***BRCA1* alterations are associated with endometriosis, but *BRCA2* alterations show no detectable endometriosis risk: a study in Indian population**

Suresh Govatati · Kiran Challa · Sunita B. Reddy ·
Kayathi Pramod · Mamata Deenadayal ·
Baidyanath Chakravarty · Sisinthy Shivaji · Manjula Bhanoori

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Abstract

Purpose To investigate the role of genetic variations and expression alterations of *BRCA1* and *BRCA2* genes in the pathophysiology of endometriosis.

Methods A genetic association study was conducted in 573 endometriosis cases and 490 controls of Indian origin. We genotyped 13 selected promoter SNPs of *BRCA1* gene and 2 selected promoter SNPs of *BRCA2* gene by PCR-sequencing analysis. In addition, to better understand genetic

Capsule *BRCA1* alterations may modify the endometriosis risk in Indian women. The analysis of *BRCA1* genetic variants and/or expression might help to identify patients at high risk for disease outcome.

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S. Govatati · S. B. Reddy · K. Pramod · M. Bhanoori (✉)
Department of Biochemistry, Osmania University, Hyderabad 500
007, India
e-mail: bhanoorim@yahoo.co.in

K. Challa
Department of Genetics, Osmania University, Hyderabad 500 007,
India

M. Deenadayal
Infertility Institute and Research Centre (IIRC), Secundrabad 500
003, India

B. Chakravarty
Institute of Reproductive Medicine (IRM), Kolkata 700 064, India

S. Shivaji
Centre for Cellular and Molecular Biology (CCMB), Hyderabad 500
007, India

S. Shivaji
Jhaveri Microbiology Centre, L V Prasad Eye Institute,
Hyderabad 500034, India

contributions to the pathophysiology of endometriosis, the expression pattern of *BRCA1* & 2 was analyzed in the eutopic endometria of endometriosis cases and controls by western-blot and immunohistochemical analysis.

Results Our results revealed significant association between *BRCA1* rs71361504 (–/GTT) SNP and endometriosis risk in Indian women ($P<0.0001$), while the remaining SNPs of both *BRCA1* & 2 genes showed no difference between cases and controls. Western-blot and immunohistochemical analysis revealed significantly decreased *BRCA1* expression levels in eutopic endometria of patients compared with controls ($P<0.05$). Furthermore, nuclear *BRCA1* was frequently lost compared with cytoplasmic *BRCA1* in eutopic endometria of patients. Expression of *BRCA2* did not differ between patients and controls.

Conclusions *BRCA1* rs71361504 SNP may modify the endometriosis risk in Indian women. In addition, decreased expression of *BRCA1* may play an important role in the pathophysiology of endometriosis. The analysis of *BRCA1* genetic variants and/or expression might help to identify patients at high risk for disease outcome.

Keywords Endometriosis · *BRCA1* · *BRCA2* · SNP · Indian women

Introduction

Endometriosis (MIM 131200) is a complex gynaecological disorder characterized by the presence of endometrium-like tissue outside the uterine cavity [1]. The disease affects ~5–10 % of women of reproductive age and up to 30–50 % of women with infertility. Although endometriosis is a benign lesion, it often displays characteristics that are associated with

malignancy. It can invade into the abdominal tissues and the viscera and has a ‘metastatic pattern’ of spread [2]. The causes of endometriosis remain uncertain despite over 100 years of hypothesis-driven research. Earlier, we had demonstrated the correlation between various candidate genes and risk of endometriosis in Indian women [3–12]. The emerging evidence strongly suggests that the disease has a polygenic and multifactorial basis [13].

BRCA1 (MIM 113705; breast cancer 1, early onset) and *BRCA2* (MIM 600185; breast cancer 2, early onset) are caretaker genes of human genome and are important tumor suppressor genes (TSGs) located at *17q21* and *13q12.3* loci respectively. Frequent allelic loss at these loci and single nucleotide polymorphisms (SNPs) and/or mutations in *BRCA1* & 2 genes have been reported in the development and progression of various human tumors [14–16], however reports related to endometriosis are very few [17].

The *BRCA1* (198.796 kDa) and *BRCA2* (384 kDa) proteins have very different primary sequences but both plays an important role in DNA repair and maintenance of genome stability [18]. *BRCA1* by interacting with other tumor suppressors, DNA damage sensors, signal transducers, RNA polymerase II, and histone deacetylase complexes, plays a critical role in DNA repair of double-stranded breaks, ubiquitination, and transcriptional regulation [18, 19]. Along with the *BRCA1*, *BRCA2* binds the single strand DNA and directly interacts with the recombinase *RAD51* to stimulate strand invasion, a vital step of homologous recombination. By repairing DNA, *BRCA1* & 2 plays a role in maintaining the stability of the human genome and prevents dangerous gene rearrangements that can lead to tumorigenesis. Alterations in the expression and cellular distribution of *BRCA1* & 2 have been reported in various tumors [20–24], but no reports are documented in endometriosis. The present study was conducted to analyze the association between sequence variants in the promoter region of *BRCA1* & 2 genes and endometriosis risk in two ethnically different Indian populations. In addition, to better understand genetic contributions to the pathophysiology of endometriosis, the expression pattern of *BRCA1* & 2 was analyzed in phase matched eutopic endometria of endometriosis patients and controls.

Materials and methods

Subjects and samples for genotyping of *BRCA1* & 2 genetic variants

Two independent case–control groups were recruited from two ethnically different linguistic groups of Indian subcontinent [25]. A total of 573 pre-menopausal unrelated Indian

women with moderate-severe (III–IV) endometriosis staged using the revised American Fertility Society classification system [26], were recruited at the Infertility Institute and Research Centre (IIRC), Secundrabad, India, (South Indian or Dravidian linguistic group, $n=295$) and Institute of Reproductive Medicine (IRM), Kolkata, India (North Indian or Indo-European linguistic group, $n=278$). All women had a trans-vaginal ultrasound scan (TVS) at screening followed by a laparoscopy to confirm the diagnosis (rAFS III = 211; IV = 362). All the patients had different forms of endometriosis such as peritoneal lesions, adhesions and endometrioma. Women with other ovarian cysts, adenomyosis, ovarian cancer, fibroids, and stage I and II endometriosis were excluded from the study. The aim was to focus on patients with more severe endometriosis (Stage III and IV) because the more severe forms include ovarian cystic disease, which almost certainly has a different etiology to peritoneal forms, and the diagnosis is usually unequivocal, which is not the case for Stage I and II. Their mean age $\pm$ SD was 26.8 ± 2.5 (range 20 to 40) years. All the patients complained of dysmenorrhoea (mild = 42 %; moderate 33 %; severe 25 %) and 78 % had dyspareunia. Most women (97.4 %) were infertile (primary 84 %; secondary 16 %).

Four hundred and 90 women (245 of each from IRM and IIRC) were recruited from the same clinic population and had an equal opportunity to be identified as cases, thereby meeting the criteria for appropriate controls set by Zondervan et al. [27]. The controls consisted of 428 (87.3 %) women with no evidence of endometriosis on TVS and laparoscopy and 62 (12.7 %) women with no evidence of an ovarian endometrioma on TVS (and no clinical symptoms of endometriosis) who therefore did not subsequently had a laparoscopy. Their mean age $\pm$ SD was 27.9 ± 3 (range 22 to 40). Seventy-four percent had primary and 26 % had secondary infertility. Among the symptomatic controls, 26 % complained of mild dysmenorrhoea and 25 % complained of dyspareunia.

Peripheral blood samples (5 ml) were collected from both groups in EDTA vacutainers and stored at -80°C until further use.

Subjects and samples for the analysis of *BRCA1* & 2 expression

Secretory phase endometrial tissue was obtained from the uterine fundus of endometriosis patients ($n=5$) and controls ($n=4$) by curettage at the time of laparoscopy. Each sample was divided into two portions: one was immediately immersed in 4 % paraformaldehyde in 0.05 M of Tris–HCl buffer (pH=7.4) at 4°C for at least 24 h and embedded in paraffin for histological examination. Another portion was rinsed in ice cold phosphate-buffered saline (PBS) on ice to remove excess blood and then rapidly frozen in liquid

nitrogen and stored at 70 °C for subsequent western immunoblot assay. Histological assessment of endometrial morphology was carried out using standard criteria [28].

Ethical approval

Written informed consent was obtained from all participants. The Institutional Review Board of the Centre for Cellular and Molecular Biology, Hyderabad, approved the study.

DNA extraction and determination of *BRCA1* & 2 genotypes

DNA was extracted from peripheral blood samples following method described elsewhere [29]. Genotyping was carried out in a randomized, blinded fashion by PCR-Sequencing analysis as per the protocols described earlier [6]. PCRs were carried out in a total volume of 25 µl, containing 50 ng genomic DNA, 2–6 pmole of each primer, 1X Taq polymerase buffer (1.5 mM MgCl₂) and 0.25 U of Amplitaq DNA polymerase (Perkin Elmer, USA). The primers and PCR conditions used were given in Table 1. The PCR products of 293 bp (*BRCA1*) and 230 bp (*BRCA2*) were analysed using 1.5 % agarose gel and were then sequenced with a Taq-Dye deoxy-terminator cycle sequencing kit (Applied BioSystems, USA) using an automated ABI 3770 DNA sequencer (Applied BioSystems, USA). Genotype calling was performed using Chromas V.2 software (Technelysium Ltd., Australia).

Immunohistochemical analysis

Immunohistochemical analysis of *BRCA1* and *BRCA2* was performed on the sections of secretory phase eutopic endometria from cases ($n=5$) and controls ($n=4$) as per the protocols described earlier [3]. Briefly, after incubation with primary antibody (*BRCA1* (1:200), *BRCA2* (1:250) obtained from Santa Cruz Biotechnology, USA) for 4 h at room temperature, tissue sections were washed in PBS and incubated with horse radish peroxidase-conjugated secondary goat anti-rabbit antibody (1:1,000, DAKO Envision™ + System, DAKO Corp.) for 30 min. Then the sections were washed and treated with 3-3'-

diaminobenzidine (DAB) chromogen for 5–10 min, counterstained with hematoxylin, and mounted with DPX. Negative controls were obtained by substituting primary antibodies with nonimmune rabbit serum. Staining intensity was scored for each tissue and each antibody using Image J software. All individual tissue samples were stained at one time for consistency and quantitation was done blinded to pathology information.

Western blot analysis

Protein extraction and Western blot analysis were performed as per the protocol described by Bhanoori et al. [9] using secretory phase eutopic endometria from cases ($n=5$) and controls ($n=4$). Briefly, after blocking with 5 % (w/v) nonfat milk in TBST (Tween 0.1 % (v/v) in TBS) for 1 h at room temperature, the membranes were washed and incubated with the primary antibody (*BRCA1* (1:1,000), *BRCA2* (1:1,000) obtained from Santa Cruz Biotechnology, USA) prepared in TBST containing BSA 1 % (w/v) for 2 h. After the incubation period, the membranes were washed and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (1:10,000, Sigma) for 1 h at room temperature. Then, the membranes were washed and the antigen–antibody complexes were detected using a chemiluminescence reagent kit (GE health care, USA). Band intensity in the western blots was determined using imageJ software.

Statistical analysis

Statistical analysis was performed using SPSS statistical package (V 11.0). The genotype distribution among subjects was tested for Hardy–Weinberg equilibrium (HWE) using Fisher's exact test. The allele ratios and genotype distributions of cases and controls were analysed with Pearson χ^2 or Fisher's exact test. The odds ratio and 95 % confidence interval (CI) values were calculated using the online Vassar Stats Calculator (www.faculty.vassar.edu/lowry/Vassar-Stats.html). The Student's *t*-test was used to analyze differences between immunoblot band densities; $P<0.05$ was considered statistically significant based on three or more independent experiments.

Table 1 Primers and PCR conditions used for the genotyping of *BRCA1* and *BRCA2* promoter polymorphisms

Gene	Primers (5' to 3')	Amplicon size	PCR conditions
<i>BRCA1</i>	F: CCACTCTTTCCGCCCTAA R: AAGGCCTAGTTTCTGCTT	293 bp	5 min at 95 °C; “40 s at 94 °C, 30 s at 56 °C, 50 s at 72 °C (35 cycles)”; and 10 min at 72 °C
<i>BRCA2</i>	F: AAGCGTGAGGGGACAGA R: GACCTTTCTCTCAGGCATG	230 bp	5 min at 95 °C; “40 s at 94 °C, 30 s at 58 °C, 50 s at 72 °C (35 cycles)”; and 10 min at 72 °C

Results

Genotyping of *BRCA1* polymorphisms

In the present study we genotyped 13 selected promoter polymorphisms of *BRCA1* gene (Table 2) by PCR-sequencing analysis. We identified polymorphism at 2 (rs71361504 and rs3092986) out of 13 SNP sites (Fig. 1a and b) while all the remaining variants (11/13) were monomorphic in both patients and controls (Table 2).

rs71361504 (-/GTT) polymorphism

The genotype and allele distribution of *BRCA1* rs71361504 (-/GTT) SNP revealed significant differences between patients and controls (all '*P*' values <0.0001; Table 3). There was significant reduction of the wild genotype (-/-) frequency and elevation of the mutant genotype (GTT/GTT) frequency in patients as compared to controls (Table 3). The allele frequency also showed a similar trend indicating that the addition of 'GTT' allele might confer risk to endometriosis.

rs3092986 (T/C) polymorphism

The frequencies of genotypes (*P*=0.059) and alleles (*P*=0.109) of the rs3092986 (T/C) polymorphism were not significantly different between cases and controls (Table 3). The genotype and allele distribution showed high prevalence of wild type allele (T) both in cases and controls.

The people from Indian subcontinent have diverse ethnicity [25], so we performed statistical analysis independently for both North (Indo-European) & South Indian (Dravidian) women (Supplemental Tables 1 and 2). Our study provides very good evidence of ethnic differences leading to different association statuses. The analysis surprisingly revealed two different scenarios in the two groups. We found no association between the rs3092986 (T/C) polymorphism and endometriosis in Indo-European group (Supplemental Table 1); however, the Dravidian group showed strong association of this polymorphism with endometriosis (*P*=0.0115; Supplemental Table 2). The study, apart from presenting useful data for these populations of India, also brings forward important issue of variation in association levels due to ethnicity.

Genotyping of *BRCA2* polymorphisms

In the present study we genotyped 2 selected promoter SNPs of *BRCA2* gene by PCR-sequencing analysis. We identified polymorphism at rs206118 A/G (13:32889792) SNP site while the remaining variant (rs56211573 G/C; 13:32889829) was monomorphic in both patients and controls (Table 2).

rs206118 (A/G) polymorphism

Among both cases and controls the genotype distributions as well as the allele system of rs206118 (A/G) polymorphism were all in Hardy–Weinberg equilibrium (*P*>0.05). Sequence analysis of 230 bp PCR product of the rs206118 (A/G)

Table 2 SNPs genotyped in the present study

Serial number	dbSNP ID	Location	NT ^a	Ambiguity code	MAF ^b	SNP Status
BRCA1						
1	rs199689700	17:41278010	A/G	R	–	Absent
2	rs71361504	17:41278009–41278010	–/GTT	–	–	Present
3	rs8176071	17:41278005–41278006	–/TGT/T	–	–	Absent
4	rs111292942	17:41278005–41278006	–/TGT	–	–	Absent
5	rs34085552	17:41278004–41278005	–/GT	–	–	Absent
6	rs73625095	17:41277998	G/A	R	–	Absent
7	rs3092986	17:41277996	T/C	Y	0.0285	Present
8	rs8176072	17:41277944	A/T	W	0.0014	Absent
9	rs184820044	17:41277854	G/A	R	0.0005	Absent
10	rs73625094	17:41277853	G/T/C	B	0.0014	Absent
11	rs192782245	17:41277816	G/C	S	0.0005	Absent
12	rs144689288	17:41277812–41277813	–/G	–	–	Absent
13	rs8176074	17:41277765	C/T	Y	0.0018	Absent
BRCA2						
1	rs206118	13:32889792	A/G	R	0.156	Present
2	rs56211573	13:32889829	G/C	S	–	Absent

^a Nucleotide change

^b Minor allele frequency

Fig. 1 Genotyping of the *BRCA* polymorphisms: **a** *BRCA1* rs71361504 (–/GTT); **b** *BRCA1* rs3092986 (T/C); **c** *BRCA2* rs206118 (A/G; Reverse strand sequence shown)

Table 3 Genotype and allele distribution of *BRCA1* (rs71361504; rs3092986) and *BRCA2* (rs206118) promoter polymorphisms in endometriosis patients and controls of Indian origin

Genotypes/Alleles	Cases			Controls <i>n</i> =490 (%)	<i>P</i> - value	Odds ratio	95 % CI
	Stage III <i>n</i> =211(%)	Stage IV <i>n</i> =362(%)	Total <i>n</i> =573(%)				
rs71361504							
Genotypes					<0.0001 ^a		
W/W	65 (30.8)	103 (28.5)	168 (29.3)	191 (39)			
W/M	102 (48.3)	151 (41.7)	253 (44.2)	237 (48.4)			
M/M	44 (20.9)	108 (29.8)	152 (26.5)	62 (12.6)			
Alleles					<0.0001 ^b	0.616	0.5182 to 0.7339
W	232 (55)	357 (49.3)	589 (51.4)	619 (63.2)			
M	190 (45)	367 (50.7)	557 (48.6)	361 (36.8)			
rs3092986							
Genotypes					0.059 ^a		
T/T	197 (93.4)	331 (91.4)	528 (92.2)	456 (93.1)			
T/C	07 (3.3)	19 (05.2)	26 (04.5)	28 (05.7)			
C/C	07 (3.3)	12 (3.4)	19 (03.3)	06 (01.2)			
Alleles					0.109 ^b	0.719	0.48 to 1.0782
T	401 (95.0)	681 (94.1)	1082 (94.4)	940 (95.9)			
C	21 (05.0)	43 (05.9)	64 (05.6)	40 (04.1)			
rs206118							
Genotypes					0.412 ^a		
A/A	154 (72.9)	258 (71.3)	412 (71.9)	358 (73.1)			
A/G	46 (21.8)	80 (22.1)	126 (21.9)	111 (22.6)			
G/G	11 (5.2)	24 (6.6)	35 (6.1)	21 (4.3)			
Alleles					0.353 ^b	0.896	0.7117 to 1.1298
A	354 (83.9)	596 (82.3)	950 (82.9)	827 (84.4)			
G	68 (16.1)	128 (17.7)	196 (17.1)	153 (15.6)			

CI Confidence Interval, W Wild, M Mutant ('GTT' addition)

^a Fisher's exact test (3×2 table at 2 df) *P*<0.05

^b Fisher's exact test (2×2 table at 1 df) *P*<0.05

polymorphism is shown in Fig. 1c. Homozygotes (*A/A* & *G/G*) appear as a single peak, whereas heterozygote (*A/G*) as a double peak. In both patients and controls, the allelic pattern showed dominance of the 'A' allele than 'G'. There was no statistically significant difference in the frequencies of genotypes ($P=0.412$) and alleles ($P=0.353$) between the cases and controls (Table 3).

Furthermore, we performed statistical analysis independently for both North (Indo-European) & South Indian (Dravidian) women (Supplemental Tables 1 and 2). The genotype and allele frequencies were not much different between two groups and were not significantly associated with endometriosis.

Expression analysis of *BRCA1* & 2

Immunohistochemical analysis

Our results showed different pattern of BRCA1 staining between case–control tissues (Fig. 2a). BRCA1 immunoreactivity was strong and nearly homogeneous in eutopic endometria of controls, while less and heterogeneous in patients ($P<0.0001$). Furthermore, nuclear BRCA1 was frequently lost compared with cytoplasmic BRCA1 in eutopic endometria of patients. BRCA2 immunoreactivity was predominantly detected in cytoplasm of endometrial glands compared with stroma. The degree of BRCA2 immunostaining did not appear to differ in cases versus controls ($P>0.05$; Fig. 2b).

Western blot analysis

Western blot results confirmed our immunohistochemistry data. The level of BRCA1 expression in eutopic endometria of patients was significantly lower than in controls ($P=0.044$;

Fig. 3a), while the level of BRCA2 did not appear to vary between case and control tissues ($P>0.05$; Fig. 3b).

Discussion

Single nucleotide polymorphisms (SNPs) are common DNA sequence variations among individuals, which play a significant role in the development of several human diseases including cancer. SNPs particularly in protein encoding regions and promoters play an important role in altering gene function and/or transcriptional efficiency. Mutations and SNPs of *BRCA1* and *BRCA2* genes have been reported in various human diseases [14, 30], however only one report was documented in endometriosis. Goumenou et al. [17] have analyzed *BRCA1* (exons 2, 5, 11, 18, 20 and 24) *BRCA2* genes (exons 2, 3, 9, 10 and 11) in endometriosis patients and reported no significant association. In the present study, we detected significant association between *BRCA1* rs71361504 (–/GTT) SNP and endometriosis risk in Indian women (Table 3). rs71361504 (–/GTT) SNP is located at the upstream of the transcriptional start site of *BRCA1* gene. Several cis-acting elements such as E boxes, CAAT box, SP1 binding site etc. are present within the proximity of above site. Hence it may have significant effect on the transcriptional activation of *BRCA1* gene. However, the exact role of this variant on pathogenesis of endometriosis is currently unknown. Further extensive biochemical and molecular functional studies are necessary to validate the role of this SNP in endometriosis.

In the present study, for the first time we report significantly decreased BRCA1 expression in eutopic endometria of patients compared to controls (Figs. 2a and 3a). Increased proliferative nature of endometriotic cells may be correlated with

Fig. 2 Immunohistochemical analyses of BRCA1 and BRCA2 in secretory phase eutopic endometria of Stage IV endometriosis cases and controls. **a** – **b** Show representative endometrial tissue sections from cases and controls that were stained for BRCA1 and BRCA2, respectively. **a'** – **b'** Show the quantitative analysis of staining intensity by Image-J software. Asterisk (*) indicates significant difference ($P<0.05$) between cases and controls

Fig. 3 Western blot analyses of BRCA1 and BRCA2 in secretory phase eutopic endometria of Stage IV endometriosis cases and controls. **a – b** Show representative immunoblots of BRCA1 and BRCA2, respectively. **a' – b'** Show the graphical representation of the immunoblots A–B, which was obtained by densitometry analysis. The semi-quantitative data of A–B immunoblots was normalized by relative intensity of β -actin. Asterisk (*) indicates significant difference ($P < 0.05$) between cases and controls

this finding. The loss of *BRCA1* expression may be a crucial mechanism in the pathogenesis of endometriosis. Down-regulation of BRCA1 has also been reported in various tumors [20, 22, 24]. In addition, advanced stage tumors generally have the lowest levels of BRCA1 [31] and exhibit higher proliferation rates [32]. Some studies indicated an adverse effect on disease-free survival of low levels of BRCA1 [33]. Similar studies have correlated decreased BRCA1 levels with development of sporadic ovarian cancer [22]. This would be in accordance with the common function of BRCA1 as a tumor suppressor in these tissues. Overall, these findings suggest a model in which loss of BRCA1 activity, either by germ-line mutations or by down-regulation of gene expression, leads to tumor formation in appropriate target tissues.

Currently, the subcellular localization and function of the BRCA1 protein, its role in normal cells, and its role in tumorigenesis are under active investigation. In the present study, we observed frequent loss of nuclear BRCA1 in eutopic endometria of patients when compared to controls (Fig. 2a). The sub-cellular localization of BRCA1 is controversial, and two conflicting views exist. According to Jensen et al. [34] BRCA1 belongs to the granin family of proteins and is found in the cytoplasm, localizing to secretory vesicles and functioning as a secreted growth inhibitor. However, Chen et al. [35] reported that BRCA1 localized primarily to the nucleus in a variety of normal breast cell lines, as well as in tumor cell lines derived from organs other than breast or ovary. In contrast, BRCA1 localized mainly in the cytoplasm or had dual localization to both cytoplasm and nucleus in most breast and ovarian cancer cell lines and in other fixed tissue sections [32]. Reconciling the reasons for the conflicting localization found in published studies has been difficult; but, because most of the data originate from cell lines and some studies have used monoclonal rather than polyclonal antibodies, differences in antibody specificity and fixation techniques may be responsible.

A significant finding of our study was the frequent loss of nuclear BRCA1 expression in eutopic endometria of patients compared to controls. This may be due to a somatic mutation in the wild-type allele in these tissues. Alternatively, decreased expression may be the result of mRNA instability associated with nonsense mutations or it may be due to the presence of splice variants that are expressed only in the cytoplasm. Alternative splicing events may affect localization and expression of the BRCA1 protein. The RNA from the *BRCA1* splice variant, BRCA1- γ has been shown to localize exclusively to the cytoplasm, whereas *BRCA1* mRNA localizes to the nucleus [36]. However, the exact role of this finding on pathogenesis of endometriosis is currently unknown. Although our results are very interesting, we are not going to conclude them as direct cause of endometriosis because with this small sample size, it is very difficult to decipher their impact. Further studies with increased sample size are necessary to validate our findings.

Furthermore, our findings revealed no significant difference in BRCA2 expression between patients and controls. Although, inconsistent reports have been documented regarding BRCA2 expression in various tumors [23], most of them revealed over-expression in tumor tissues compared to controls [21]. In addition, a significant link was observed between BRCA2 over-expression and advanced stage tumors, suggesting that over-expression of BRCA2 plays a role in the aggressiveness of tumors. It can be attributable to nuclear polymorphism and mitotic index, which respectively reflect extent of genetic changes in tumors and the proliferation rate. The results obtained in vivo are thus in agreement with reports from several authors [37, 38] showing that BRCA2 expression is up-regulated in rapidly proliferating cells in vitro. These observations are consistent with a model in which BRCA2 exerts a positive effect on proliferation. Such a model is difficult to reconcile with the genetic evidence that BRCA2

is a tumor-suppressor gene whose over-expression should inhibit proliferation and vice versa. Rajan et al. [37] suggested the possible existence of a regulatory loop in which proliferation induces BRCA2 expression that, in turn, inhibits proliferation. The existence of such a feedback loop would imply that the up-regulation of BRCA2 expression induced by proliferation is a protective response tending to decrease tumor progression. However, in the present study we did not find significant difference in BRCA2 expression between eutopic endometria of patients and controls. Further larger studies may be required to validate the role of BRCA2 expression in pathophysiology of endometriosis.

In conclusion, we identified a significant association between *BRCA1* promoter SNP (rs71361504) and endometriosis risk in Indian population. In addition, we also detected significantly decreased *BRCA1* expression in eutopic endometria of patients compared to controls. Although further work is necessary to understand the potential link between these findings and endometriosis, our findings may lead to new insights into the pathogenesis of endometriosis.

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Conflict of interest The authors declare that they have no conflict of interest.

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