The sex hormone system in carriers of *BRCA1/2* mutations: a case-control study

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Summary

Background Penetrance for breast cancer, ovarian cancer, or both in carriers of *BRCA1/BRCA2* mutations is disproportionately high. Sex hormone dysregulation and altered end-organ hormone sensitivity might explain this organ-specific penetrance. We sought to identify differences in hormone regulation between carriers of *BRCA1/2* and women who are negative for *BRCA1/2* mutations.

Methods We assessed endometrial thickness for each menstrual cycle day (as an index of hormone regulation) in 393 scans from 228 women in the UK Familial Ovarian Cancer Screening Study (UK FOCSS) known to carry either mutation and 1573 scans from 754 women known to be negative for the mutations. To quantify differences in endometrial thickness we focused on days 10–14 and days 21–26, and calculated the area under the curve. We then compared serum oestradiol and progesterone titres during these days of the menstrual cycle in the same groups. Follicular and luteal oestradiol and progesterone serum titres were grouped into quartiles and odds ratios were calculated with logistic regression.

Findings Follicular phase endometrial thickness of carriers of the mutations adjusted for age and day of the menstrual cycle was higher (odds ratio [OR] 1·11, 95% CI 1·03–1·20; p=0·0063) and luteal phase endometrial thickness lower (0·90, 0·83–0·98; p=0·027) than for women negative for the mutations. Median luteal phase titres of progesterone were 121% higher (p=0·00037) in carriers than in women negative for the mutations, and for oestradiol were 33% higher (p=0·007)—ie, 59% of carriers had concentrations of serum progesterone that would have been in the top quartile of concentrations in the control group (OR 8·0, 95% CI 2·1–52·57; p=0·008).

Interpretation Carriers of *BRCA1/BRCA2* mutations are exposed to higher titres of oestradiol and progesterone—known risk-factors for breast cancer. Higher titres of oestradiol in carriers are compatible with this hormone having a role in ovarian carcinogenesis in such women. Our findings could not be explained by differential contraceptive pill use.

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Introduction

In all inherited cancer syndromes the germline mutation is thought to have a so-called local effect in an organ that is predisposed to the development of cancer, because these mutations do not cause cancers in all organs. For example, the increased cancer risk in carriers of the *BRCA1* and *BRCA2* mutations is predominantly that of breast cancer, ovarian cancer, or both.[1] These mutations are thought to cause cancer via a defect in DNA damage response or in the DNA repair pathway.[2] However, this defect does not explain the organ-specific cancer penetrance. That removal of both ovaries and Fallopian tubes reduces not only the risk of ovarian but also breast cancer[3] implicates a systemic dysregulation of hormone production in carriers of the mutation, which affects both the Müllerian epithelium, as the cell of origin for ovarian cancer,[4] and breast epithelium. Evidence from preclinical models suggests that both hormone production and hormone sensitivity of end organs is altered in carriers of the *BRCA1* mutation. Studies in animals[5–7] showed that mice carrying a *Brca1* mutation in the steroid-hormone-producing granulosa cells had a longer pro-oestrus phase, corresponding with the oestrogen-dominant follicular phase of the human menstrual cycle. Furthermore, serum oestradiol titres in mutant mice were higher than those of wild-type mice when both groups were stimulated with exogenous gonadotropins. Also, the insulin-like growth factor system has a fundamental role in endometrial biology, acting via autocrine and paracrine mechanisms.[8] There are strong interactions between the insulin-like growth factor and *BRCA1* signalling pathways, which also involve oestrogen signalling;[9] hence, it is possible that the endometrium of carriers of the *BRCA1* mutation has altered sensitivity to hormones.

Cyclical change in oestradiol and progesterone titres alters endometrial thickness and menstrual bleeding in premenopausal women. We postulate that both endometrial thickness as a functional index of hormonal activity in a target organ that undergoes cyclic changes, and serum ovarian steroid hormone titres at particular stages of the menstrual cycle, would differ between carriers of the *BRCA1/BRCA2* mutations and women who are known to have no *BRCA1/BRCA2* mutations.

The UK Familial Ovarian Cancer Screening Study (UK FOCSS; registered with Current Controlled Trials, number ISRCTN32794457)[10] has accrued sufficient

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For more on the **UK FOCSS** see http://www.instituteforwomenshealth.ucl.ac.uk/academic_research/gynaecologicalcancer

prospective data and samples to allow assessment of our hypotheses. Because many women in the study had undergone clinical genetic testing for *BRCA1/2* mutations, we had a cohort of women known to carry the mutation and a cohort known to be negative for the mutation to compare. However, most of the women in the study had not undergone clinical genetics testing. To establish the mutation status of these women, high-throughput next generation sequencing provided the fastest and most cost-effective method of rapidly detecting carriers of the mutations, albeit with potentially lower sensitivity than clinical testing, which in the UK uses a combination of Sanger sequencing (including limited Ashkenazi mutation screening where appropriate) and multiplex ligation probe amplification.

Methods

Participants

After ethical approval (Eastern MREC 97/5/007), UKFOCSS recruited from 44 UK regional centres. Between June, 2002, and September, 2010, women older than 35 years, at an estimated minimum 10% lifetime risk of ovarian cancer based on family history or predisposing germline gene mutations (appendix) were recruited and screening data and outcomes were collected prospectively. So far, about 25% of the study population have undergone clinically initiated testing for *BRCA1* and *BRCA2* mutations (either before or after recruitment).

After ethical approval (Joint University College London/ University College London Hospital Ethics Committee, ref 06/Q0505/102), we selected all premenopausal UKFOCSS participants with no previous or subsequent history of cancer (to avoid including women on hormonal therapy or women with a subclinical cancer, which could have triggered an altered hormonal environment and which could have confounded our analyses) and no intrauterine device.

Procedures

Ovarian cancer screening was done with transvaginal sonography to assess ovarian morphology and serum CA125 tumour marker measurement. All centres scanned study participants and all scans were done by sonographers, radiologists, or gynaecologists approved by the UK's National Health Service (NHS), employed by the local hospital for gynaecological scanning, and subject to local NHS quality control. Initially, CA125 tests were done annually, but after 2007 these tests were done every 4 months. Samples were taken in EDTA-containing tubes at women's primary care practices and posted to our laboratory for aliquoting, CA125 testing, and storage at –80°C. Samples were discarded if they reached the laboratory more than 56 h after venepuncture or were haemolysed. Endometrial thickness and date of last menstrual period were routinely recorded during sonography. Information on use of oral contraceptive

pills was collected in a general health questionnaire sent to all UK FOCSS participants in 2011, asking whether they had used the oral contraceptive pill in each of their third, fourth, and fifth decades.

We assessed UK FOCSS volunteers for germline mutations in the coding sequence of *BRCA1* and *BRCA2* with the Illumina HiSeq2000 sequencer (San Diego, CA USA; appendix). Participants were included if they matched our inclusion criteria and had provided a DNA sample.

Oestradiol and progesterone were measured with automated immunoassays on the Elecsys 2010 analyser (Roche Diagnostics GmbH, Mannheim, Germany). The measuring range for oestradiol was 18–15 800 pmol/L and for progesterone was 0·095–191 nmol/L. Standard Westgard rules were applied. Mutation carriers and controls were randomly mixed in batches and analysis was masked. Single lot numbers of reagent and calibrator were used. The oestradiol intra-assay coefficient of variability (CV) is 1·6–5·7% and interassay CV is 2·3–6·2%. The progesterone intra-assay CV is 1·5–2·7% and interassay CV is 3·7–5·4%.

Statistical analysis

Endometrial thickness for each group was averaged for each day of menstrual cycle and smoothed using the averaging running window of 5 days. For example, if five women had samples from cycle day 10, we first took the mean result for these five women. We then took the mean of the means for days 8–12 and plotted this as the value for day 10. On start and end days of the menstrual cycle the dependencies were prolonged—eg, for day 1, endometrial thickness was averaged over days 30, 31, 1, 2, and 3. To quantify differences we focused on days 10–14 and days 21–26 (because these were the times in the cycle when differences were most pronounced), and calculated the area under the curve (AUC). Differences in AUC and in distributions represented in boxplots were estimated with the Mann-Whitney *U* test. To construct the distributions for AUCs, the endometrial thickness values were bootstrapped: for each cycle day the thickness value was sampled with replacement, then the obtained dependence was averaged using a window of 5 days and the AUC was calculated for the obtained dependence between days 10–14 and 21–26. The odds ratios (ORs) for *BRCA* status were obtained for endometrial thickness, treated as a continuous predictor variable, and adjusted in the logistic regression for age and the specific menstrual cycle day as continuous variables.

Follicular and luteal oestradiol and progesterone serum titres were grouped into quartiles and ORs were calculated with logistic regression. Because endometrial thickness, oestradiol, and progesterone data did not differ significantly between carriers of *BRCA1* and *BRCA2* mutations (appendix), we combined the data of the mutation carriers to increase statistical power with
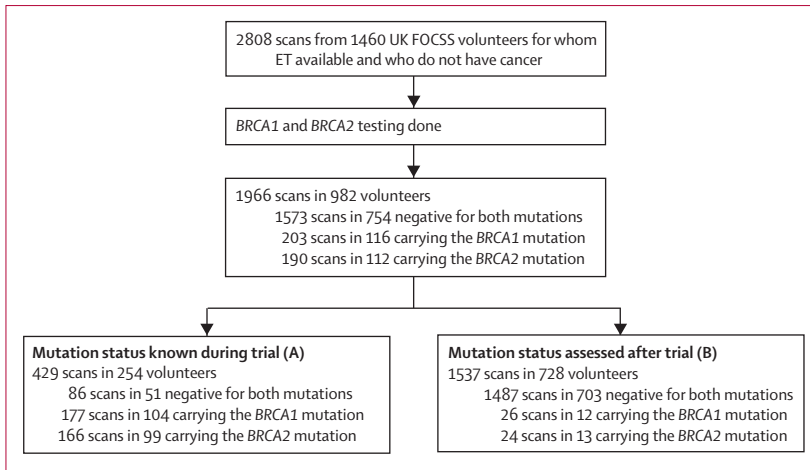
Figure 1: Availability of transvaginal ultrasound scans

Scans from women within the UK Familial Ovarian Cancer Screening Study (UK FOCSS) who had clinically initiated *BRCA* testing before or during the trial (A) or who had *BRCA* testing by next generation sequencing after the trial finished (B). In the UK, clinically initiated testing is done primarily on women with stronger family histories and affected living relatives. Therefore the only difference between B and A is that the former would have had a lower a-priori risk of being mutation carriers—this explains the lower prevalence of mutations recorded in B versus A. However, there was no difference in ultrasound methods, data collection, sample collection, or storage for these women, so this is not a source of bias. Consequently no statistical adjustment was deemed necessary. ET=endometrial thickness.

	BRCA1 and BRCA2 negative	BRCA1 or BRCA2 mutation	p value*
All scans			
No contraceptive pill use	1099 (77%)	219 (78%)	0·61
Contraceptive pill use	332 (23%)	61 (22%)	
Mutation status known during the trial†			
No contraceptive pill use	46 (81%)	191 (79%)	0·73
Contraceptive pill use	11 (19%)	52 (21%)	
Mutation status assessed after the trial‡			
No contraceptive pill use	1053 (77%)	28 (76%)	0·89
Contraceptive pill use	321 (23%)	9 (24%)	

Use of oral contraceptive pills in the same decade as endometrial thickness scan was available for 87% of the 1966 endometrial thickness scans done. *From χ² test. †Women whose mutation status was established with clinical genetics testing. ‡Women whose mutation status was established with next generation sequencing.

Table 1: Proportions of endometrial thickness scans with known contraceptive pill use during decade of ultrasound scan

the limited number of samples available. ORs for *BRCA* status were obtained for endometrial thickness, treated as a continuous predictor variable, and adjusted for age and the specific menstrual cycle day. Software R version 2.11.1 was used for statistics.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

2808 endometrial-thickness measurements and associated last menstrual period dates were available from 1460 eligible women, including 1573 endometrial thickness measurements in women negative for both mutations, 203 in carriers of *BCRA1*, and 190 in carriers of *BRCA2* (baseline data shown in the appendix). 254 women had *BRCA* testing done during the course of the UK FOCSS and 728 women (who had provided DNA) had their *BRCA* status assessed after the trial by next generation sequencing (figure 1). Information on use of oral contraceptive pills during the decade when the transvaginal scan was done was available for 1711 (87%) of 1966 endometrial thickness measurements and was not significantly different in any of the groups assessed (table 1).

409 stored serum samples from cycle days 10–14 and days 21–26 were available from known carriers of *BRCA1* (n=38) and *BRCA2* (n=32) mutations and negative controls (n=339) for oestradiol and progesterone testing.

Figure 2 and table 2 show endometrial thickness in the study groups by menstrual cycle day. Endometrial thickness was higher in the follicular phase in carriers than in controls negative for the mutations, but lower than that of controls in the luteal phase. The AUC of endometrial thickness in both follicular and luteal phase differed significantly when comparing carriers of either mutation with those who were carriers of neither mutation (p<0·0001 for both comparisons) and endometrial thickness results did not differ significantly between carriers of the mutations (appendix). In view of this statistically similar endometrial thickness pattern in carriers of the mutations, we combined these groups, and noted clear differences between the combined carrier group and the group negative for mutations (figure 2 and table 2). In the follicular phase, the carrier group's endometrial thickness was significantly higher, whereas in the luteal phase endometrial thickness was significantly lower than that of non-carriers, even after using logistic regression analysis and adjusting for age and menstrual cycle day. To assess whether a woman's knowledge of her mutation status might affect the results (eg, by changing her lifestyle in an attempt to minimise her cancer risk), we separately analysed the 728 women in the next generation sequencing group who did not know their mutation status during the trial. The same endometrial thickness pattern between mutation carriers and non-mutation carriers was noted (appendix). To be certain that the endometrial thickness differences were not due to differential oral contraceptive pill use, we analysed the 1318 endometrial thickness scans for which the women had reported no oral contraceptive pill use in the decade the scan was done (table 1). Again, we noted the same endometrial thickness patterns as before (appendix), with higher follicular phase endometrial thickness and lower luteal phase endometrial thickness in carriers of the mutations than for women negative for the mutations.

The differences in endometrial thickness between carriers and those negative for the mutations could be a consequence of different hormonal sensitivity of the target tissue (ie, the endometrium) in carriers of the mutations, triggered by different titres of the steroid hormones known to regulate endometrial biology, or a combination of the two. Although it is difficult to assess hormonal sensitivity directly, to assess the triggering threshold we analysed oestradiol and progesterone in stored serum samples from all premenopausal carriers of the mutations who provided samples between days 10–14 (follicular phase) and 21–26 (luteal phase; n=59, mean age 40·6 years, 70 samples), and all women negative for the mutations (n=283, mean age 43·5 years, 339 samples). Again, there were no differences between carriers of either mutation (appendix). Oestradiol titres did not differ significantly between the carrier and mutation-negative groups during days 10–14 (appendix). Progesterone titres during day 10–14 were not measured in all women because pilot data in 38 carriers and 44 controls showed such low titres (median 1·27 nmol/L in carriers and controls) that no significant differences would become apparent on testing all available samples. Median luteal phase titres of progesterone were 121% higher (p=0·00034) in carriers than in women negative for the mutations, and oestradiol titres were 33% higher (p=0·007)—ie, 59% of carriers had concentrations of serum progesterone that would have been in the top quartile of concentrations in the control group (OR 8·0, 95% CI 2·1–52·57; p=0·008; appendix, figure 3).

Discussion

Our findings show clear differences in cyclical endometrial thickness in carriers of *BRCA1/2* mutations compared with wild-type controls. We have also shown congruent changes in sex-hormone titres, which are one of the probable explanations for this difference. Our cohort of well matched premenopausal carriers and controls with combined endometrial thickness information, and serum samples with known menstrual cycle data, was ideal for testing the hypothesis that the organ-specific cancer penetrance in carriers is due to hormonal dysregulation. To our knowledge, the UK FOCSS population is the largest cohort for whom this information is available (panel).

Our findings of high luteal phase progesterone titres in carriers of *BRCA* mutations, associated with decreased endometrial thickness, are in complete concordance with each other, and might relate to a defect in steroid-hormone regulation in carriers that results in an end-organ effect. We speculate that the high luteal phase oestradiol we recorded in carriers triggers increased expression of progesterone receptors,[11] thus potentiating any possible mutagenic effect of the higher luteal progesterone. Our findings support those from studies in mice carrying a *Brca1* mutation in ovarian granulosa cells.[6,7] Although data on endogenous premenopausal progesterone exposure
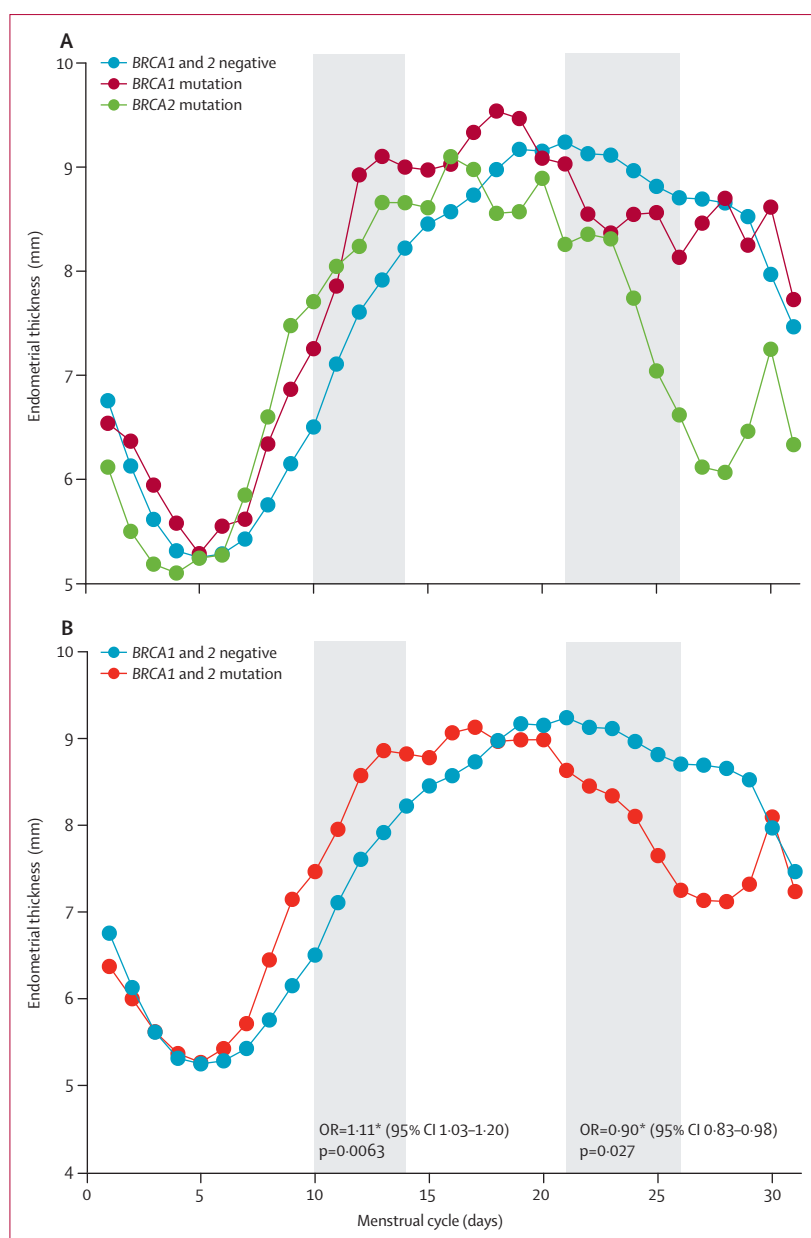

Figure 2: Endometrial thickness as a function of the menstrual cycle
(A) Endometrial thickness calculated from 1573 transvaginal ultrasound scans from 754 women negative for both mutations, 203 scans from 116 carriers of the *BRCA1* mutation, and 190 scans from 112 carriers of the *BRCA2* mutation. (B) Endometrial thickness in 754 women negative for both mutations (1573 scans) and the combined 228 women who were carriers of either mutation (393 scans). OR=odds ratio. *Adjusted for menstrual cycle day and age.

and cancer risk is less extensive and less conclusive,[12] postmenopausal exogenous progesterone exposure is a well established risk factor for breast cancer.[11,13–15] We were recently part of a collaborative report suggesting that progestogens cause breast cancer by inducing expression of the receptor activator of NF-κB ligand (RANKL), and that deleting the receptor for this ligand delays the onset of progestogen-driven breast cancers.[16] There is already an

	Area under curve				p value
	Days 10–14 of cycle		Days 21–26 of cycle		
	n	AUC	n	AUC	
Separate *BRCA* analysis					<0·0001
BRCA1 mutation	40	32	31	43	
BRCA2 mutation	42	32	32	41	
BRCA1/2 negative	308	28	274	45	
Combined *BRCA* analysis					<0·0001
BRCA1/2 mutated	82	32	63	41	
BRCA1/2 negative	308	28	274	45	

AUC=area under curve. n=number of volunteers.

Table 2: **Endometrial thickness as a function of menstrual cycle in women by *BRCA1* and *BRCA2* mutation status**
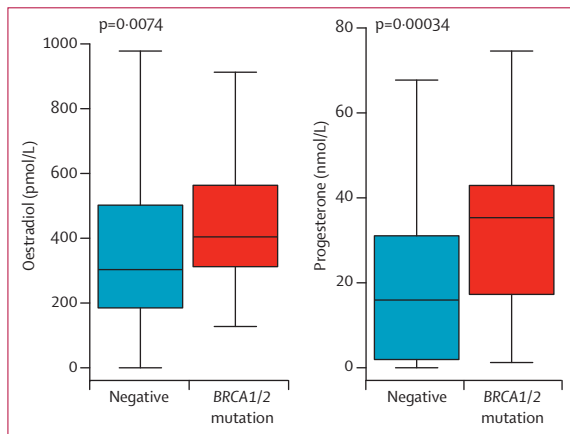

Figure 3: **Serum progesterone and oestradiol analysis during luteal phase of the menstrual cycle**
Boxplots with horizontal line show 25%, 50%, and 75% quartiles. Error bars show 95% CIs.

Panel: **Research in context**

Systematic review

We searched PubMed for studies on *BRCA* mutation and alterations in the female premenopausal reproductive hormone system published in English between Sept 1, 1993, and Feb 28, 2013. We used the search terms ("BRCA1" or "BRCA2") and ("endocrine" or "hormones" or "endometrium" or "menstrual"). We established that studies in animals showed aberrant hormonal regulation upon loss of BRCA1 in granulosa cells and that premenopausal surgical resection of the ovaries in women carrying the *BRCA1* or *BRCA2* mutation led to a substantial reduction in the risk of breast cancer.[3] Systemic endocrine effects of *BRCA1* or *BRCA2* mutations have not previously been assessed in humans.

Interpretation

Our findings suggest that *BRCA1/2* germline mutations are driving carcinogenesis only in part via altered molecular pathways (eg, those involved in DNA repair) in the organ at risk, and that *BRCA1/2*-associated changes in the endocrine system are additional factors. These insights could act as a major impetus for novel chemoprevention trials using strategies that can exploit the hormonal dysregulation in carriers of *BRCA1/2* mutations. Potential agents for these trials include selective oestrogen or progesterone receptor modulators, and the anti-RANKL (receptor activator of NF-κB ligand) antibody denosumab (currently used for osteoporosis treatment, but known to block the downstream carcinogenic effects of progestogens).

antibody against RANKL (denosumab), which is used at present for the treatment of osteoporosis. Our findings might therefore provide the basis for trials of this antibody as a chemopreventive agent for *BRCA1/2*-triggered breast cancer. In conjunction with data that show the potential of a progesterone antagonist to prevent *BRCA1*-mediated mammary tumorigenesis in mice,[17] our findings provide an additional rationale for treatment that interferes with progesterone signalling to prevent breast cancer in carriers of *BRCA1/BRCA2* mutations. One such compound (the progesterone receptor modulator, ulipristal acetate) has been used successfully for treatment of uterine fibroids, with fewer side-effects than agonists of luteinising-hormone-releasing hormone.[18,19] We speculate that such drugs could be used as chemoprophylaxis for women at high risk of breast cancer.

The recorded higher follicular phase endometrial thickness in carriers of the *BRCA1/2* mutations was not associated with any differences in follicular phase titres of oestradiol and progesterone between carriers and controls. We speculate that the higher follicular phase endometrial thickness in carriers might be a consequence of altered endometrial sensitivity to steroid hormones. Evidence on endometrial cancer penetrance in carriers[20] suggested that excess endometrial cancer risk was explained by use of tamoxifen. This finding suggests that the endometrium of carriers might be more sensitive to oestrogen-receptor agonists.

Oestrogen replacement usage[21] and obesity (a hyper-oestrogenic state)[22] are established epidemiological risk factors for ovarian cancer, implicating hormonal dysregulation in its pathogenesis. Our data, suggesting higher oestradiol titres in the luteal phase of the menstrual cycle in women who are carriers of the *BRCA1/2* mutations compared with women negative for the mutations supports this hypothesis. We speculate that the higher titres of progesterone with concordant reduced endometrial thickness recorded in the luteal phase in the carrier group compared with the control group might explain why the penetrance for the third hormonally triggered cancer—namely endometrial cancer—in carriers is much lower than that for ovarian and breast cancer; it is well recognised that progestogens suppress endometrial proliferation,[23] resulting in a thinner endometrial thickness and lower lifetime risk of endometrial cancer.[24]

Our study has certain limitations. Although we excluded women with a levonorgestrel-releasing intrauterine device, we had limited information on use of oral contraceptive pills. We only recorded use of the pill in the same decade as the endometrial scan. However, these data were available on 87% of the women for whom we had endometrial thickness data. Based on these data, oral contraceptive use during the same decade as the ultrasound scan did not differ between carriers and controls. Furthermore, in view of the age of the cohort (all were >35 years) and their known increased risk of breast cancer, it is probable the proportion using the contraceptive pill at the time of sample donation would have been even lower than the data above. Most importantly, excluding women with unreported pill use and those known to have taken the pill in the same decade as the scan did not reduce the statistical significance of the endometrial thickness differences between carriers and controls.

The control group could have included participants that carry *BRCA1/2* mutations who were missed using our next generation sequencing mutation-detection methods (eg, mutations in regions of low sequence coverage or large genomic rearrangements). Although it is not possible to estimate the frequency of missed mutations, the individuals screened were unaffected and therefore had at most a 50% chance of inheriting a germline mutation even if it were present in their family. Therefore, the frequency of *BRCA1/BRCA2*-positive individuals in the screen-negative group is probably very low. Any mutation carriers in the screen-negative group would bias our finding towards the null, suggesting that our study has underestimated rather than overestimated the strength of the recorded effects.

We had insufficient numbers of scans with last menstrual period dates of a cycle length greater than 28 days to draw any conclusions about whether carriers of *BRCA1/BRCA2* mutations might have a longer menstrual cycle than women negative for the mutations, as was evident in the previously described mouse model.[5–7] If this were the case, then we speculate that longer cumulative exposure, and the observed higher absolute titres of progesterone and to a lesser extent oestradiol, might contribute to the excess breast cancer risk of carriers of the mutations.

Our study cannot address whether endometrial thickness and hormone titres are respectively a marker and effector of breast cancer risk within a *BRCA1/2*-mutant population. Women with such a mutation have a very high (up to 85%) lifetime risk of breast cancer. Therefore many women undergo risk-reducing salpingo-oophorectomy, mastectomy, or both. As a result addressing the question as to whether altered endometrial thickness and steroid hormone titres in premenopausal women are markers for subsequent breast cancer risk or not would be difficult to do. It would need a substantial prospective long-term study of premenopausal *BRCA*-

carriers who were unwilling to undergo risk-reducing surgery but willing to be followed up for decades. Our findings suggest that sex steroids are one of the major drivers for development of breast cancer in this population.

We deliberately excluded patients who had a previous diagnosis of breast or ovarian cancer because they could have been on antiendocrine therapy or chemotherapy, which would have altered their ovarian function and biased our findings. We also excluded the few women who developed breast or ovarian cancer subsequent to their scan or serum sample donation. It is well known that sex steroids are locally produced within invasive but also within non-invasive breast carcinoma,[25] which might not have been clinically apparent at the time of sample donation. Furthermore, small subclinical ovarian tumours might potentially interfere with normal hormone production. Hence we decided a priori not to include women with a diagnosis of breast or ovarian cancer subsequent to sample donation to avoid any bias that would favour the hypothesis of an aberrant endocrine system being associated with cancer development in high-risk women. It could be argued that by excluding cases of subsequent breast cancer, we have diluted the breast cancer risk profile of our study group. However, despite this, we have noted hormonal changes that would be expected to increase breast cancer risk (ie, raised oestradiol and progesterone in carriers *vs* controls). Furthermore, because of the young age of the study group (all were premenopausal), most have not yet reached the age at which their breast cancer would be likely to occur, so most of the excess breast cancer risk of the study population has yet to accrue. Excluding the small number of women who we know developed breast cancer subsequent to sample donation would be likely to minimise differences between carriers and controls, making our findings more compelling.

To improve statistical power, we combined carriers of *BRCA1* and *BRCA2* mutations into a single study group. Although we acknowledge that it is possible that there are biological differences between these groups relevant to our investigation, we did not identify statistical differences between them with respect to endometrial thickness or hormone titres (appendix). Furthermore, figure 2 clearly shows that both groups have a similar pattern of higher follicular phase endometrial thickness and lower luteal phase endometrial thickness compared with women negative for the mutations.

Although we have in effect analysed multiple cross-sectional data rather than longitudinal data, we have still identified statistically significant differences between carriers and controls. Furthermore, longitudinal data would need a prospective study of a similar number of mutation carriers and controls, requiring daily venepuncture and transvaginal sonography. We feel such a study would be logistically challenging and recruitment for it would be extremely difficult.

The control group was not a random sample from the general population, but rather was deliberately selected for high familial risk of ovarian cancer (to minimise any differences between mutation carriers and controls). The control group's results might not therefore be representative of the general population. However, their shared increased ovarian cancer risk makes them the most appropriate control group to compare with carriers of *BRCA1*/*BRCA2* mutations.

In conclusion, our findings provide novel insights into the high penetrance for breast cancer (via higher progesterone and oestrogen titres) and also possibly ovarian cancer (via higher oestrogen titres and potentially lower titres of anti-Müllerian hormone[26]) in carriers of *BRCA1*/*BRCA2* mutations. Our findings provide a rationale for novel cancer prevention strategies in these women. They also provide a possible explanation for the absence (via higher progesterone titres) of high penetrance for endometrial cancer in carriers.

Contributors
MW had the idea and together with ANR did the literature search, data collection, data analysis, and data interpretation; designed the study; prepared the figures; and wrote the report. SP, IR, LF, EOF, UM, and IJJ collected, analysed, and interpreted the data. LD interpreted the data. JH, MPI, CKE, SJR, and SAG did the next generation sequencing. AZ did the statistics and generated figures and table.

Conflicts of interest
We declare that we have no conflicts of interest.

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