+45T>C	None	0.0053	0.0043	Novel
3'-UTR	c.816+162T>C	None	0.0027	0	Novel
3'-UTR	c.816+216A>G	None	0.0027	0.0021	Novel
3'-UTR	c.816+413delT	None	0	0.0021	Novel
3'-UTR	c.816+897_898GC>AT	None	0.0027	0	Novel

Abbreviations: *IL1A*, interleukin-1 alpha gene; NCBI, National Center for Biotechnology Information; UTR, untranslated region.

^aDatabase search was performed using NCBI dbSNP Build 137.

Table 2 Allele frequencies of the *IL1A* polymorphisms in Japanese endometriosis patients and controls

SNP	Localization	Variation	Risk allele	Risk allele frequency			
				Cases (n = 377)	Controls (n = 457)	OR (95% CI)	P-value
rs17561	Exon 5	G/T	G	0.951	0.911	1.88 (1.26–2.82)	0.0024
rs1304037	3'-UTR	A/G	A	0.951	0.911	1.88 (1.26–2.82)	0.0024
rs2856836	3'-UTR	T/C	T	0.951	0.909	1.94 (1.30–2.89)	0.0014
rs3783553	3'-UTR	-TTCA	—	0.804	0.746	1.40 (1.10–1.76)	0.0061

Abbreviations: CI, confidence interval; *IL1A*, interleukin-1 alpha gene; OR, odds ratio; SNP, single-nucleotide polymorphisms; UTR, untranslated region.

Table 3 Meta-analysis of results from original and replication studies for the association between *IL1A* SNP rs17561 and endometriosis

Original			Replication			Combined ^a			Heterogeneity	
OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value	Cochran's Q	I^2
1.88	1.26–2.82	2.4×10^{-3}	1.91	1.41–2.61	4.0×10^{-5}	1.90	1.49–2.43	2.5×10^{-7}	0.95	0.0

Abbreviations: CI, confidence interval; *IL1A*, interleukin-1 alpha gene; OR, odds ratio; SNP, single-nucleotide polymorphisms; UTR, untranslated region.

^aResults from fixed-effects model and DerSimonian-Laird random effects model meta-analyses were identical because of low between-study heterogeneity.

(rs1304037, rs2856836, and rs3783553) in the 3'-untranslated region between cases and controls. It is possible that SNPs in the 3'-untranslated region can alter protein expression by affecting stability of the mRNA.²⁹ We focused on the nonsynonymous variant of *IL1A* (rs17561) showing strong significant association with endometriosis ($P = 2.5 \times 10^{-7}$; OR, 1.90; 95% CI, 1.49–2.43 in the meta-analysis). We found that the SNP showed stronger association signal for patients with moderate-to-severe endometriosis (rAFS stage 3 and 4) ($P = 1.5 \times 10^{-6}$; OR, 2.06; 95% CI, 1.53–2.77). The variant results in A114S, which is a substitution at proform sequence. Although three dimensional structure of IL1a mature protein is known, functional significance of the proform variant is not clear. The amino acid substitution (p.A114S) was predicted to be damaging by PolyPhen-2.²¹ The A114S might be possible to affect processing efficiency but the functional approach was not applied in the current study. Extensive studies on protein function in endometriotic cells are necessary to clarify the roles of IL1a in endometriosis.

Patients with endometriosis not only suffer from various symptoms such as severe dysmenorrhea, deep dyspareunia, chronic pelvic pain, ovulation pain and infertility but also have the risk of malignant transformation of endometriosis.^{1,31} In fact, ~1.0% of women with endometriosis have lesions that undergo malignant transformation.³² White *et al.*³³ recently reported that inherited inflammatory related variants are associated with ovarian cancer risk in a multisite case-control study. Very intriguingly, *IL1A* rs17561, which is the susceptibility to endometriosis, is also associated with increased risk of clear cell, mucinous and endometrioid histologic type, but not with the most common serous subtype.³³ Especially, it is well known that there is a strong link between clear cell or endometrioid histologic type and endometriosis,³⁴ *IL1A* rs17561 might be associated with the risk of malignant transformation of endometriosis.

Clarifying biological significance of variants of *IL1A* in endometriosis may lead to understanding of the pathogenesis of endometriosis and can be utilized as one of targets for the therapeutic development strategies in clinical management of endometriosis.

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- Giudice, L. C. & Kao, L. C. Endometriosis. *Lancet* **364**, 1789–1799 (2004).
- Simpson, J. L., Elias, S., Malinak, L. R. & Buttram, V. C. Jr Heritable aspects of endometriosis. I. Genetic studies. *Am. J. Obstet. Gynecol.* **137**, 327–331 (1980).
- Moen, M. H. & Magnus, P. The familial risk of endometriosis. *Acta. Obstet. Gynecol. Scand.* **72**, 560–564 (1993).
- Treloar, S. A., O'Connor, D. T., O'Connor, V. M. & Martin, N. G. Genetic influences on endometriosis in an Australian twin sample. *Fertil. Steril.* **71**, 701–710 (1999).
- dos Reis, R. M., de Sa, M. F., de Moura, M. D., Nogueira, A. A., Ribeiro, J. U., Ramos, E. S. *et al.* Familial risk among patients with endometriosis. *J. Assist. Reprod. Genet.* **16**, 500–503 (1999).
- Kashima, K., Ishimaru, T., Okamura, H., Suginami, H., Ikuma, K., Murakami, T. *et al.* Familial risk among Japanese patients with endometriosis. *Int. J. Gynaecol. Obstet.* **84**, 61–64 (2004).

- Montgomery, G. W., Nyholt, D. R., Zhao, Z. Z., Treloar, S. A., Painter, J. N., Missmer, S. A. *et al.* The search for genes contributing to endometriosis risk. *Hum. Reprod. Update* **14**, 447–457 (2008).
- Rahmiloglu, N., Missmer, S. A., Montgomery, G. W. & Zondervan, K. T. Insights into Assessing the Genetics of Endometriosis. *Curr. Obstet. Gynecol. Rep.* **1**, 124–137 (2012).
- Manolio, T. A., Brooks, L. D. & Collins, F. S. A HapMap harvest of insights into the genetics of common disease. *J. Clin. Invest.* **118**, 1590–1605 (2008).
- Altshuler, D., Daly, M. J. & Lander, E. S. Genetic mapping in human disease. *Science* **322**, 881–888 (2008).
- Uno, S., Zembutsu, H., Hirasawa, A., Takahashi, A., Kubo, M., Akahane, T. *et al.* A genome-wide association study identifies genetic variants in the CDKN2BAS locus associated with endometriosis in Japanese. *Nat. Genet.* **42**, 707–710 (2010).
- Adachi, S., Tajima, A., Quan, J., Haino, K., Yoshihara, K., Masuzaki, H. *et al.* Meta-analysis of genome-wide association scans for genetic susceptibility to endometriosis in Japanese population. *J. Hum. Genet.* **55**, 816–821 (2010).
- Painter, J. N., Anderson, C. A., Nyholt, D. R., Macgregor, S., Lin, J., Lee, S. H. *et al.* Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. *Nat. Genet.* **43**, 51–54 (2011).
- Bulun, S. E. Endometriosis. *N. Engl. J. Med.* **360**, 268–279 (2009).
- American Society for Reproductive Medicine. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil. Steril.* **67**, 817–821 (1997).
- Nakaoka, H. & Inoue, I. Meta-analysis of genetic association studies: methodologies, between-study heterogeneity and winner's curse. *J. Hum. Genet.* **54**, 615–623 (2009).
- DerSimonian, R. & Laird, N. Meta-analysis in clinical trials. *Control. Clin. Trials* **7**, 177–188 (1986).
- Cochran, W. G. The combination of estimates from different experiments. *Biometrics* **10**, 101–129 (1954).
- Higgins, J. P. & Thompson, S. G. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* **21**, 1539–1558 (2002).
- Higgins, J. P., Thompson, S. G., Deeks, J. J. & Altman, D. G. Measuring inconsistency in meta-analyses. *BMJ* **327**, 557–560 (2003).
- Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P. *et al.* A method and server for predicting damaging missense mutations. *Nat. Methods* **7**, 248–249 (2010).
- Hindorf, L. A., Sethupathy, P., Junkins, H. A., Ramos, E. M., Mehta, J. P., Collins, F. S. *et al.* Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc. Natl Acad. Sci. USA* **106**, 9362–9367 (2009).
- Cirulli, E. T. & Goldstein, D. B. Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nat. Rev. Genet.* **11**, 415–425 (2010).
- Coppola, G., Chinnathambi, S., Lee, J. J., Dombroski, B. A., Baker, M. C., Soto-Ortolaza, A. I. *et al.* Evidence for a role of the rare p.A152T variant in MAPT in increasing the risk for FTD-spectrum and Alzheimer's diseases. *Hum. Mol. Genet.* **21**, 3500–3512 (2012).
- Sidransky, E., Nalls, M. A., Aasly, J. O., Aharon-Peretz, J., Annesi, G., Barbosa, E. R. *et al.* Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N. Engl. J. Med.* **361**, 1651–1661 (2009).
- Kajihara, H., Yamada, Y., Kanayama, S., Furukawa, N., Noguchi, T., Haruta, S. *et al.* New insights into the pathophysiology of endometriosis: from chronic inflammation to danger signal. *Gynecol. Endocrinol.* **27**, 73–79 (2011).
- Sikora, J., Mielczarek-Palacz, A. & Kondera-Anasz, Z. Imbalance in cytokines from interleukin-1 family - role in pathogenesis of endometriosis. *Am. J. Reprod. Immunol.* **68**, 138–145 (2012).
- Kondera-Anasz, Z., Sikora, J., Mielczarek-Palacz, A. & Jonca, M. Concentrations of interleukin (IL)-1alpha, IL-1 soluble receptor type II (IL-1 sRII) and IL-1 receptor antagonist (IL-1 Ra) in the peritoneal fluid and serum of infertile women with endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **123**, 198–203 (2005).
- Nackley, A. G., Shabalina, S. A., Tchivileva, I. E., Satterfield, K., Korchynskyi, O., Makarov, S. S. *et al.* Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* **314**, 1930–1933 (2006).
- Koumantakis, E., Matalliotakis, I., Neonaki, M., Froudarakis, G. & Georgoulas, V. Soluble serum interleukin-2 receptor, interleukin-6 and interleukin-1a in patients with endometriosis and in controls. *Arch. Gynecol. Obstet.* **255**, 107–112 (1994).
- de Ziegler, D., Borghese, B. & Chapron, C. Endometriosis and infertility: pathophysiology and management. *Lancet* **376**, 730–738 (2010).
- Higashiura, Y., Kajihara, H., Shigetomi, H. & Kobayashi, H. Identification of multiple pathways involved in the malignant transformation of endometriosis (Review). *Oncol. Lett.* **4**, 3–9 (2012).
- White, K. L., Schildkraut, J. M., Palmieri, R. T., Iversen, E. S. Jr, Berchuck, A., Vierkant, R. A. *et al.* Ovarian cancer risk associated with inherited inflammation-related variants. *Cancer Res.* **72**, 1064–1069 (2012).
- Pearce, C. L., Templeman, C., Rossing, M. A., Lee, A., Near, A. M., Webb, P. M. *et al.* Association between endometriosis and risk of histological subtypes of ovarian cancer: a pooled analysis of case-control studies. *Lancet Oncol.* **13**, 385–394 (2012).

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