# Exploring the role of homologous recombination deficiency and BRCA1/2 mutations in endometrial cancer

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# Chapter 3

## Linking uterine serous carcinoma to *BRCA1/2*-associated cancer syndrome; a meta-analysis and case report

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## Abstract

**Background**

Uterine serous carcinoma (USC) shows greater morphological, clinical and molecular similarities to high-grade ovarian tubal serous carcinoma than to other types of endometrial cancer. As high-grade ovarian tubal serous carcinoma is known to be associated with *BRCA1/2* pathogenic germline mutations (PMs), we aimed to explore whether USC is also a constituent of hereditary breast and ovarian cancer syndrome.

**Methods**

Pubmed, EMBASE and Web of Science were searched in July-2016 for articles assessing the association between USC and germline *BRCA1/2*-PMs. Pooled analysis and comparisons were performed using a random effects logistic model, stratifying for ethnicity (Ashkenazi versus non-Ashkenazi). In addition, tumour tissue from an USC case with a hereditary *BRCA1*-PM was analysed for loss of heterozygosity at the *BRCA1* locus and was functionally analysed for homologous recombination proficiency.

**Results**

The search yielded 1893 citations, 10 studies were included describing 345 USC patients. For Ashkenazi Jews, the pooled odds ratio of having a germline *BRCA1/2*-PM was increased in USC patients compared with the general Ashkenazi population: odds ratio: 5.4 (95%-confidence interval: 2.2-13.1). In the patient with USC, we identified the known germline *BRCA1*-PM in the tumour DNA. Furthermore, we showed both loss of heterozygosity of the wild-type allele and a deficiency of homologous recombination.

**Conclusion**

This study suggests that USC may be an overlooked component of *BRCA1/2*-associated hereditary breast and ovarian cancer syndrome. Screening for germline *BRCA1/2*-PMs should be considered in patients diagnosed with USC, especially in cases with a positive first-degree family history for breast and/or ovarian cancer.

## Introduction

Uterine serous carcinoma (USC) is an aggressive subtype of endometrial cancer (EC) which constitutes 5-10% of all uterine carcinomas,[1] accounting for almost 40% of EC-related deaths.[2, 3] Treatment options for USC are limited and consist of complete surgical staging or debulking either after or followed by (neo)adjuvant platinum-based chemotherapy and/or adjuvant radiotherapy depending on tumour stage.[4, 5] Despite aggressive treatment approaches, little progress in survival benefit has been achieved in the last decade.



Next-generation sequencing has improved the understanding of the molecular alterations that underlie USC, showing that USC is different from the more common endometrioid endometrial carcinoma at the molecular level while showing striking similarities with the molecular landscape of high-grade ovarian tubal serous carcinomas (HGOTSC). Both USC and HGOTSC show frequent *TP53* mutations (91% and 96%) and a high degree of somatic copy number alterations (SCNA) with similar focal SCNA patterns.[5-10] These similar SCNAs may be related to homologous recombination deficiency (HRD), known to be present in almost 50% of HGOTSC and often caused by *BRCA1/2* defects.[11, 12]

Moreover, USC and HGOTSC show similar histomorphologic and clinical features, as both have the tendency to spread over peritoneal surfaces, are associated with poor survival rates and show good responsiveness to platinum-based chemotherapy, although the latter could not be confirmed by all studies.[5, 11, 13, 14]

HGOTSC is associated with hereditary breast and ovarian cancer syndrome (HBOCS) caused by hereditary pathogenic mutations (PMs) in the *BRCA1* or *BRCA2* genes, which are present in approximately 15% of all HGOTSC.[11] Currently, USC is not considered as a manifestation of HBOCS. Given the many similarities between these two entities, it has been suggested by some that USC is indeed a feature of *BRCA1/2*-associated HBOCS,[15, 16] which might influence genetic counselling and treatment strategies. However, literature on this association has not yet been systematically reviewed.[15, 17]

The aim of this study was to assess whether USC is a component of *BRCA1/2*-associated HBOCS. To address this question, we present a systematic review and meta-analysis and also describe a case report as proof of concept. Furthermore, we determined whether USC patients with a germline *BRCA1/2*-PM showed a higher frequency of either a positive family history and/or personal history for *BRCA1/*2-associated malignancies.

# Methods

## Systematic review and meta-analysis

### *Eligibility criteria, literature search and data collection*

We searched for studies investigating *BRCA1* and/or *BRCA2* germline mutations in association with USC. We aimed to include case-control studies and cohort-studies/trial-designs. However, examination of the literature failed to identify any case-control study (comparing germline *BRCA1/2*-PM status in USC patients and controls without USC). Also, no cohort study formally compared USC incidence in germline *BRCA1/2*-PM carriers versus non-carriers. We therefore adapted our inclusion criteria so that single-arm case-only studies (studying *BRCA1/2*-PM prevalence in USC patients) and single-arm cohort studies (studying USC frequency in carriers) were also eligible. To allow a comparison, a control group to establish population frequencies of germline *BRCA1/2*-PMs in women with the same ethnic background was extracted from the literature.

For the purposes of this systematic review all studies which investigated at least one mutation in one of the *BRCA*-genes in relation to USC were considered eligible. In these studies, USC was defined by having at least 10% serous histology, with the uterus as primary site of origin. Studies on carcinosarcomas and studies in which no distinction was made between histologic subtypes of EC were excluded. Single-arm cohort studies were only included when patients had a proven germline *BRCA1/2*-PM and the cohort was not enriched for a particular malignancy (thereby preventing selection bias).

Relevant studies were identified by literature search in the PubMed, EMBASE and Web of Science databases using a search strategy which was devised in collaboration with a trained librarian. The search strategy consisted of a combination of Medical Subject Heading (MeSH) and free text words with the following combined keywords: 'BRCA' and 'uterine neoplasms', including all relevant keyword variations (Appendix A). The search was performed in July 2016. No limits or filters were placed on the searches. Reference lists of papers were checked for additional citations to ensure that no references were omitted. Two authors (MJ and AM) independently reviewed the titles and abstracts of the citations to identify studies eligible for inclusion. Articles published in languages other than English, German or Dutch were excluded. Data were reported using the PRISMA checklist (Appendix B).

### *Data extraction*

Data were extracted by two authors (MJ and AM) independently. For the single-arm case-only studies, data on germline *BRCA1/2*-prevalence were extracted. Germline *BRCA1/2*-PM prevalence rates vary among populations and are especially high in Ashkenazi Jews.[15, 18-22]. To avoid bias, in studies describing an ethnic Jewish population, only the data on Ashkenazi Jews were extracted if possible. To reduce the probability of false positive results due to

population stratification, the literature was searched for a control group of the same ethnicity. Data on personal history of breast cancer and first-degree family history of breast and/or ovarian cancer were extracted. For the single-arm cohort studies, USC incidence risk during follow-up was assessed.

***Risk of bias assessment***

Adequateness of USC diagnosis (revised by expert pathologist, indicated whether mixed-USC cases were also included), risk for population stratification (did studies define the ethnic groups included and were these groups extractable) and potential for selection bias (tamoxifen use) were determined for every study included. For case-only studies, *BRCA1/2*-PM testing (full coverage of the genes or just founder mutations) was assessed. For cohort studies, follow-up was considered sufficient if the mean or median age of the study participants plus the mean or median follow-up together equalled the average age of USC development (age 70 years).



***Data synthesis and statistical analysis***

To determine whether germline *BRCA1/*2-PMs are more common in women with USC compared with women without USC, first the pooled proportion with 95% confidence interval (CI) of a germline *BRCA1/2*-PM was estimated for patients with USC and for population controls without USC. These pooled proportions were subsequently compared and a pooled odds ratio (OR) with 95% CI was estimated to compare presence of a germline *BRCA1/2*-PM. These estimates were obtained from a logistic regression with a random effect at the study level.

To determine whether germline *BRCA1/2*-PMs are more common in women with USC who have a positive first-degree family history and/or personal history for *BRCA1/*2-associated malignancies compared with the women with no such history, data on personal and family history were extracted from single-arm case studies and a pooled risk ratio (RR) was estimated.

For the single-arm cohort studies, no suitable control group was found, therefore meta-analysis could not be performed.

Statistical analyses were performed in STATA (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP).

## Case report

***Molecular and functional assays***

After obtaining informed consent, normal and tumour DNA was isolated from formalin-fixed paraffin-embedded tissue blocks. Three 0.6-mm cores were taken from normal and tumour tissue. Fully automated DNA isolation was performed as described previously[23] followed by

'next generation sequencing' using a modified two pool version of the Ion AmpliSeq *BRCA1* and *BRCA2* community panel. The Ion Proton (Thermo Fisher Scientific Inc.) system was used for sequencing according to manufacturers' recommendations. Loss of heterozygosity (LOH) was determined by frequency analysis of the pathogenic germline variants and single nucleotide variants in the *BRCA* genes.

The functional RAD51 assay was performed on fresh USC tissue which was obtained from the Pathology department at the Leiden University Medical Centre directly after resection. The research sample was prepared for analysis of RAD51 focus formation by immunofluorescence microscopy as previously described.[24] In brief, cancer tissue was irradiated *ex vivo* with 5 Gy ionising radiation to induce DNA double strand breaks. After 2 h of incubation at 37°C, the tissue was fixed in formalin and embedded in paraffin. As a functional read out for homologous recombination (HR) proficiency, the ability of the cells to recruit RAD51 protein to sites of DNA damage was measured. Tumour samples are considered HRD if less than 20% of the replicating tumour cells form RAD51 foci.[24]

# Results

## Systematic review and meta-analysis

### *Search results*

The literature search yielded a total of 1893 citations (Pubmed; 778, EMBASE; 700, Web of Science; 415), of which 1365 were unique. Forty-two articles were retrieved for full-text review (See flow-chart, Fig. 1). Of these, thirty-two publications were excluded for reasons described in the flow-chart. Finally, ten publications were included for analysis, of which seven were case-only studies (Table 1) and three were single-arm cohort studies (Table 2). All included studies were identified via the initial database search. Included studies were published between 2000 and 2016.

### *Risk of bias assessment*

Risk of bias assessment is provided in Table 3. Regarding USC diagnosis, in 40% of the studies, USC cases were revised by an expert pathologist and 40% stated whether included USC cases were of pure serous histology or contained mixed-histologic elements. Only one single-arm case study fully covered *BRCA1/2*, whereas five of seven studies only tested for the most common founder mutations in the Ashkenazi Jewish population. For two studies that contained a predominantly ethnic Jewish population,[21, 25] specific data were not extractable for Ashkenazi Jews alone. Data on previous tamoxifen use were given for four of ten studies. Follow-up was inadequate for all studies according to our formulated definition for adequacy (mean or median age study participants plus mean or median follow-up equalled the average age of USC development (age 70 years).

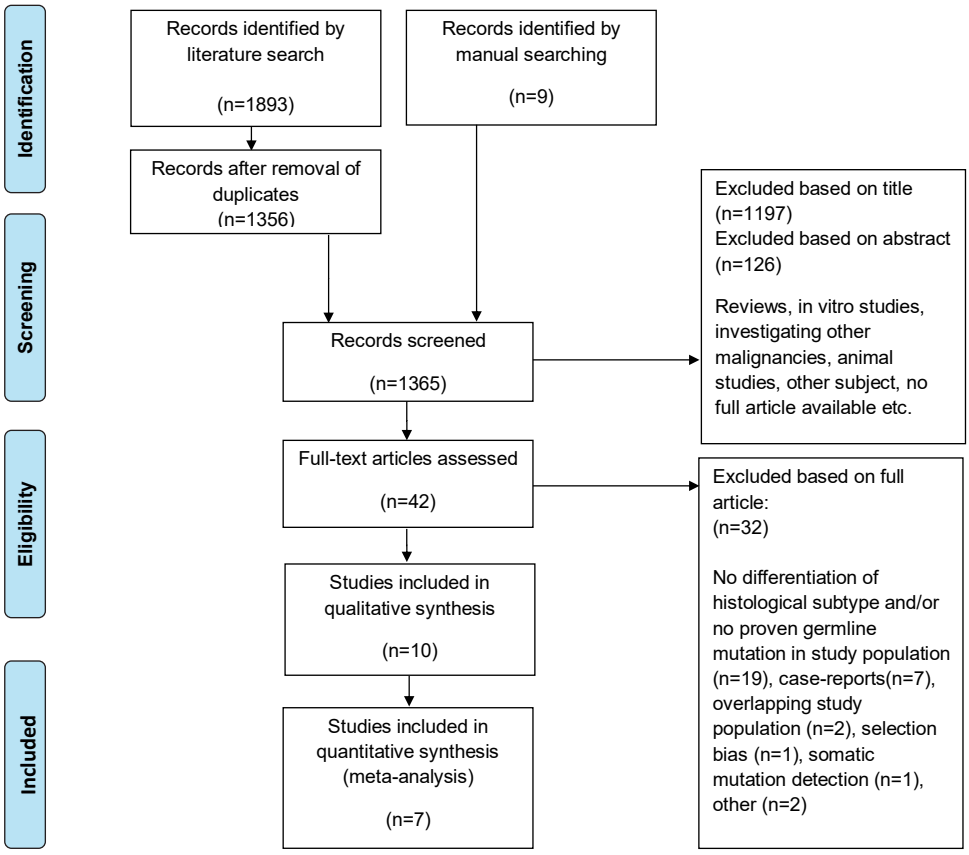


**Figure 1: Flowchart illustrating the selection of studies.**

***Germline BRCA1/2-PM prevalence in USC patients compared to population cohorts***

Seven single-arm case studies are summarised in Table 1. Five of the seven reports studied Jewish patients, mainly of Ashkenazi origin, and 2 reports considered an admixed, western population.

Sixteen germline *BRCA1/2*-PMs were identified in 134 Jewish women (mainly of Ashkenazi origin) with USC (Fig. 2). Reported prevalence of germline *BRCA1/*2-PM ranged from 0% to 26.1%, with the pooled proportion being 11.9% (95% CI: 5.1-25.6). Three cases with germline *BRCA1/2*-PMs were found in a total of 207 women from Western origin (mainly Caucasian) with USC. In this group, the pooled proportion was 1.5 (95% CI 0.5-4.1). Of the *BRCA1/2*-PMs found, most were *BRCA1*-PMs (14/19, 74%).

Literature was searched for control groups for both Ashkenazi Jewish and Western women to determine the prevalence of germline *BRCA1/2*-PM. Based on three studies, the

**Table 1. Reported prevalence rates of pathogenic germline *BRCA1/2* mutations in uterine serous carcinoma in single-arm case studies**

| Study | Cases carrier/ Total (%) | *BRCA1/2*-PM detected | LOH[a] | Personal history | Family history case carrier[b] | Mutations tested | Population | Country |
|---|---|---|---|---|---|---|---|---|
| Barak et al, 2010 | 0/34 (0.0) | - | n.a. | - | - | *BRCA1* (185delAG, 5382InsC, Tyr978X) *BRCA2* (6174delT) | 75% Ashkenazi Jew, 25% non-Ashkenazi Jews | Israel |
| Bruchim et al, 2010 | 6/23 (26.1)[c] | *BRCA2*-6174delT | n.a. | No | Breast | *BRCA1* (185delAG and 5382insC), *BRCA2* (6174delT) | 100% Ashkenazi Jew | Israel |
| | | *BRCA1*-185delAG | | No | Breast | | | |
| | | *BRCA2*-6174delT | | Breast | No | | | |
| | | *BRCA1*-5382insC | | No | Breast | | | |
| | | *BRCA2*-6174delT | | No | No | | | |
| | | *BRCA2*-6174delT | | No | No | | | |
| Goshen et al, 2000 | 0/56 (0.0) | - | - | - | - | *BRCA1* (exon 11, 185delAG, 5382insC, and dup(ex13)) and *BRCA2* (exons 10, 11, 6174delT)[d] | Admixed, not specified, probably admixed Canadian | Canada |
| Lavie et al, 2000 | 2/9 (22.2)[e] | *BRCA1*-5382insC | yes | No | Ovarian | *BRCA1* (185delAG and 5382insC), *BRCA2* (6174delT) | 100% Ashkenazi Jew | Israel |
| | | *BRCA1*-185delAG | yes | Breast | Ovarian, Prostate | | | |
| Lavie et al, 2010 | 8/51 (15.7)[f] | *BRCA1*-185delAG | 3/4[g] | 3/8 (37,5) breast | Breast and/or Ovarian[h] | *BRCA1* (185delAG and 5382insC), *BRCA2* (6174delT) | 100% Ashkenazi Jew | Israel |
| | | *BRCA1*-185delAG | | | Breast and/or Ovarian[h] | | | |
| | | *BRCA1*-185delAG | | | Breast and/or Ovarian[h] | | | |
| | | *BRCA1*-185delAG | | | Breast and/or Ovarian[h] | | | |
| | | *BRCA1*-185delAG | | | Breast and/or Ovarian[h] | | | |
| | | *BRCA1*-5382insC | | | Breast and/or Ovarian[h] | | | |
| | | *BRCA1*-5382insC | | | Breast and/or Ovarian[h] | | | |
| | | *BRCA2*-6174delT | | | Breast and/or Ovarian[h] | | | |
| Levine et al, 2001 | 0/17 (0.0) | - | - | - | - | *BRCA1* (185delAG and 5382insC), *BRCA2* (6174delT) | >90% Ashkenazi Jew | United States |
| Pennington et al, 2013 | 3/151 (2.0) | *BRCA1*-p.E1535X | n.a. | Breast | | | | |
| | | *BRCA1*-c.2594delC | | Ovarian (synchronous), Breast | Breast, Ovarian | *BRCA1*, chr 17:41191313-41282500 *BRCA2*, chr13:32884617-32978809 | Admixed; 66% Caucasian, 26% African American, 1% Asian, 2% Other, 5% Unknown | United States |
| | | *BRCA1*-c.713-2A>C | | Esophageal | Cervix | | | |

Abbreviations: PM: pathogenic mutation, LOH: loss of heterozygosity, n.a.: Not available/not extractable. [a] LOH: Partial or complete loss of heterozygosity of the wild-type *BRCA* allele. [b] Family history: first-degree relatives; parents, children, full siblings. [c] Non-Ashkenazi Jews excluded (n=8). [d] According to the authors, the protein truncation assay (*BRCA1* (exon 11) and *BRCA2* (exons 10 and 11) detects approximately 70% of pathogenic mutations. [e] Non-Ashkenazi Jews were excluded (n=3). [f] Non-Ashkenazi Jews were excluded (n=8). [g] Data extracted from study of Lavie *et al*, 2004. [h] Only mentioned that at least 1 first-degree relative showed a history of breast or ovarian cancer.

**Table 2. Single-arm cohort studies: incidence of uterine serous carcinoma in women with a pathogenic germline *BRCA1/2* mutation**

| Study | *BRCA*-PM in USC cases | USC/Total (%) | Expected number of EC[a] | Mean/Median age at enrolment, years (range) | Mean/Median Follow-up, years (range) | Short description |
|---|---|---|---|---|---|---|
| Beiner et al, 2006 | n.a. | 0/857 (0) | 1.13[b] | 54.4* (45–70) | 3.3* (0.01–9.6) | Women from 11 countries (North America, Europe and Israel) with a germline *BRCA1* (n=619) or *BRCA2 (*n=236) mutation or both (n=2) and an intact uterus were followed until diagnosis of endometrial cancer, ovarian cancer, hysterectomy, death, age of 70 or the date of completion of the last questionnaire. |
| Reitsma et al, 2013 | n.a. | 0/315 (0) | 0.94[b] | 43# (30–71) | 6 # (0-27) | Women with a *BRCA1* (n=201) or *BRCA2* (n=144) mutation in the Netherlands who had undergone a risk-reducing salpingo-oophorectomy without hysterectomy from January 1996 until March 2012 at the University Medical Center Groningen were analysed for endometrial cancer using the Dutch nationwide pathology database, PALGA. |
| Shu et al, 2016 | 3x *BRCA1*<br>1x *BRCA2* | 4/1083 (0.4[d]) | 4.3[b]<br>0.34[c] | 45.6# (40.9-52.5) | 5.1# (3.0-8.4) | Women with a deleterious *BRCA1* (n=627) or *BRCA2* (n=453) mutation or both (n=3) who had undergone a risk-reducing salpingo-oophorectomy without hysterectomy from January 1995 to December 2011 at 9 academic medical centers in the United states. Censoring occurred at uterine cancer diagnosis, hysterectomy, last follow-up, or death. |

Abbreviations: EC; endometrial carcinoma, PM; pathogenic mutation, USC; Uterine serous carcinoma, n.a.: not applicable, * Mean, # Median. [a] Data extracted from article. [b] Expected number of EC. [c] Expected number of serous/serous-like EC. [d] Carcinosarcoma with serous epithelial component (n=1) was excluded.

**Table 3. Risk of bias assessment single-arm case studies and single-arm cohort studies**

| Study | Diagnosis of uterine serous carcinoma | | *BRCA1/2* pathogenic mutation detection | | Risk population stratification | Selection bias | Follow-up |
|---|---|---|---|---|---|---|---|
| | All cases revised by expert pathologist | Stated number of pure serous and/ or serous-like/ mixed with serous carcinoma | Full coverage *BRCA1/2* | Only founder mutations tested (*BRCA1* (185delAG, 5382InsC) *BRCA2* (6174delT)) | Different ethnic group(s) extractable | Data on previous tamoxifen use available | Adequate follow-up for developing uterine serous carcinoma[a] |
| **Single-arm case studies** | | | | | | | |
| Barak et al, 2010 | N | N | N | Y | N | N | n.a. |
| Bruchim et al, 2010 | Y | Y | N | Y | Y | Y | n.a. |
| Goshen et al, 2000 | Y | U | N | N | N | N | n.a. |
| Lavie et al, 2000 | N | U | N | Y | Y | N | n.a. |
| Lavie et al, 2010 | N | U | N | Y | Y | N | n.a. |
| Levine et al, 2001 | Y | Y | N | Y | N | N | n.a. |
| Pennington et al, 2013 | Y | Y | Y | N | N | N | n.a. |
| **Single-arm cohort studies** | | | | | | | |
| Beiner et al, 2006 | N | N | n.a. | n.a. | Y | Y | N |
| Reitsma et al, 2013 | N | N | n.a. | n.a. | N | Y | N |
| Shu et al, 2016 | N | Y | n.a. | n.a. | N | Y | N |

Abbreviations: Y = yes, N = no, U = unclear, n.a.= not applicable..[a] Follow-up was considered adequate if the mean or median age of the study plus the mean or median follow-up comprised the average age of uterine serous carcinoma development (age 70 years).

reported prevalence of the three most common (founder) germline *BRCA1/2*-PMs [*BRCA1* (185delAG: NM_007294.3:c.68_69delAG, 5382insC: NM_007294.3:c.5266dupC), *BRCA2* (6174delT:NM_000059.3:c.5946delT)] in the general Ashkenazi Jewish population is estimated to be between 1.9 and 2.7%.[18-20] Based on the pooled germline *BRCA1/2*-PM prevalence in the Ashkenazi Jewish population and the pooled germline *BRCA1/*2-PM prevalence in USC cases, the OR for a germline *BRCA1/2*-PM was increased for women with USC: 5.4 (95% CI 2.2-13.1).



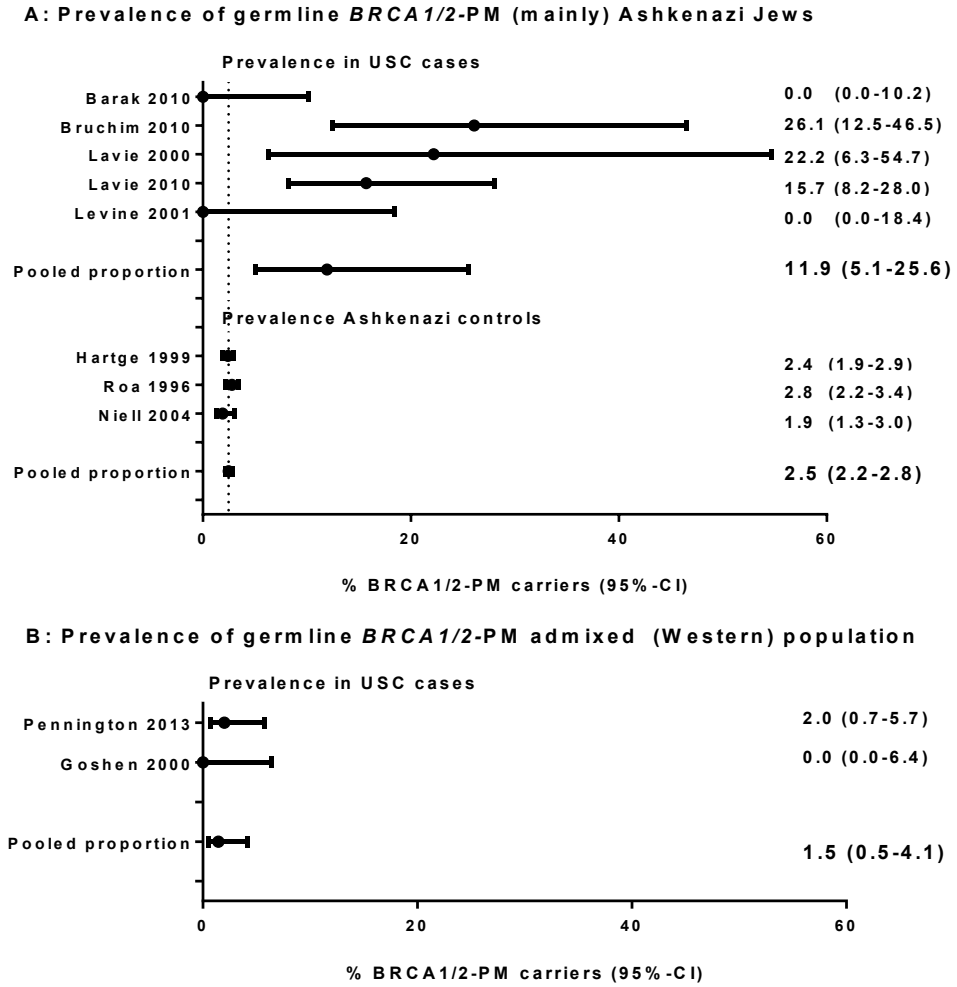


**Figure 2. Meta-analysis of data extracted from single-arm case studies: germline *BRCA1/*2-pathogenic mutation prevalence in USC stratified for ethnicity.** A: Pooled proportion of germline *BRCA1/2*-PM prevalence in (mainly) Ashkenazi Jewish women with USC compared to the pooled proportion of general Ashkenazi Jewish population. The germline *BRCA1/2*-PM prevalence is significantly higher in Ashkenazi Jews with USC compared to the general population. B: Pooled proportion of germline *BRCA1/2*-PM prevalence in an admixed population of women with USC from Canada and the United States of America. Abbreviations: PM; pathogenic mutation, USC: uterine serous carcinoma

For the general admixed Western population, reported germline *BRCA1/*2-PM prevalence varied between 0.23% and 0.32% based on estimates in women from the UK.[26-28] Because no measures of uncertainty were provided, no formal OR could be estimated. However, since these germline *BRCA1/2*-PM prevalence estimations do not lie within the 95% CI (0.5-4.1) of the pooled proportion of USC patients in the admixed population, this is suggestive for an increased prevalence of germline *BRCA1/2*-PMs in USC women.

Data from three single-arm cohort studies in *BRCA1/2*-PM carriers are summarised in Table 2.[16, 29, 30] The mean follow-up periods in the single-arm cohort studies ranged from 3.3 to 6 years. The median/mean ages at enrolment varied from 43 to 54.4 years.[16, 29, 30] No USCs occurred in two of the single-arm cohort studies. In one study,[16] 4 USCs/mixed USCs occurred in a population of 1083 women in which the expected number of serous/serous-like EC (e.g., serous, undifferentiated, carcinosarcoma) was 0.3.

***Personal history and family history in USC patients with germline BRCA1/2-PM***

Data on personal and first-degree family history of USC patients correlated to germline *BRCA1/2*-PM status were available in seven studies (Supplementary Table 1). The pooled RR for carriage of a germline *BRCA1/2*-PM in women with USC and a positive first-degree family history for breast and/or ovarian cancer, 4.0 (95% CI: 2.1-7.5), increased compared to women with no such family history (Fig. 3a). In terms of a personal history of breast cancer, the pooled RR for having a germline *BRCA1/2*-PM was 2.1 (95% CI: 0.9-4.9) (Fig. 3B).

## Case report

A 53-year-old Caucasian women, with a first-degree family history positive for *BRCA1*-associated breast cancer, was found to be a carrier of this germline *BRCA1*-PM in exon 13 (NM_007294.3:c.4327C>T (p.[Arg1443*]). She presented with postmenopausal bleeding and endometrial curettage showed EC suggestive for USC.

There was no personal history of cancer. A RRSO followed by a prophylactic bilateral mastectomy was performed approximately three years before the onset of symptoms. No (pre)malignancy was diagnosed in either sample.

The patient underwent a total abdominal hysterectomy and dissection of the iliac and para-aortal lymph nodes to achieve maximum cytoreduction.

Pathological examination by a gynaecopathologist revealed USC (<50% of the myometrial thickness) with substantial lymph-vascular space invasion and involvement of 16 of 22 removed iliac and para-aortal lymph nodes. Wilm's tumour 1-IHC was negative in the tumour cells, supporting the primary endometrial origin.[31, 32] One month after surgery, positron emission tomography demonstrated multiple remaining FDG-avid lymph nodes from the

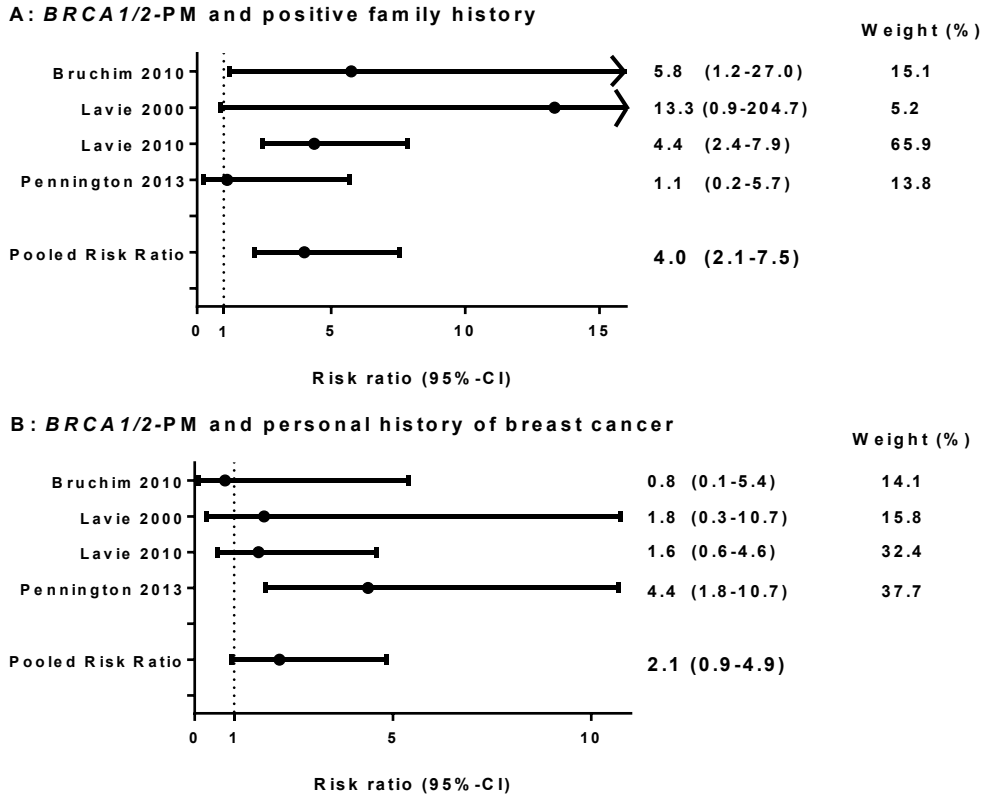


**Figure 3. The association of family and personal history with germline *BRCA1/2*-pathogenic mutations in women with uterine serous carcinoma.** A: The relative risk for having a germline *BRCA1/2*-PM in women with USC and a positive first-degree family history (parents, children, full-siblings) for breast and/or ovarian cancer was significantly increased compared to women with no such history. B: The relative risk for having a germline *BRCA1/2*-PM in women with USC and a positive personal history for breast cancer is increased, although not significantly. Abbreviations: PM; pathogenic mutation, USC: uterine serous carcinoma

renal vein until the bifurcation of the internal and external iliac artery at both sides. Also, a positive lymph node at the left supraclavicular fossa was detected (FIGO stage IV). After completion of six cycles of adjuvant carboplatin and paclitaxel chemotherapy, computed tomography demonstrated complete radiological remission of residual disease.

The known germline *BRCA1*-PM was detected in the tumour DNA. The tumour showed complete LOH of the *BRCA1* wild-type allele.

In addition, the functional *ex vivo* RAD51 assay showed complete absence of RAD51 ionising radiation induced foci formation in replicating tumour cells, supporting homologous recombination deficiency due to the absence of functional BRCA1 protein (Fig. 4).

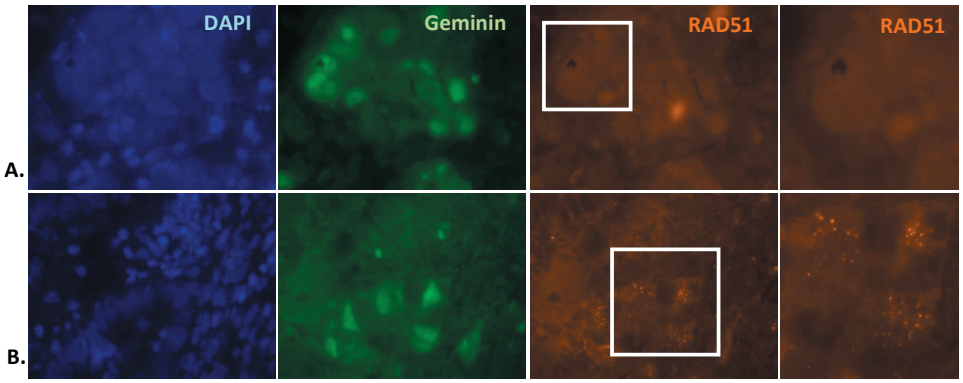


**Figure 4: Homologous recombination capacity determined by *ex vivo* RAD51 assay.** A: Absence of RAD51 accumulation after ionising radiation in replicating tumor cells stained by Geminin (a marker that is expressed during S and G2 phases of the cell cycle), indicating a homologous recombination deficiency in the tumor cells. DAPI stains cell nuclei. B: RAD51 accumulation was present in replicating tumor cells from an endometroid endometrial carcinoma. In the last column a magnification the RAD51 staining of a subset of the Geminin positive cells is shown.

## Discussion

The morphological, molecular and clinical similarities between USC and HGOTSC suggest common etiologies and raise the question of whether USC is a component of *BRCA1/2*-associated HBOCS. In this study, combining a systematic review and a case report, we provide epidemiological, molecular and functional support for the concept of USC as a *BRCA1/2*-associated HBOCS disease.

The main limitation of this study was the absence of case-control studies comparing germline *BRCA1/2*-PM frequencies in USC patients and healthy controls. In addition, no cohort studies formally comparing USC incidence in germline *BRCA1/2*-PM carriers versus non-carriers were available. Control groups were therefore borrowed from the literature. For Ashkenazi Jews, the prevalence of germline *BRCA1/2*-PMs could be estimated based on three large series of Ashkenazi Jews.[18-20] However, data on the Western admixed population were less solid[26-28] and therefore only comparisons of proportions could be performed. Another potential limitation is that, especially in advanced disease, HGOTSC can mimic USC[31, 32] making the ascertainment of the primary site of origin sometimes problematic. Of all USC cases with a germline *BRCA1/2*-PM, only one study reported an USC-case with synchronous ovarian cancer.[33] A Wilm's tumour 1-IHC staining to assist with determination of the primary site of origin,[31, 32] was not routinely performed in these studies. Misclassification of HGOTSC as USC can potentially give bias as it is known that germline *BRCA1/2*-PMs are prevalent in HGOTSC.[11]

Furthermore, substantial heterogeneity was found in the germline *BRCA1/2*-PM prevalence between studies, which can possibly be explained by incomplete analysis of the *BRCA1/*2-genes. *BRCA1* and *BRCA2* are very large genes which, with the exception of population-specific founder mutations, lack mutational hotspots.[34-36] Two of five studies with a Jewish population did not make the distinction between Ashkenazi Jews and other Jews. Since only specific founder mutations characteristic for Ashkenazi Jews were analysed, this may have led to an underestimation of the prevalence of germline *BRCA1/2*-PMs. In addition, one of the two single-arm case studies considering the admixed population used a test only capable of detecting approximately 70% of deleterious *BRCA1/*2-PMs.[17] This approach may have missed 2 of the 3 *BRCA1* mutations identified in the study by Pennington *et al.* [33]. Finally, data on the use of previous tamoxifen-treatment were only available for a subset of studies.[16, 22, 37, 38] Although some studies demonstrate a potential relationship between previous tamoxifen use and the development of USC, which might give bias,[39-41] this relationship remains controversial. Future studies need to be performed to clarify whether tamoxifen indeed increases the risk for non-endometrioid ECs or that this effect has been biased by unknown germline *BRCA1/2*-PMs in the included study-participants with previous breast cancer.



Despite these limitations, this meta-analysis supports a relationship between *BRCA1/2*-PM and USC, especially focussing on studies investigating the prevalence of *BRCA1/2*-PMs in USC patients and comparing this to the prevalence of *BRCA1/2*-PMs in the ethnicity-specific population control groups. In the follow-up studies, this relationship could not be established by two of three studies.[16, 29, 30] However, given that USC develops at a median age of 70 years,[3] all follow-up studies had insufficient follow-up (mean/median 3.3-6 years) relative to the age at enrolment (median/mean age 43-54.4 years) to meaningfully address the question of whether germline *BRCA1/2*-PM carriers have an increased risk of developing USC. Despite this shortcoming, one of the three single-arm cohort studies[16] reported a positive association between germline *BRCA1*-PMs and serous/serous-like carcinoma.

In addition, we described a case of a woman carrying a germline *BRCA1*-PM who developed an USC three years after RRSO. Molecular and functional analysis of tumour DNA demonstrated complete LOH of the *BRCA1* wild-type allele, causing a functional defect in HR, supporting a causal relationship. Of the included studies that additionally analysed LOH, the majority of cases (7/9, 77.8%) demonstrated LOH[15, 16, 38] of the *BRCA1* wild-type allele, further stressing a potential causal relationship between germline *BRCA1/2*-PMs and USC.

Aforementioned relationship has potentially important clinical implications. First, a clinical genetic consultation should be considered for USC patients who have not yet undergone germline *BRCA1/2*-PM testing, especially in the context of a positive first-degree family history, as shown in our meta-analysis.

Second, a prophylactic hysterectomy has to be considered. Since USC is a rare disease, even the 5-fold increased USC risk found in this study does not necessarily carry clinical consequences as the absolute USC risk remains low. However, due to the poor prognosis associated with USC, clinicians should be aware of this relationship and they should inform patients.

Third, it might open up ways for new systemic treatment options, such as use of PARP inhibitors, currently only registered for recurrent platinum-sensitive HGOTSC with germline or somatic *BRCA1/2*-PMs.[42-44] Although platinum derivates might have less effect on USC than HGOTSC,[11, 14, 45] there seems to be a subgroup of USC that is platinum-sensitive.[13] The smaller effect of platinum-based chemotherapy on USC compared with HGOTSC might be explained by the fact that USC more often present with mixed histology. Furthermore, (germline) *BRCA1/2*-PMs are less common in USC compared with HGOTSC. To the best of our knowledge, this is the first study to demonstrate HRD in USC using a functional RAD51 assay. The absence of HR in the tumour cells suggest that USC patients with germline *BRCA1/2*-PM may benefit from PARP inhibitor treatment. Women with a *BRCA1/2*-associated USC should be considered as potential candidates for future trials of PARP inhibitors.

In conclusion, data from our systematic review and meta-analysis support the view that USC is a component of *BRCA1/*2-associated HBOCS. This, together with our case report documenting LOH and HRD in USC, suggests a causal relationship between germline *BRCA1/2*-PMs and the development of USC. As germline *BRCA1/2*-PMs in USC may have therapeutic consequences in terms of use of PARP inhibitors and potentially risk-reducing surgery for patients and family members, clinicians should be aware of this association. Most importantly, this study supports the notion that women with USC should be offered screening for germline *BRCA1/2*-PMs when there is a positive family history for malignancies associated with HBOCS.

## Acknowledgments

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## Funding

The authors did not receive any funding for this study.

## Conflicts of interest statement

None declared.

# Appendix A: Search string

## PubMed

("Genes, BRCA1"[Mesh] OR "BRCA1"[all fields] OR "BRCA-1"[all fields] OR "BRCA1 Protein"[mesh] OR "Breast Cancer 1 Protein"[all fields] OR "Breast Cancer 1 Gene Product"[all fields] OR "Genes, BRCA2"[Mesh] OR "BRCA2"[all fields] OR "BRCA-2"[all fields] OR "BRCA2 Protein"[Mesh] OR "FANCD1 Protein"[all fields] OR "BRCA2 Gene Product"[all fields] OR brca*[all fields] OR "Breast Neoplasms/genetics"[mesh]) AND ("Uterine neoplasms"[mesh] OR "Uterus Neoplasm"[all fields] OR "Uterus Neoplasms"[all fields] OR "Uterine Neoplasm"[all fields] OR "Cancer of Uterus"[all fields] OR "Uterus Cancers"[all fields] OR "Cancer of the Uterus"[all fields] OR "Uterus Cancer"[all fields] OR "Uterine Cancer"[all fields] OR "Uterine Cancers"[all fields] OR "Uterus tumor"[all fields] OR "Uterine tumor"[all fields] OR "Uterus tumour"[all fields] OR "Uterine tumour"[all fields] OR "Uterus tumors"[all fields] OR "Uterine tumors"[all fields] OR "Uterus tumours"[all fields] OR "Uterine tumours"[all fields] OR "Uterus carcinoma"[all fields] OR "Uterine carcinoma"[all fields] OR "Uterus carcinomas"[all fields] OR "Uterine carcinomas"[all fields] OR "Uterus adenocarcinoma"[all fields] OR "Uterine adenocarcinoma"[all fields] OR "Uterus adenocarcinomas"[all fields] OR "Uterine adenocarcinomas"[all fields] OR "Endometrial Neoplasms"[mesh] OR "endometrium carcinoma"[all fields] OR "endometrium adenocarcinoma"[all fields] OR "endometrium carcinomas"[all fields] OR "endometrium adenocarcinomas"[all fields] OR "endometrium cancer"[all fields] OR "endometrium cancers"[all fields] OR "endometrial carcinoma"[all fields] OR "endometrial adenocarcinoma"[all fields] OR "endometrial carcinomas"[all fields] OR "endometrial adenocarcinomas"[all fields] OR "endometrial cancer"[all fields] OR "endometrial cancers"[all fields] OR "endometrial tumor"[all fields] OR "endometrium tumor"[all fields] OR "endometrial tumors"[all fields] OR "endometrium tumors"[all fields] OR "endometrial tumour"[all fields] OR "endometrium tumour"[all fields] OR "endometrial tumours"[all fields] OR "endometrium tumours"[all fields] OR "endometrioid adenocarcinoma"[all fields] OR "endometrioid adenocarcinomas"[all fields] OR "endometrioid carcinoma"[all fields] OR "endometrioid carcinomas"[all fields] OR endometrium serous carcinoma OR endometrium serous adenocarcinoma OR endometrium serous carcinomas OR endometrium serous adenocarcinomas OR endometrium serous cancer OR endometrium serous cancers OR endometrial serous carcinoma OR endometrial serous adenocarcinoma OR endometrial serous carcinomas OR endometrial serous adenocarcinomas OR endometrial serous cancer OR endometrial serous cancers OR endometrial serous tumor OR endometrium serous tumor OR endometrial serous tumors OR endometrium serous tumors OR endometrial serous tumour OR endometrium serous tumour OR endometrial serous tumours OR endometrium serous tumours OR endometrioid serous adenocarcinoma OR endometrioid serous adenocarcinomas OR endometrioid serous carcinoma OR endometrioid serous carcinomas OR "Hysterectomy"[Mesh] OR "Hysterectomy"[all fields] OR Hysterectom*[all fields])

## Embase

(("BRCA1".mp OR "BRCA-1".mp OR "BRCA1 Protein"/ OR "Breast Cancer 1 Protein".mp OR "Breast Cancer 1 Gene Product".mp OR "BRCA2".mp OR "BRCA-2".mp OR "BRCA2 Protein"/ OR "FANCD1 Protein".mp OR "BRCA2 Gene Product".mp OR brca*.mp OR "hereditary breast and ovarian cancer syndrome"/ OR ("Breast cancer"/ AND "Cancer genetics"/)) AND (exp *"Uterus Tumor"/ OR exp *"Uterus Cancer"/ OR "Uterus Neoplasm".ti,ab OR "Uterus Neoplasms".ti,ab OR "Uterine Neoplasm".ti,ab OR "Cancer of

Uterus".ti,ab OR "Uterus Cancers".ti,ab OR "Cancer of the Uterus".ti,ab OR "Uterus Cancer".ti,ab OR "Uterine Cancer".ti,ab OR "Uterine Cancers".ti,ab OR "Uterus tumor".ti,ab OR "Uterine tumor".ti,ab OR "Uterus tumour".ti,ab OR "Uterine tumour".ti,ab OR "Uterus tumors".ti,ab OR "Uterine tumors".ti,ab OR "Uterus tumours".ti,ab OR "Uterine tumours".ti,ab OR "Uterus carcinoma".ti,ab OR "Uterine carcinoma". ti,ab OR "Uterus carcinomas".ti,ab OR "Uterine carcinomas".ti,ab OR "Uterus adenocarcinoma".ti,ab OR "Uterine adenocarcinoma".ti,ab OR "Uterus adenocarcinomas".ti,ab OR "Uterine adenocarcinomas". ti,ab OR "endometrium carcinoma".ti,ab OR "endometrium adenocarcinoma".ti,ab OR "endometrium carcinomas".ti,ab OR "endometrium adenocarcinomas".ti,ab OR "endometrium cancer".ti,ab OR "endometrium cancers".ti,ab OR "endometrial carcinoma".ti,ab OR "endometrial adenocarcinoma". ti,ab OR "endometrial carcinomas".ti,ab OR "endometrial adenocarcinomas".ti,ab OR "endometrial cancer".ti,ab OR "endometrial cancers".ti,ab OR "endometrial tumor".ti,ab OR "endometrium tumor". ti,ab OR "endometrial tumors".ti,ab OR "endometrium tumors".ti,ab OR "endometrial tumour".ti,ab OR "endometrium tumour".ti,ab OR "endometrial tumours".ti,ab OR "endometrium tumours".ti,ab OR "endometrioid adenocarcinoma".ti,ab OR "endometrioid adenocarcinomas".ti,ab OR "endometrioid carcinoma".ti,ab OR "endometrioid carcinomas".ti,ab OR (endometr* AND serous AND (carcinoma* OR cancer OR tumor* OR tumour* OR adenocarcinom*)).ti,ab OR exp *"Hysterectomy"/ OR "Hysterectomy". ti,ab OR Hysterectom*.ti,ab)) OR (("BRCA1".ti,ab OR "BRCA-1".ti,ab OR *"BRCA1 Protein"/ OR "Breast Cancer 1 Protein".ti,ab OR "Breast Cancer 1 Gene Product".ti,ab OR "BRCA2".ti,ab OR "BRCA-2".ti,ab OR *"BRCA2 Protein"/ OR "FANCD1 Protein".ti,ab OR "BRCA2 Gene Product".ti,ab OR brca*.ti,ab OR *"hereditary breast and ovarian cancer syndrome"/ OR (*"Breast cancer"/ AND *"Cancer genetics"/)) AND (exp "Uterus Tumor"/ OR exp "Uterus Cancer"/ OR "Uterus Neoplasm".mp OR "Uterus Neoplasms". mp OR "Uterine Neoplasm".mp OR "Cancer of Uterus".mp OR "Uterus Cancers".mp OR "Cancer of the Uterus".mp OR "Uterus Cancer".mp OR "Uterine Cancer".mp OR "Uterine Cancers".mp OR "Uterus tumor".mp OR "Uterine tumor".mp OR "Uterus tumour".mp OR "Uterine tumour".mp OR "Uterus tumors".mp OR "Uterine tumors".mp OR "Uterus tumours".mp OR "Uterine tumours".mp OR "Uterus carcinoma".mp OR "Uterine carcinoma".mp OR "Uterus carcinomas".mp OR "Uterine carcinomas".mp OR "Uterus adenocarcinoma".mp OR "Uterine adenocarcinoma".mp OR "Uterus adenocarcinomas".mp OR "Uterine adenocarcinomas".mp OR "endometrium carcinoma".mp OR "endometrium adenocarcinoma". mp OR "endometrium carcinomas".mp OR "endometrium adenocarcinomas".mp OR "endometrium cancer".mp OR "endometrium cancers".mp OR "endometrial carcinoma".mp OR "endometrial adenocarcinoma".mp OR "endometrial carcinomas".mp OR "endometrial adenocarcinomas".mp OR "endometrial cancer".mp OR "endometrial cancers".mp OR "endometrial tumor".mp OR "endometrium tumor".mp OR "endometrial tumors".mp OR "endometrium tumors".mp OR "endometrial tumour". mp OR "endometrium tumour".mp OR "endometrial tumours".mp OR "endometrium tumours".mp OR "endometrioid adenocarcinoma".mp OR "endometrioid adenocarcinomas".mp OR "endometrioid carcinoma".mp OR "endometrioid carcinomas".mp OR (endometr* AND serous AND (carcinoma* OR cancer OR tumor* OR tumour* OR adenocarcinom*)).mp OR exp "Hysterectomy"/ OR "Hysterectomy". mp OR Hysterectom*.mp))

## Web of science

TS=(("BRCA1" OR "BRCA-1" OR "BRCA1 Protein" OR "Breast Cancer 1 Protein" OR "Breast Cancer 1 Gene Product" OR "BRCA2" OR "BRCA-2" OR "BRCA2 Protein" OR "FANCD1 Protein" OR "BRCA2 Gene Product" OR brca* OR "hereditary breast and ovarian cancer syndrome" OR ("Breast cancer" AND "Cancer genetics")) AND ("Uterus Tumor" OR "Uterus Cancer" OR "Uterus Neoplasm" OR "Uterus

Neoplasms" OR "Uterine Neoplasm" OR "Cancer of Uterus" OR "Uterus Cancers" OR "Cancer of the Uterus" OR "Uterus Cancer" OR "Uterine Cancer" OR "Uterine Cancers" OR "Uterus tumor" OR "Uterine tumor" OR "Uterus tumour" OR "Uterine tumour" OR "Uterus tumors" OR "Uterine tumors" OR "Uterus tumours" OR "Uterine tumours" OR "Uterus carcinoma" OR "Uterine carcinoma" OR "Uterus carcinomas" OR "Uterine carcinomas" OR "Uterus adenocarcinoma" OR "Uterine adenocarcinoma" OR "Uterus adenocarcinomas" OR "Uterine adenocarcinomas" OR "endometrium carcinoma" OR "endometrium adenocarcinoma" OR "endometrium carcinomas" OR "endometrium adenocarcinomas" OR "endometrium cancer" OR "endometrium cancers" OR "endometrial carcinoma" OR "endometrial adenocarcinoma" OR "endometrial carcinomas" OR "endometrial adenocarcinomas" OR "endometrial cancer" OR "endometrial cancers" OR "endometrial tumor" OR "endometrium tumor" OR "endometrial tumors" OR "endometrium tumors" OR "endometrial tumour" OR "endometrium tumour" OR "endometrial tumours" OR "endometrium tumours" OR "endometrioid adenocarcinoma" OR "endometrioid adenocarcinomas" OR "endometrioid carcinoma" OR "endometrioid carcinomas" OR (endometr* AND serous AND (carcinoma* OR cancer OR tumor* OR tumour* OR adenocarcinom*)) OR "Hysterectomy" OR "Hysterectomy" OR Hysterectom*))



## Appendix B: PRISMA-checklist

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## References


1. Plataniotis G, Castiglione M. Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010;21 Suppl 5:v41-5.
2. Hendrickson M, Ross J, Eifel P, Martinez A, Kempson R. Uterine papillary serous carcinoma: a highly malignant form of endometrial adenocarcinoma. Am J Surg Pathol 1982;6(2):93-108.
3. Hamilton CA, Cheung MK, Osann K, Chen L, Teng NN, Longacre TA, et al. Uterine papillary serous and clear cell carcinomas predict for poorer survival compared to grade 3 endometrioid corpus cancers. Br J Cancer 2006;94(5):642-6 doi 10.1038/sj.bjc.6603012.
4. Integraal Kankercentrum Nederland. Endometriumcarcinoom. Landelijke richtlijn met regionale toevoegingen. 2011, Versie: 3.0, www.oncoline.nl. Accessed: 22-05-2016.
5. Colombo N, Preti E, Landoni F, Carinelli S, Colombo A, Marini C, et al. Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24 Suppl 6:vi33-8.
6. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, et al. Integrated genomic characterization of endometrial carcinoma. Nature 2013;497(7447):67-73.
7. Kuhn E, Wu RC, Guan B, Wu G, Zhang J, Wang Y, et al. Identification of molecular pathway aberrations in uterine serous carcinoma by genome-wide analyses. J Natl Cancer Inst 2012;104(19):1503-13.
8. Zhao S, Choi M, Overton JD, Bellone S, Roque DM, Cocco E, et al. Landscape of somatic single-nucleotide and copy-number mutations in uterine serous carcinoma. Proc Natl Acad Sci USA 2013;110(8):2916-21.
9. Lax SF, Kendall B, Tashiro H, Slebos RJ, Hedrick L. The frequency of p53, K-ras mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathways. Cancer 2000;88(4):814-24.
10. Lax SF, Kurman RJ. A dualistic model for endometrial carcinogenesis based on immunohistochemical and molecular genetic analyses. Verh Dtsch Ges Pathol 1997;81:228-32.
11. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD. Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer. Cancer Discov 2015;5(11):1137-54.
12. Marquard AM, Eklund AC, Joshi T, Krzystanek M, Favero F, Wang ZC, et al. Pan-cancer analysis of genomic scar signatures associated with homologous recombination deficiency suggests novel indications for existing cancer drugs. Biomark res 2015;3:9.
13. Frimer M, Levano KS, Rodriguez-Gabin A, Wang Y, Goldberg GL, Horwitz SB, et al. Germline mutations of the DNA repair pathways in uterine serous carcinoma. Gynecol Oncol 2016;141(1):101-7.
14. Hogberg T, Signorelli M, de Oliveira CF, Fossati R, Lissoni AA, Sorbe B, et al. Sequential adjuvant chemotherapy and radiotherapy in endometrial cancer—results from two randomised studies. Eur J Cancer 2010;46(13):2422-31.
15. Lavie O, Ben-Arie A, Segev Y, Faro J, Barak F, Haya N, et al. BRCA germline mutations in women with uterine serous carcinoma—still a debate. Int J Gynecol Cancer 2010;20(9):1531-4.

16. Shu CA, Pike MC, Jotwani AR, Friebel TM, Soslow RA, Levine DA, et al. Uterine Cancer After Risk-Reducing Salpingo-oophorectomy Without Hysterectomy in Women With BRCA Mutations. JAMA oncol 2016 Nov 1;2(11):1434-1440.
17. Goshen R, Chu W, Elit L, Pal T, Hakimi J, Ackerman I, et al. Is uterine papillary serous adenocarcinoma a manifestation of the hereditary breast-ovarian cancer syndrome? Gynecol Oncol 2000;79(3):477-81.
18. Niell BL, Rennert G, Bonner JD, Almog R, Tomsho LP, Gruber SB. BRCA1 and BRCA2 founder mutations and the risk of colorectal cancer. J Natl Cancer Inst 2004;96(1):15-21.
19. Hartge P, Struewing JP, Wacholder S, Brody LC, Tucker MA. The prevalence of common BRCA1 and BRCA2 mutations among Ashkenazi Jews. Am J Hum Genet 1999;64(4):963-70.
20. Roa BB, Boyd AA, Volcik K, Richards CS. Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. Nature genet 1996;14(2):185-7.
21. Barak F, Milgrom R, Laitman Y, Gemer O, Rabinovich A, Piura B, et al. The rate of the predominant Jewish mutations in the BRCA1, BRCA2, MSH2 and MSH6 genes in unselected Jewish endometrial cancer patients. Gynecol oncol 2010;119(3):511-5.
22. Bruchim I, Amichay K, Kidron D, Attias Z, Biron-Shental T, Drucker L, et al. BRCA1/2 germline mutations in Jewish patients with uterine serous carcinoma. Int J Gynecol Cancer 2010;20(7):1148-53.
23. van Eijk R, Stevens L, Morreau H, van Wezel T. Assessment of a fully automated high-throughput DNA extraction method from formalin-fixed, paraffin-embedded tissue for KRAS, and BRAF somatic mutation analysis. Exp Mol Pathol 2013;94(1):121-5.
24. Naipal KA, Verkaik NS, Ameziane N, van Deurzen CH, Ter Brugge P, Meijers M, et al. Functional ex vivo assay to select homologous recombination-deficient breast tumors for PARP inhibitor treatment. Clin Cancer Res 2014;20(18):4816-26.
25. Levine DA, Lin O, Barakat RR, Robson ME, McDermott D, Cohen L, et al. Risk of endometrial carcinoma associated with BRCA mutation. Gynecol Oncol 2001;80(3):395-8.
26. Antoniou AC, Cunningham AP, Peto J, Evans DG, Lalloo F, Narod SA, et al. The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. Br J Cancer 2008;98(8):1457-66.
27. Peto J, Collins N, Barfoot R, Seal S, Warren W, Rahman N, et al. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. J Natl Cancer Inst 1999;91(11):943-9.
28. Anglian Breast Cancer Study Group. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Br J Cancer 2000;83(10):1301-8.
29. Reitsma W, Mourits MJ, de Bock GH, Hollema H. Endometrium is not the primary site of origin of pelvic high-grade serous carcinoma in BRCA1 or BRCA2 mutation carriers. Mod Pathol 2013;26(4):572-8.
30. Beiner ME, Finch A, Rosen B, Lubinski J, Moller P, Ghadirian P, et al. The risk of endometrial cancer in women with BRCA1 and BRCA2 mutations. A prospective study. Gynecol Oncol 2007;104(1):7-10.

31. Al-Hussaini M, Stockman A, Foster H, McCluggage WG. WT-1 assists in distinguishing ovarian from uterine serous carcinoma and in distinguishing between serous and endometrioid ovarian carcinoma. Histopathology 2004;44(2):109-15.
32. Goldstein NS, Uzieblo A. WT1 immunoreactivity in uterine papillary serous carcinomas is different from ovarian serous carcinomas. Am J Clin Pathol 2002;117(4):541-5.
33. Pennington KP, Walsh T, Lee M, Pennil C, Novetsky AP, Agnew KJ, et al. BRCA1, TP53, and CHEK2 germline mutations in uterine serous carcinoma. Cancer 2013;119(2):332-8.
34. Trujillano D, Weiss ME, Schneider J, Koster J, Papachristos EB, Saviouk V, et al. Next-generation sequencing of the BRCA1 and BRCA2 genes for the genetic diagnostics of hereditary breast and/or ovarian cancer. J Mol Diagn 2015;17(2):162-70.
35. Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat 2011;32(5):557-63.
36. Ferla R, Calo V, Cascio S, Rinaldi G, Badalamenti G, Carreca I, et al. Founder mutations in BRCA1 and BRCA2 genes. Ann Oncol 2007;18 Suppl 6:vi93-8.
37. Hornreich G, Beller U, Lavie O, Renbaum P, Cohen Y, Levy-Lahad E. Is uterine serous papillary carcinoma a BRCA1-related disease? Case report and review of the literature. Gynecol Oncol 1999;75(2):300-4.
38. Lavie O, Hornreich G, Ben Arie A, Renbaum P, Levy-Lahad E, Beller U. BRCA1 germline mutations in women with uterine serous papillary carcinoma. Obstet Gynecol 2000;96(1):28-32.
39. Cohen I. Endometrial pathologies associated with postmenopausal tamoxifen treatment. Gynecol Oncol 2004;94(2):256-66.
40. Segev Y, Iqbal J, Lubinski J, Gronwald J, Lynch HT, Moller P, et al. The incidence of endometrial cancer in women with BRCA1 and BRCA2 mutations: an international prospective cohort study. Gynecol Oncol 2013;130(1):127-31.
41. Jones ME, van Leeuwen FE, Hoogendoorn WE, Mourits MJ, Hollema H, van Boven H, et al. Endometrial cancer survival after breast cancer in relation to tamoxifen treatment: pooled results from three countries. Breast cancer res 2012;14(3):R91.
42. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med 2012;366(15):1382-92.
43. Ledermann JA, Drew Y, Kristeleit RS. Homologous recombination deficiency and ovarian cancer. Eur J Cancer 2016;60:49-58.
44. Liu JF, Konstantinopoulos PA, Matulonis UA. PARP inhibitors in ovarian cancer: current status and future promise. Gynecol Oncol 2014;133(2):362-9.
45. Colombo N, Creutzberg C, Amant F, Bosse T, Gonzalez-Martin A, Ledermann J, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: Diagnosis, Treatment and Follow-up. Int J Gynecol Cancer 2016;26(1):2-30.

# Supplementary table

**Supplementary Table S1. Personal and family histories of germline *BRCA1/2*-PM carriers with USC compared to non-*BRCA1/2*-PM carriers in single-arm case studies**

| | USC and germline *BRCA1/2*-PM | | | USC without germline *BRCA1/2*-PM | | |
|---|---|---|---|---|---|---|
| | Total | Personal history breast cancer (%) | Family history breast/ovarian cancer[a] (%) | Total | Personal history breast cancer (%) | Family history breast/ovarian cancer[a] (%) |
| **Barak et al, 2010** | 0 | - | - | 56 | n.a. | n.a. |
| **Bruchim et al, 2010** | 6 | 1 (16.7) | 3 (50.0) | 23[b] | 5 (21.7) | 2 (8.7) |
| **Goshen et al, 2000** | 0 | - | - | 56 | 6 (10.7) | 16 (28.6) |
| **Lavie et al, 2000** | 2 | 1 (50) | 2 (100) | 7 | 2 (28.6) | 0 (0.0) |
| **Lavie et al, 2010** | 8 | 3 (37.5) | 8 (100) | 43 | 10 (23.3) | 9 (20.9) |
| **Levine et al, 2001** | 0 | - | - | 17 | n.a. | n.a. |
| **Pennington et al, 2013** | 3[c] | 2 (66.7) | 1 (33.3) | 131[c] | 20 (13.2) | 39 (29.8) |
| **Total** | 19 | 7 (36.8) | 14 (73.7) | 260 | 38 (14.6) | 66 (25.4) |

Abbreviations: PM: pathogenic mutation, USC: Uterine serous carcinoma, n.a.: not available/not extractable. [a]: Family history only includes first degree-relatives; parents, children, full-siblings. [b]: Considers Ashkenazi Jews and non-Ashkenazi Jews, data not extractable for Ashkenazi Jews alone. [c]: Personal and family history data available for 134/151 women (including women with germline *BRCA1/2*-PM).