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Does serous tubal intraepithelial carcinoma (STIC) metastasize? The clonal relationship between STIC and subsequent high-grade serous carcinoma in BRCA1/2 mutation carriers several years after risk-reducing salpingo-oophorectomy

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HIGHLIGHTS

- Clonal relationship between temporally distant STICs and HGSCs was established in five of the seven cases.
- Similar *TP53* mutations were detected by performing next generation targeted sequencing.
- Median interval for developing HGSC after risk-reducing salpingo-oophorectomy was 59 months.
- These findings support the hypothesis that STIC lesions may metastasize.

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ABSTRACT

Objective. The majority of high-grade serous carcinomas (HGSC) of the ovary, fallopian tube, and peritoneum arise from the precursor lesion called serous tubal intraepithelial carcinoma (STIC). It has been postulated that cells from STICs exfoliate into the peritoneal cavity and give rise to peritoneal HGSC several years later. While co-existent STICs and HGSCs have been reported to share similarities in their mutational profiles, clonal relationship between temporally distant STICs and HGSCs have been infrequently studied and the natural history of STICs remains poorly understood.

Methods. We performed focused searches in two national databases from the Netherlands and identified a series of BRCA1/2 germline pathogenic variant (GPV) carriers ($n = 7$) who had STIC, and no detectable invasive carcinoma, at the time of their risk-reducing salpingo-oophorectomy (RRSO), and later developed peritoneal HGSC. The clonal relationship between these STICs and HGSCs was investigated by comparing their genetic mutational profile by performing next-generation targeted sequencing.

Results. Identical pathogenic mutations and loss of heterozygosity of *TP53* were identified in the STICs and HGSCs of five of the seven patients (71%), confirming the clonal relationship of the lesions. Median interval for developing HGSC after RRSO was 59 months (range: 24–118 months).

Conclusion. Our results indicate that cells from STIC can shed into the peritoneal cavity and give rise to HGSC after long lag periods in BRCA1/2 GPV carriers, and argues in favor of the hypothesis that STIC lesions may metastasize.

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1. Introduction

1.1. Serous tubal intraepithelial carcinoma (STIC) is a recognized precursor of high-grade serous carcinoma (HGSC)

HGSC of the ovary or fallopian tube is known to be one of the most lethal gynecological malignancies, with a five-year survival rate of 47% [1,2]. Clinico-pathological data from the last two decades indicate that most HGSCs arises in the fallopian tubes and not in the ovary, from a precursor lesion called STIC [3–5]. STIC is considered as an early morphologically recognizable step in HGSC development, and its status as an HGSC precursor has been established on the basis of similarities in morphological, immunophenotypical and transcriptomic profiles with HGSC [6]. >90% of STICs harbor pathogenic mutations of the *TP53* gene, which is known to be the earliest DNA sequence alteration to occur during HGSC carcinogenesis [6]. Potential fallopian tube precursor lesions are histologically classified into three different entities: p53 signatures, serous tubal intraepithelial lesions (STILs) and STICs. These entities are distinguished on the basis of the differences in their morphological and immunohistochemical (p53 and Ki-67) features. A p53 signature is defined by a *TP53* missense mutation in a small number of epithelial cells with a normal morphology and no increase in proliferative activity (Ki-67 < 10%). A STIL is a lesion where some, but not all, of the criteria for STIC are met, for example *TP53* mutation in cells with morphological abnormalities but no increased proliferative activity (<10%). In a STIC there is cytological atypia, *TP53* mutation and increased Ki-67 activity (>10%) [6–9].

1.2. Ovarian cancer risk in BRCA1/2-gene germline pathogenic variants carriers

Cumulative life-time risk for ovarian cancer up to the age of 80 years is 44% (95% CI 36–53%) for BRCA1 GPV carriers and 17% (95% CI, 11–25%) for BRCA2 GPV carriers, compared to 1.3% in the general population [10]. As there are no effective screening strategies for HGSC, BRCA1/2 GPV carriers are advised to undergo risk-reducing salpingo-oophorectomy (RRSO) by the age of 35–40 and 40–45 years respectively [11–13]. However, BRCA1/2 GPV carriers still remain at a risk of developing HGSC of the peritoneum, especially when the RRSO is performed after the advised age. The estimated cumulative risk is 3.9% for BRCA1 and 1.9% for BRCA2 carriers, up to 20 years post-RRSO [14,15]. For women with a STIC at RRSO, the 5- and 10-year risks of developing HGSC of the peritoneum are10.5% and 27.5% respectively, whereas for women without a STIC, the risk would be 0.3% and 0.9% respectively [16].

1.3. Focus of the current study

Currently, there is limited understanding of the mechanisms by which peritoneal HGSCs develop in BRCA1/2 GPV carriers after RRSO. It has been posited that cells from STICs, or lesions preceding the development of STICs harboring *TP53* mutations, may disseminate into the peritoneal cavity and potentially remain biologically quiescent until they manifest as a HGSC several years later [17,18].

To better understand the biology of STICs and the relationship between the STIC and subsequent HGSC, we identified BRCA1/2 GPV carriers from two Dutch national databases, who had an isolated STIC at RRSO, and subsequently developed HGSC (at least >12 months post-RRSO). We investigated the clonal relationship between the STICs and subsequent HGSCs by studying their genetic profiles by performing next-generation targeted sequencing.

Our hypothesis is that if the STIC and subsequent HGSC share a clonal relationship evidenced by the presence of identical *TP53* mutation, the concept of peritoneal dissemination of STIC lesions and progression to malignancy later on in life will be supported.

2. Materials and methods

2.1. Patient selection

Patients were retrospectively selected from the Hereditary Breast Ovarian Cancer Research Group Netherlands (HEBON) cohort, the database of Dutch Nationwide Pathology Databank (PALGA) and from the electronic records of the Department of Gynecologic Oncology, Erasmus MC. The HEBON database was searched for potential inclusions between 2000 and 2011. The HEBON database is a national database containing >35,000 patients with breast- and ovarian cancer, of which 7102 female BRCA1/2 GPV carriers are registered. The PALGA database was searched for potential inclusions from 1994 to 2020. The PALGA database annually accrues ~3 million pathology records from all the Dutch centers. From the PALGA database, 147 patients were fulfilled our search criteria. The databases were search using the Dutch terms and synonyms for BRCA, STIC and HGSC of the peritoneum.

Patients from the above-mentioned databases who fulfilled the following criteria were included:

1. Proven *BRCA1/2* GPV carrier status
2. Underwent RRSO
3. Presence of only STIC and no invasive carcinoma in the RRSO specimen
4. Developed HGSC at least 12 months after RRSO (the cut-off of 12 months was chosen to exclude the possibility that the HGSC was already present at the time of RRSO)

2.2. Clinical data collection and analysis

The following information was collected for all included patients: age, type of *BRCA* mutation [1 or 2], menopausal status at RRSO, date of RRSO, date of STIC diagnosis, date of HGSC diagnosis, FIGO stage of HGSC at diagnosis, history of breast carcinoma, treatment, and follow-up information. Descriptive analysis of data was performed using SPSS Statistics version 25 (Armonk, NY: IBM Corp).

2.3. Histopathology

For all included patients, the tubal tissue was completely enclosed and examined. Due to the retrospective nature of this study, we were not able to determine whether the *S*ectioning and *E*xtensively *E*xamining the *Fim*bria (SEE-FIM) protocol was followed for handling the tubal specimens [19]. The histology of all included lesions (STIC and HGSC) was reviewed by an experienced gyneco-pathologist (PEG) to confirm the diagnosis of a STIC.

2.4. Immunohistochemistry

For patients where immunohistochemistry (IHC) with p53 and/or Ki-67 had been conducted for the STIC lesion at the time of diagnosis, the results were extracted from the pathology reports. For the remaining patients, if sufficient material was available, 4 μm sections were prepared from the formalin fixed paraffin embedded (FFPE) tissues, and IHC was conducted using p53 (BP53–11, Ready to use, Ventana) and Ki-67 (clone 30–9, Ready to use, Ventana) on Benchmark Ultra Immunostainer (Roche), following manufacturer's instructions. Abnormal p53-expression, i.e., continuous strong nuclear expression, or complete absence of expression, along with a Ki-67 labeling index ≥10% were considered confirmatory for the histological diagnosis of STIC.

2.5. Next-generation targeted sequencing

Clonal relationship between the STICs and HGSCs was investigated using next-generation targeted sequencing (NGTS). To extract DNA for NGTS, lesional tissue was manually micro-dissected from sections

prepared from the FFPE material. Dissected tissue fragments were subjected to proteinase K digestion for 16 h at 56 °C in the presence of 5% Chelex 100 resin (BioRad Laboratories, Hercules, CA). The extracted DNA was used without further purification after inactivation of proteinase K and the removal of cell debris and the Chelex resin. DNA concentration was measured with Qubit 2.0 fluorometer (Thermo Fisher Scientific Inc., Wilmington, DE) following manufacturer's instructions. NGS was performed using a custom cancer hotspot AmpliSeq panel consisting of 1028 amplicons on the Ion Torrent platform (Supplementary Table 1). The library was prepared with the AmpliSeq Library Kit Plus (ThermoFisher Scientific Inc.). Template preparation and chip loading were done on the Ion Chef and sequencing was performed using the Ion Genestudio S5 prime system on Ion 540 chips (ThermoFisher Scientific, Waltham, MA).

A commercially available software package for variant calling (Sequence Pilot v5.2.0, JSI Medical Systems, Ettenheim, Germany, https://www.jsi-medisys.de/products/sequence-pilot/seqnext/) was used to select the potentially reliable variants. Variants reported in the ESP6500si or 1000 genomes databases and occurring at frequencies higher than 1% were filtered out. For inclusion, variants needed to (i) have a minimum coverage of 100 reads per amplicon, (ii) be present in at least 20% of the called reads and/or correspond to the tumor cell percentage. The following alterations were included: nonsynonymous point mutations, splice site alterations, and insertions and deletions changing the protein amino acid sequence. Additionally, the intuitive web-application SNPitty was used to detect loss of heterozygosity (LOH) [20].

2.6. Ethical clearance

Approval of the Institutional Review Board of Erasmus MC (MEC-2020-0326) and all participating institutions was obtained for this study. All patient data were anonymized and patient materials were handled in accordance with the guidelines of Foundation Federation of Dutch Medical Scientific Societies (federa.org/codes-conduct).

3. Results

Seven patients from the HEBON (2000—2011) and PALGA databases (1994–2020) fulfilled our inclusion criteria, of which three patients were excluded as for various reasons. In one patient, no STIC lesion could be detected in the RRSO specimen on pathology review and in two other patients, there was insufficient STIC tissue available to perform NGS. Three additional patients fulfilling our inclusion criteria were identified from the database of Erasmus MC. This resulted in a total of seven patients for final inclusion, which were histologically reviewed by an experienced gyneco-pathologist (PEG) and fulfilled the criteria for a STIC [8]. A graphical study flowchart of performed analyses is shown in Fig. 1 and the clinical data is presented in Table 1. The immunohistochemistry results of p53 and Ki-67 are reported in Supplementary Table 2. For patient 2 and 3 the Ki-67 index was missing in the original pathology report and there was insufficient material available after performing NGS to perform Ki-67 immunohistochemistry.

3.1. Clinical characteristics

The mean age of the included women was 57 years (range: 46–69 years). All women were post-menopausal at the time of RRSO. The median interval for developing HGSC after RRSO was 59 months (range: 24–118 months). Four of the seven women died from HGSC. The median interval to death after diagnosis was 30 months (range: 21–63 months). Of the three women who are still alive, one had recurrent disease 33 months after the diagnosis of HGCS (patient number 5), which was stable at the last visit, and two women (case number 6 and 7) had no signs of recurrence 4 and 18 years following RRSO, respectively. Three of the included women had a history of breast cancer.

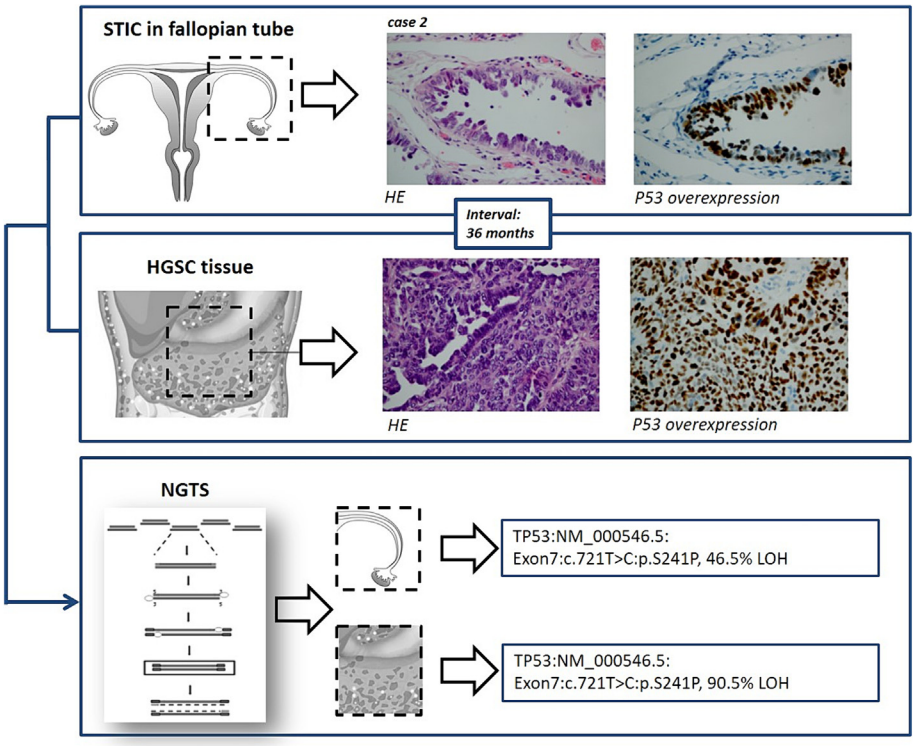

Fig. 1. Graphical flowchart of study design.
Abbreviations: STIC, serous tubal intraepithelial carcinoma, HGSC, high-grade serous carcinoma, HE, hematoxylin and eosin, NGTS, next-generation sequencing; P53, tumor protein 53; LOH, loss of heterozygosity.

Table 1
Clinical characteristics of study population.

Patient number	Age at diagnosis of STIC	BRCA1/2	History of breast cancer	Interval between STIC and HGSC (months)	FIGO stage of HGSC at diagnosis	Treatment of HGSC	Surgical outcome	Progressive disease	Death of disease	Interval between HGSC and date of death (months)
1	57	BRCA2	No	59	IV	Interval CRS and 6 courses of chemotherapy	Complete CRS	Yes	Yes	21
2	57	BRCA1	No	36	IV	Primary CRS and 6 courses of chemotherapy	Optimal CRS	Yes	Yes	23
3	61	BRCA1	Yes	33	IV	Hormonal therapy and chemotherapy (initially interpreted as a metastasis of breast cancer)	N/A	Yes	Yes	63
4	62	BRCA1	No	24	IIIC	Interval CRS and 6 courses of chemotherapy	Complete CRS	Yes	Yes	36
5	46	BRCA1	No	118	IIIC	Interval CRS and 6 courses of chemotherapy	Optimal CRS	No	N/A	N/A
6	53	BRCA1	Yes	80	IIC	Interval CRS and 6 courses of chemotherapy	Complete CRS	No	N/A	N/A
7	69	BRCA2	Yes	72	IIIC	Interval CRS and 6 courses of chemotherapy	Complete CRS	No	N/A	N/A

Abbreviations: STIC, serous tubal intraepithelial carcinoma; BRCA, breast cancer gene; FIGO, The International Federation of Gynecology and Obstetrics; HGSC, high-grade serous cancer; CRS, cytoreductive surgery; N/A, not applicable.

None of the patients underwent surgical staging or systemic therapy after diagnosis of the STIC.

3.2. Next-generation targeted sequencing results

Identical pathogenic mutations of *TP53* were identified in the STICs and HGSCs of five patients (71%; Table 2). Of these, three patients [1,2,6] and also showed loss of heterozygosity (LOH) of the *TP53* gene in both the STIC and HGSC. Based on the presence of identical *TP53* mutations and LOH affecting the *TP53* gene, we deduced a clonal relationship between the STIC and HGSC of five patients in our series (Table 2). Patient 5 showed retention of homozygosity (ROH) in the STIC and LOH in the peritoneal HGSC. No clonal relationship could be established between the STIC and HGSC of patients 3 and 7 on the basis of CNV analyses, *TP53* or other genetic mutations. Both of these patients had a history of breast cancer. On performing NGTS on the breast tumor tissue, we detected a different *TP53* mutation in one patient and a *PIK3CA* mutation in the other patient (Table 2). Patient number 7 had a STIC with 18% Ki-67 expression (lower limit 10%).

4. Discussion

4.1. Main findings and clinico-pathological significance

To the best of our knowledge, this is the first study to investigate the clonal relationship in a series of STICs, and subsequent HGSCs, post RRSO in BRCA1/2 GPV carriers by performing a comprehensive search in two large national databases. In five of the seven (71%) included patients, a clonal relationship between the STICs and HGSCs could be established by detection of identical mutations and loss of heterozygosity of the *TP53* gene. These findings support the origin of HGSC from a tubal precursor, and also suggest that lesional cells that

Table 2
Results of next-generation sequencing.[a]

Patient number	NGS STIC lesion	CNV-analysis	NGS HGSC	CNV-analysis	NGS breast cancer	CNV-analysis
1	*TP53*:NM_000546.5:exon4: c.320dupA:p.Y107_G108delinsX, variant allele frequency: 57%	LOH	*TP53*:NM_000546.5:exon4:c.320dupA:p.Y107_G108delinsX, variant allele frequency: 23.5%	LOH	Not applicable	Not applicable
2	*TP53*:NM_000546.5:exon7: c.721 T > C:p.S241P, variant allele frequency: 46.5%	LOH	*TP53*:NM_000546.5:exon7:c.721 T > C: p.S241P, variant allele frequency: 90.5%	LOH	Not applicable	Not applicable
3	*TP53*:NM_000546.5:exon7: c.722C > G:p.S241C, variant allele frequency: 52%	LOH	*TP53*:NM_000546.5:exon6:c.590 T > A: p.V197E, variant allele frequency: 30.2%	Poor quality	*TP53*:NM_000546.5:exon6: c.659 A > C:p.Y220S, variant allele frequency: 24%	LOH
4	*TP53*: NM_000546.5: exon 1:c.-29 + 1G > T (p.?), variant allele frequency: 33%	Not available	*TP53*: NM_000546.5: exon 1:c.-29 + 1G > T (p.?), variant allele frequency: 42%	Not available	Not applicable	Not applicable
5	*TP53*:NM_000546.5:exon8: c.817C > T:p.R273C, variant allele frequency: 33%	ROH	*TP53*:NM_000546.5:exon8:c.817C > T: p.R273C, variant allele frequency: 38%	LOH	Not applicable	Not applicable
6	*TP53*:NM_000546.5:exon4: c.249_250delGG, variant allele frequency: 44%	LOH	*TP53*:NM_000546.5:exon4: c.249_250delGG, variant allele frequency: 77%	LOH	Not available	Not available
7	*TP53* (NM_000546) exon 5: c.476C > A, p.A159D, variant allele frequency: 52%.	Not available	*TP53* (NM_000546) exon 7: c.722C > A; p.S241Y, variant allele frequency: 71% (T2) and 92% (T3)	LOH	PIK3CA (NM_006218) exon 21: c.3140 A > G; p.H1047R, variant allele frequency: 56%	LOH

Abbreviations: STIC, serous tubal intraepithelial carcinoma; HGSC, high-grade serous cancer; NGS, next-generation sequencing; TP53, tumor protein 53; CNV, copy number variation; LOH, loss of heterozygosity; ROH, retention of heterozygosity.

[a] In patient 1,2, 4, 5 and 6 the STIC lesions were clonally related to HGSC tissue; in patient 3 and 7 the STIC lesions and HGSC tissue were not clonally related. For these patients their breast cancer tissue was additionally sequenced and was neither clonally related to the STIC or to the HGSC tissue.

exfoliate from STIC may remain quiescent for years on the peritoneal surface, giving rise to HGSC after long lag periods. Furthermore, our results also argue in favor of the theory that peritoneal HGSC is essentially a metastatic disease, in which the primary lesion lies in the fallopian tube. This potential metastatic capability of STICs, highlights the importance of extensive sampling of the fallopian tubes of BRCA1/2 carriers to detect STICs.

Interestingly, clonal relationship between STIC and HGSC could not be established for two patients, both of whom had a history of breast carcinoma (detailed information of these cases has been described in Supplementary Table 3). Judging from the *TP53* mutational profile, their breast carcinoma, although *TP53* mutant, was not clonally related to either the STIC or the HGSC. Additionally, in one of these patients, a lesion histologically similar to STIC was observed on the ovarian surface on histology review. None of the other patients had in-situ/dysplastic lesions in the ovary. Although this lesion was diagnosed as a STIC at the time of the RRSO, this lesion cannot be strictly called a STIC as it was not tubal. However, as we know from an anatomical perspective, there is a close connection of the fimbriated end of the fallopian tube and the ovary. Therefore, there is a possibility that the ovarian lesion may have originated from cells that detached from the fimbriated end and implanted on the ovarian surface. It is also conceivable that in these patients a different pathophysiological mechanism may have played a role in the development of HGSC. Although several studies support the tubal origin of ovarian cancer and HGSC, there may be a minority of patients in which this theory does not apply [3–6,16,21–23]. Nevertheless, it is important to acknowledge that lack of identical *TP53* mutations does not necessarily disprove a clonal relationship. The original clone may have been subsumed by overgrowing cancer cells or could have been outcompeted for survival by other clones. Another explanation may be that these cases had a second/separate STIC, which was missed by the pathologist. Even by after applying the SEE-FIM protocol, STIC lesions can be missed if they are very small.

4.2. Comparison of our data with existent literature

To explain the development of invasive carcinoma from quiescent precursor cells after long lag periods, several hypotheses have been postulated, most of which are based on the principles of gradual genetic evolution. An alternative theory of a more catastrophic and complex genomic event was recently proposed by Yoon et al. The catastrophic genetic event is called chromothripsis, which is common in cancers associated with p53 dysfunction. [24]. According to this theory, the precursor cells escape into the peritoneal cavity years prior to the catastrophic genomic event, during which one or a few chromosomes undergo reassembly and trigger the carcinogenesis process [24]. We were not able to investigate this in our study as we performed targeted sequencing on our samples. Furthermore, reliable calling of chromothripsis in archival tissue samples can be challenging as formalin fixation is known to degrade DNA quality and introduce artifacts into the genetic data.

In one of our previous studies on a 16-year cohort of BRCA1/2 GPV carriers who underwent RRSO ($n = 527$), we observed that of the four patients who later developed HGSC, two had an isolated STIC at the time of their RRSO [25]. The STIC and HGSC from both patients harbored identical *TP53* mutations, which indicated that the lesions were clonally related [25]. Additional studies have reported on the association of an isolated STIC at RRSO with subsequent HGSCs at rates varying between 0 and 50% [16,26–29]. Nevertheless, studies investigating the clonal relationship between STIC and subsequent HGSCs remain sparse because of the rarity of finding an isolated STIC at RRSO and the prolonged follow-up required to detect subsequent HGSCs [6].

The majority of the current evidence on the clonal relationship between of STIC and HGSC comes from studies with small number of patients, due to the rarity of the disease [4,30–33]. However, most of these studies actually investigated clonality of STIC and the *concurrent* HGSC, and not spatially distinct lesions as in our study. Labidi-Galy et al. performed whole-exome sequencing and copy number analyses of STIC and concurrent ovarian HGSC in nine patients. They reported identical somatic alterations in the STIC and ovarian HGSC, and computed a window of seven years between development of a STIC and initiation of ovarian HGSC using evolutionary analyses [4]. In our unique case-series, the median interval of developing HGSC after the detection of STIC at RRSO was 59 months (range: 24–118 months). This is similar to that reported in a recently published individual patient-data meta-analysis of 17 studies comprising 3121 BRCA1/2 GPV carriers (48 months; 18–118 months) [16]. In addition to small sample sizes, lengths of follow-up in previous studies have been quite short, and in some cases, have not even been provided.

A recent retrospective study investigating 2557 women showed HGSC in respectively 1.5% and 0.6% of the RRSO tissue from asymptomatic BRCA1 and 2 GPV carriers [23]. Of these women, 73.3% had a focus of invasive carcinoma in the fallopian tubes along with a concurrent STIC. As far as could be investigated, none of our patients had invasive disease at RRSO, as this was an exclusion criterion.

4.3. Strengths and limitations

Our case-series carefully assembled by searching several large national databases provides the unique opportunity to investigate genomic changes at different time-points in STICs already present at RRSO, and HGSCs that develop several years later. Detailed clinical and pathological annotations were available for all included patients and high-quality NGTS could be performed. The main limitation is that this is still a small sample size. Larger, international cohorts would be essential to strengthen the evidence. In addition, functional studies on in-vivo and in-vitro models would be valuable to understand the precise genomic changes that drive the transformation of STIC to HGSC and shed light on the dissimilarities in the mutational profiles of the smaller subset of STICs and HGSCs. Finally, a limitation of our study was that the archival tissue material yielded DNA of insufficient quantity and quality to perform more advanced sequencing techniques like shallow whole genome sequencing. STIC-lesions in general are very small with low cellularity, which makes it challenging to use advanced sequencing techniques and generate reliable data. As the main focus of our study was to investigate *TP53*-mutations, we considered the AmpliSeq panel was to be sufficient to address our research question.

4.4. What our study adds and future perspectives

Our study is unique in proving the clonality of STIC and HGSC that develop several years after RRSO. HGSC has a high mortality rate, and pathologists and clinicians can benefit from a better understanding of tumor pathogenesis. This study supports the tubal hypothesis regarding the origin of HGSC in the fallopian tube and provides evidence that the so-called 'primary' peritoneal serous carcinoma (PSC) should be regarded as a metastatic spread from a tubal primary. As such, PSC can be considered as a misnomer and should rather be named HGSC. Although the nature of STIC is not fully elucidated, it can be concluded that the biological behavior of STIC is not indolent, since STICs have the possibility to shed tumor cells to the peritoneal cavity, leading to the development HGSC. Knowing this, it highlights the importance of extensive sampling of the fallopian tubes of BRCA1/2 carriers to detect STICs. Understanding the pathophysiology of STIC and its significance for the individual patient is important, also for non-BRCA1/2 carriers who have their tubes removed because of other genetic mutations or during elective surgery as a preventive measure (opportunistic salpingectomy). From a patient's perspective, it might be difficult to know that despite undergoing RRSO, they are still at a risk of developing the lethal disease of HGSC, especially when a STIC is found. However, in this context, it must be noted that all the patients in our study underwent RRSO after the recommended age. This emphasizes the

importance of undergoing a timely preventative surgery following the national and international guidelines which recommend RRSO at the age of 35–40 years and 40–45 years respectively in BRCA1 and 2 GPV carriers [23,34]. Based on the knowledge of the tubal origin of the majority of HGSC, an alternative risk-reducing procedure has been proposed, which comprises an earlier (premenopausal) salpingectomy and delayed oophorectomy. Ongoing clinical trials are evaluating the safety of this approach [35,36], while other studies are investigating the impact on quality-of-life and psychosocial wellbeing of a delayed oophorectomy [37–39].

Although not mandated by current guidelines, our findings do raise the question whether stricter and longer follow-up protocols need to be evaluated for BRCA1/2 GPV carriers if a STIC is detected in their RRSO specimens. Currently there is a lack of consensus regarding the characterization of clinical behavior of STICs and the appropriate management strategy. While some pathologists have proposed that STICs should be viewed as lesions of 'uncertain malignant potential', others consider STICs to be lesions with 'low metastatic potential', in view of their capacity of exfoliating into the peritoneal cavity and giving rise to HGSC [6]. Regarding management, the European Society for Medical Oncology and European Society of Gynecological Oncologists Consensus Conference state that peritoneal staging should be considered for isolated STIC [40], whereas the National Comprehensive Cancer Center (NCCN) recommends observation alone with or without CA-125 testing in the absence of invasive cancer. There is currently no evidence that surgical staging and/or adjuvant chemotherapy offers sufficient benefit for women with isolated STIC [29,41]. Nevertheless, to further examine management protocols for isolated STIC at RRSO, large, multi-center studies with a long post-RRSO follow-up of BCRA1/2 GPV carriers will be of paramount importance.

Elucidating pathogenic steps in early cancer development is fundamental to identifying biomarkers for early detection and to exploring effective strategies for cancer prevention. Epigenetic changes such as DNA methylation and chromatin modification may also contribute to aberrantly expressed driver genes that activate the signaling pathways that may transform tubal epithelial cells.

It might be also worth investigating in future studies whether STICs in non-BRCA GPV carriers behave in the same way as STICs in BRCA GPV carriers. Based on the tubal hypothesis, we assume that the same biological process plays a role in non-BRCA GPV carriers and that STIC is the precursor lesion of HGSC in these cases as well. Because of the low prevalence [42] this would need assembling large, international cohorts, especially for investigating metachronous or temporally separate STIC and HGSC.

5. Conclusion

This study indicates that cells from STIC can shed into the peritoneal cavity, which can give rise to HGSC after a long lag period. Clonal relationship between STIC discovered at RRSO and the subsequent peritoneal HGSC was could be established for five of the seven patients (71%), which supports the hypothesis that HGSC after RRSO in BRCA1/2 GPV carriers is not an independent entity, but a metastatic disease of which the origin lies in the fallopian tube.

CRediT authorship contribution statement

C.B. van den Berg: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **S. Dasgupta:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **P.C. Ewing-Graham:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **J. Bart:** Writing – review & editing. **J. Bulten:** Writing – review & editing. **K.N. Gaarenstroom:** Writing – review & editing. **J.A. de Hullu:** Writing – review & editing. **C.H. Mom:** Writing – review & editing. **M.J.E. Mourits:** Writing – review & editing. **M.P. Steenbeek:** Writing – review & editing. **R. van Marion:** Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Writing – original draft, Writing – review & editing. **H.J. van Beekhuizen:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] GLOBOCAN, Ovarian Cancer Report, 2018.
[2] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, CA Cancer J. Clin. 69 (1) (2019) 7–34.
[3] J.M. Piek, P.J. van Diest, R.P. Zweemer, J.W. Jansen, R.J. Poort-Keesom, F.H. Menko, et al., Dysplastic changes in prophylactically removed fallopian tubes of women predisposed to developing ovarian cancer, J. Pathol. 195 (4) (2001) 451–456.
[4] S.I. Labidi-Galy, E. Papp, D. Hallberg, N. Niknafs, V. Adleff, M. Noe, et al., High grade serous ovarian carcinomas originate in the fallopian tube, Nat. Commun. 8 (1) (2017) 1093.
[5] I.M. Shih, Y. Wang, T.L. Wang, The origin of ovarian cancer species and precancerous landscape, Am. J. Pathol. 191 (1) (2021) 26–39.
[6] R. Vang, I.M. Shih, Serous tubal intra-epithelial carcinoma: what do we really know at this point? Histopathology 81 (5) (2022) 542–555.
[7] M. Wisztorski, S. Aboulouard, L. Roussel, M. Duhamel, P. Saudemont, T. Cardon, et al., Fallopian tube lesions as potential precursors of early ovarian cancer: a comprehensive proteomic analysis, Cell Death Dis. 14 (9) (2023) 644.
[8] K. Visvanathan, R. Vang, P. Shaw, A. Gross, R. Soslow, V. Parkash, et al., Diagnosis of serous tubal intraepithelial carcinoma based on morphologic and immunohistochemical features: a reproducibility study, Am. J. Surg. Pathol. 35 (12) (2011) 1766–1775.
[9] J.M.A. Bogaerts, M.P. Steenbeek, M.H.D. van Bommel, J. Bulten, J. van der Laak, J.A. de Hullu, et al., Recommendations for diagnosing STIC: a systematic review and meta-analysis, Virchows Arch. 480 (4) (2022) 725–737.
[10] K.B. Kuchenbaecker, J.L. Hopper, D.R. Barnes, K.A. Phillips, T.M. Mooij, M.J. Roos-Blom, et al., Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers, JAMA 317 (23) (2017) 2402–2416.
[11] Richtlijn Hereditair Mamma/Ovariumcarcinoom, Available from http://www.oncoline.nl/hereditair-mamma-ovariumcarcinoom.
[12] M.B. Daly, T. Pal, M.P. Berry, S.S. Buys, P. Dickson, S.M. Domchek, et al., Genetic/familial high-risk assessment: breast, ovarian, and pancreatic, version 2.2021, NCCN clinical practice guidelines in oncology, J. Natl. Compr. Cancer Netw. 19 (1) (2021) 77–102.

[13] Practice Bulletin No. 182 summary: hereditary breast and ovarian cancer syndrome, Obstet. Gynecol. 130 (3) (2017) 657–659.
[14] A.P. Finch, J. Lubinski, P. Møller, C.F. Singer, B. Karlan, L. Senter, et al., Impact of oophorectomy on cancer incidence and mortality in women with a BRCA1 or BRCA2 mutation, J. Clin. Oncol. 32 (15) (2014) 1547–1553.
[15] C. Marchetti, M. Arcieri, L. Vertechy, R. Ergasti, G. Russo, G.F. Zannoni, et al., Risk reducing surgery with peritoneal staging in BRCA1-2 mutation carriers. A prospective study, Eur. J. Surg. Oncol. 48 (12) (2022) 2539–2544.
[16] M.P. Steenbeek, M.H.D. van Bommel, J. Bulten, J.A. Hulsmann, J. Bogaerts, C. Garcia, et al., Risk of peritoneal carcinomatosis after risk-reducing Salpingo-oophorectomy: a systematic review and individual patient data meta-analysis, J. Clin. Oncol. 40 (17) (2022) 1879–1891.
[17] C.P. Crum, J.Y. Yoon, C.M. Feltmate, Clinical commentary extra-uterine high-grade serous carcinoma: two pathways, two preventions? Gynecol. Oncol. 169 (2022) 1–3.
[18] T.R. Soong, B.E. Howitt, A. Miron, N.S. Horowitz, F. Campbell, C.M. Feltmate, et al., Evidence for lineage continuity between early serous proliferations (ESPs) in the fallopian tube and disseminated high-grade serous carcinomas, J. Pathol. 246 (3) (2018) 344–351.
[19] F. Medeiros, M.G. Muto, Y. Lee, J.A. Elvin, M.J. Callahan, C. Feltmate, et al., The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome, Am. J. Surg. Pathol. 30 (2) (2006) 230–236.
[20] J. van Riet, N.M.G. Krol, P.N. Atmodimedjo, E. Brosens, W.F.J. van Ijcken, M. Jansen, et al., SNPitty: an intuitive web application for interactive B-allele frequency and copy number visualization of next-generation sequencing data, J. Mol. Diagn. 20 (2) (2018) 166–176.
[21] M. Shih Ie, L. Chen, C.C. Wang, J. Gu, B. Davidson, L. Cope, et al., Distinct DNA methylation profiles in ovarian serous neoplasms and their implications in ovarian carcinogenesis, Am. J. Obstet. Gynecol. 203 (6) (2010) (584 e1–22).
[22] R.C. Wu, P. Wang, S.F. Lin, M. Zhang, Q. Song, T. Chu, et al., Genomic landscape and evolutionary trajectories of ovarian cancer precursor lesions, J. Pathol. 248 (1) (2019) 41–50.
[23] I.A.S. Stroot, J. Brouwer, J. Bart, H. Hollema, D.J. Stommel-Jenner, M.M. Wagner, et al., High-grade serous carcinoma at risk-reducing Salpingo-oophorectomy in asymptomatic carriers of BRCA1/2 pathogenic variants: prevalence and clinical factors, J. Clin. Oncol. 41 (14) (2023) 2523–2535.
[24] J.Y. Yoon, D.B. Chapel, E. Goebel, X. Qian, J.K. Mito, N.S. Horowitz, et al., Genomic catastrophe, the peritoneal cavity and ovarian cancer prevention, J. Pathol. 257 (3) (2022) 255–261.
[25] F. Blok, S. Dasgupta, W.N.M. Dinjens, E.M. Roes, H.J. van Beekhuizen, P.C. Ewing-Graham, Retrospective study of a 16 year cohort of BRCA1 and BRCA2 carriers presenting for RRSO: prevalence of invasive and in-situ carcinoma, with follow-up, Gynecol. Oncol. 153 (2) (2019) 326–334.
[26] W. Reitsma, G.H. de Bock, J.C. Oosterwijk, J. Bart, H. Hollema, M.J. Mourits, Support of the 'fallopian tube hypothesis' in a prospective series of risk-reducing salpingo-oophorectomy specimens, Eur. J. Cancer 49 (1) (2013) 132–141.
[27] T.R. Rebbeck, N.D. Kauff, S.M. Domchek, Meta-analysis of risk reduction estimates associated with risk-reducing salpingo-oophorectomy in BRCA1 or BRCA2 mutation carriers, J. Natl. Cancer Inst. 101 (2) (2009) 80–87.
[28] M.L. Carcangiu, B. Peissel, B. Pasini, G. Spatti, P. Radice, S. Manoukian, Incidental carcinomas in prophylactic specimens in BRCA1 and BRCA2 germ-line mutation carriers, with emphasis on fallopian tube lesions: report of 6 cases and review of the literature, Am. J. Surg. Pathol. 30 (10) (2006) 1222–1230.
[29] N.M.A. Van der Hoeven, K. Van Wijk, S.E. Bonfrer, J.J. Beltman, L.A. Louwe, C.D. De Kroon, et al., Outcome and prognostic impact of surgical staging in serous tubal intraepithelial carcinoma: a cohort study and systematic review, Clin. Oncol. (R Coll Radiol) 30 (8) (2018) 463–471.
[30] E.E. Meserve, K.C. Strickland, A. Miron, T.R. Soong, F. Campbell, B.E. Howitt, et al., Evidence of a monoclonal origin for bilateral serous tubal intraepithelial neoplasia, Int. J. Gynecol. Pathol. 38 (5) (2019) 443–448.
[31] A.S. McDaniel, J.N. Stall, D.H. Hovelson, A.K. Cani, C.J. Liu, S.A. Tomlins, et al., Next-generation sequencing of tubal intraepithelial carcinomas, JAMA Oncol. 1 (8) (2015) 1128–1132.
[32] R.J. Kurman, Origin and molecular pathogenesis of ovarian high-grade serous carcinoma, Ann. Oncol. 24 (Suppl. 10) (2013) x16–x21.
[33] E. Kuhn, R.J. Kurman, R. Vang, A.S. Sehdev, G. Han, R. Soslow, et al., TP53 mutations in serous tubal intraepithelial carcinoma and concurrent pelvic high-grade serous carcinoma–evidence supporting the clonal relationship of the two lesions, J. Pathol. 226 (3) (2012) 421–426.
[34] Dutch Guideline on Hereditary and Familiar Ovarian Cancer, https://richtlijnendatabase.nl/gerelateerde_documenten/f/26076/6.1%20Indicatie%20RL%20Erf%20Ov.pdf.
[35] M.P. Steenbeek, M.H.D. van Bommel, J. Inthout, C.B. Peterson, M. Simons, Roes KCB, et al., TUBectomy with delayed oophorectomy as an alternative to risk-reducing salpingo-oophorectomy in high-risk women to assess the safety of prevention: the TUBA-WISP II study protocol, Int. J. Gynecol. Cancer 33 (6) (2023) 982–987.
[36] A Study to Compare Two Surgical Procedures in Individuals With BRCA1 Mutations to Assess Reduced Risk of Ovarian Cancer, Available from https://clinicaltrials.gov/study/NCT04251052.
[37] Preventing Ovarian Cancer Through Early Excision of Tubes and Late Ovarian Removal (PROTECTOR) Study, UK, Available from http://protector.org.uk/information-for-health-professionals/.
[38] M.G. Harmsen, M. Arts-de Jong, N. Hoogerbrugge, A.H. Maas, J.B. Prins, J. Bulten, et al., Early salpingectomy (TUbectomy) with delayed oophorectomy to improve quality of life as alternative for risk-reducing salpingo-oophorectomy in BRCA1/2 mutation carriers (TUBA study): a prospective non-randomised multicentre study, BMC Cancer 15 (2015) 593.
[39] Women Choosing Surgical Prevention (WISP) Study, Available from https://www.mdanderson.org/patients-family/diagnosis-treatment/clinical-trials/clinical-trials-index/clinical-trials-detail.ID2015-0814.html.
[40] N. Colombo, C. Sessa, A. du Bois, J. Ledermann, W.G. McCluggage, I. McNeish, et al., ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent diseasedagger, Ann. Oncol. 30 (5) (2019) 672–705.
[41] J. van der Ven, V.C. Linz, K. Anic, M.W. Schmidt, A. Loewe, S. Krajnak, et al., A questionnaire-based survey on the diagnostic and therapeutic approaches for patients with STIC in Germany, Arch. Gynecol. Obstet. 308 (2) (2023) 527–534.
[42] E.E.K. Meserve, J. Mirkovic, J.R. Conner, E. Yang, M.G. Muto, N. Horowitz, et al., Frequency of "incidental" serous tubal intraepithelial carcinoma (STIC) in women without a history of or genetic risk factor for high-grade serous carcinoma: a six-year study, Gynecol. Oncol. 146 (1) (2017) 69–73.