UiT The Arctic University of Norway

Faculty of Health Sciences - Department of Community Medicine

Epithelial ovarian cancer

Population-based cohort studies

The NOWAC Study and Postgenome biobank

Mie Jareid

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Sammendrag

Kreft i eggstokkene er forholdsvis sjelden kreftform, som har høy dødelighet.

Denne doktoravhandlingen bygger på spørreskjema fra 172000 kvinner i Kvinner og kreft-studien, og på blodprøver fra 50000 av deltakerne. Blodprøvene utgjør en unik biobank med bevart genuttrykk fra de hvite blodlegemene.

Fra før vet man at kvinner som har brukt p-piller har lavere risiko for eggstokkreft. I dag har mange kvinner, også de yngre, begynt å bruke hormonspiral. Det er lite kunnskap om hvorvidt kvinner som bruker hormonspiral har lavere risiko for eggstokkreft slik som p-pillebrukerne. Blant de noe eldre deltakerne i Kvinner og kreft hadde kvinner som noen gang har brukt hormonspiral en halvert risiko for eggstokkreft. Fordi det var få tilfeller, har anslaget en usikkerhet som tilsvarer mellom 10% og 70% lavere risiko.

Blodprøvene i biobanken gir mulighet til å undersøke endringer i genuttrykk i immunceller opptil sju år før diagnosen ble stilt, i håp om å forstå mer om sykdomsutviklingen. Vi gjorde en utforskende analyse av blodprøver fra kvinner som hadde fått eggstokkreft, men fant ikke entydige endringer i genuttrykket.

Summary

Important gaps in population-based epidemiological research on ovarian cancer include understanding how risk factors relate to cancer subtypes and anatomical sites, identifying safe and effective preventive measures, and getting a more detailed picture of the continuum of events during ovarian carcinogenesis. This thesis used prospective exposure information from the Norwegian Women and Cancer (NOWAC) Study and blood samples from the NOWAC Postgenome biobank to explore topics within these gaps.

On the topic of risk factors, subtypes and anatomical sites, previous studies have shown that serous carcinomas of the ovary and fallopian tube cancers have similar risk factors. This thesis compared risk factors between the ovary/fallopian tube and uterine corpus. One risk factor association separated serous carcinomas of these sites, while no differences in risk factor associations were found for endometrioid and clear cell carcinomas. Possible alternative explanations of this result include few observations in the analysis of endometrioid and clear cell carcinomas, and histological misclassification of high-grade endometrioid carcinomas.

Among preventive measures, combined oral contraceptives reduce the risk of both ovarian and uterine carcinoma. Current trends in female contraception include an increase in use of progestin-only long-acting reversible contraceptives, such as the levonorgestrel-releasing intrauterine system (LNG-IUS). In the NOWAC cohort, ever use of LNG-IUS reduced the risk of ovarian carcinoma by 53% (95% CI: 22% – 68%) and the risk of uterine carcinoma by 78% (95% CI: 60% – 87%) compared to never use. These results extend current knowledge to include postmenopausal women in a sample of the general population. The association with breast cancer was also investigated and discussed.

To investigate the continuum of events during ovarian carcinogenesis, this thesis explores gene expression in peripheral blood in the years preceding ovarian cancer diagnosis. The presented study did not find strong associations. This could be because there is little association between ovarian cancer and prediagnostic gene expression in blood, but could also be due to a small sample size, or the analytic approach that was used.

List of publications

This dissertation is based on the following publications

Paper I

Jareid M, Licaj I, Olsen KS, Lund E, Bøvelstad HM. **Does an epidemiological comparison support a common cell of origin in similar subtypes of postmenopausal uterine and ovarian carcinoma? The Norwegian Women and Cancer Study.** *Int J Cancer.* 2017 141(6): 1181-1189.

Paper II

Jareid M, Thalabard JC, Aarflot M, Bøvelstad HM, Lund E, Braaten T. **Levonorgestrel-releasing intrauterine system use is associated with a decreased risk of ovarian and endometrial cancer, without increased risk of breast cancer. Results from the NOWAC Study.** *Gynecol Oncol.* 2018 149(1): 127-132.

Paper III

Jareid M, Snapkov I, Holden M, Busund LT, Lund E, Nøst TH. **The blood transcriptome prior to ovarian cancer diagnosis: A nested case-control study in the NOWAC postgenome cohort.** *PLOS ONE* 2021 16(8): e0256442.

List of abbreviations

BMI	Body mass index
EPIC	European Prospective Investigation into Cancer and Nutrition
EOC	Epithelial ovarian cancer
ECC	Endometrioid and clear cell carcinoma (these are two different subtypes)
FC	Fold change
FDR	False discovery rate
FSH	Follicle stimulating hormone
HR	Hazard ratio
HRT	Hormone replacement therapy
IUD	Intrauterine device
LH	Luteinizing hormone
LNG-IUS	Levonorgestrel-releasing intrauterine system
NOWAC	Norwegian Women and Cancer Study
OC	Oral contraceptive
OR	Odds ratio
PY	Person-years
RR	Relative risk
SIR	Standardized incidence ratio
TICE	Transcriptomics in Cancer Epidemiology
TML	Total menstrual lifespan

Table of Contents

1 Introduction ... 1
2 Background ... 3
2.1 The female reproductive system ... 3
2.2 Ovarian cancer ... 4
2.2.1 Epidemiology ... 4
2.2.2 Diagnosis ... 6
2.2.3 Prevention ... 7
2.3 Relationships between the ovaries and reproductive tract ... 8
2.3.1 Functional relationships between the ovaries and reproductive tract ... 8
2.3.2 Developmental relationships between the ovarian surface epithelium and reproductive tract epithelia ... 9
2.4 Pathogenesis of ovarian carcinomas ... 10
2.4.1 Histopathology of carcinomas that involve the ovaries and reproductive tract 10
2.4.2 Cellular lineage of ovarian carcinomas ... 12
2.5 Risk factors for ovarian carcinomas ... 12
2.5.1 Anthropometric and lifestyle factors ... 13
2.5.2 Reproductive factors ... 13
2.5.3 Gynecologic surgery and pathology ... 14
2.5.4 Hormone use and endogenous hormone levels ... 14
2.5.5 Inflammatory and immunologic factors ... 16
2.6 Ovarian cancer and gene expression in peripheral blood ... 16
2.7 Aims ... 18
3 Materials and Methods ... 19
3.1 Study population ... 19
3.1.1 The Norwegian Women and Cancer Study ... 19
3.1.2 The Postgenome biobank ... 19
3.2 Follow-up ... 21
3.2.1 Case ascertainment ... 21
3.3 Analytical samples ... 22
3.4 Data ... 23
3.4.1 Variable selection ... 23
3.4.2 Exposure assessment ... 24
3.4.3 Study design ... 28

3.5 Statistical analysis 28
3.6 Ethics 31
4 Results – summary of papers 32
4.1 Paper I 32
4.2 Paper II 32
4.3 Paper III 33
5 Discussion 35
5.1 Methodological considerations 35
5.1.1 Study design 35
5.1.2 Impact of selection bias 35
5.1.3 Impact of information bias 38
5.1.4 Impact of confounding 42
5.2 Discussion - Paper I 44
5.3 Discussion - Paper II 47
5.4 Discussion - Paper III 51
6 Conclusion 55
7 Further research 56
Works cited 57

1 Introduction

Ovarian cancer constituted 3.2% of cancer cases and 5.4% of cancer deaths among Norwegian women in 2019. Compared to other female cancers such as breast cancer (23% and 12.6%), uterine cancer (5.1% and 1.2%), and cervical cancer (2.2% and 1.7%) (Cancer Registry of Norway 2021), ovarian cancer has poor survival rates. This is not simply because it is a disease of old age: In the population as a whole, ovarian cancer constitutes 1.2 % of all cancer cases and 4% of years of life lost (Brustugun, Møller et al. 2014; Cancer Registry of Norway 2015).

One important determinant of cancer survival is the extent of metastatic spread at diagnosis. The difference between the cancers mentioned above and ovarian cancer is the proportion of cases discovered at an early stage (Cancer Registry of Norway 2021). This is because they either cause non-ambiguous symptoms or are subject to screening programs, and because the tissues can be biopsied in-office, so that malignant tumors can be distinguished from benign. Ovarian cancers, and epithelial ovarian cancers (ovarian carcinomas; EOC) in particular, are complicated because a pelvic biopsy is invasive, and it is not clear which structure to biopsy. Ovarian carcinoma subtypes appear to be a set of diseases with histologies that resemble tissues in the upper reproductive tract. Where, or in which type of cells, these histological subtypes arise is not completely clear (Sun and Auersperg 2019). Furthermore, the most common EOC subtype, serous carcinoma, seems to spread first and grow subsequently (Brown and Palmer 2009). This constellation of difficulties places primary prevention at the center of opportunities for reducing ovarian cancer deaths.

Among factors that modulate risk of EOC, childbearing and breastfeeding are protective, while ‘natural childlessness’ (including some infertility-related conditions) increases risk. ‘Artificial childlessness’ by certain contraceptive modalities decreases risk; this is best demonstrated in users of combined OC. New contraceptive types are introduced continuously, and in order to assess how they impact ovarian cancer risk, epidemiological studies are necessary (Doherty, Jensen et al. 2017).

Another goal in ovarian cancer research is to understand ovarian carcinogenesis across the cancer continuum (Tworoger and Doherty 2017). To understand carcinogenesis, it is necessary to investigate pre-clinical cancer. In the human, observation is the available method. The population-based approach relies on collecting information on risk factors and possibly biological samples, and making estimates of associations with clinicopathologic

endpoints. The interpretation of these observations relies on biological basal research (Lund and Dumeaux 2008).

This thesis reports the results of research undertaken in the evolving landscape of population health and biomedical science. The setting for these studies is the prospective, population-based Norwegian Women and Cancer (NOWAC) Study and the NOWAC Postgenome Biobank for functional molecular epidemiology (Dumeaux, Borresen-Dale et al. 2008; Lund, Dumeaux et al. 2008). Three studies are presented:

I. A comparison of how risk factors relate to extrauterine (ovarian/fallopian) and intrauterine (endometrial) carcinoma subtypes

II. An estimate of the association between use of levonorgestrel-releasing intrauterine system (LNG-IUS), a hormonal contraceptive for which there is a paucity of cancer data, and risk of ovarian, endometrial and breast cancer in the general population.

III. A characterization of blood gene expression prior to a diagnosis of EOC. This study reports the results for ovarian cancer in the EU grant TICE (Transcriptomics in Cancer Epidemiology).

2 Background

2.1 The female reproductive system

The upper female reproductive tract begins at the inner cervix (neck of the uterus) and includes the uterine body and the fallopian (uterine) tubes (Fig 1) (Peric, Weiss et al. 2019). The ovaries are ovoid in shape with a volume of 1.2–9.4 cm^3 (Refaey and Yu Jin 2008) and are attached to the sides of the uterus by ligaments, in close proximity to the open ends of the fallopian tubes. Ligaments attached to the pelvis connect the ovaries to the circulatory, lymph, and autonomic nervous system (Sobotta 1994). The parenchyma (functional tissue) of the ovaries are called follicles, which consist of one oocyte surrounded by granulosa cells (Fig 1). The stroma (supportive tissue) of the ovaries is a collagenous, vascularized connective tissue. Stromal cells types, functions and significance are poorly understood (Kinnear, Tomaszewski et al. 2020). The ovaries are covered by a mesothelium, referred to as the ovarian surface epithelium (Auersperg, Wong et al. 2001).

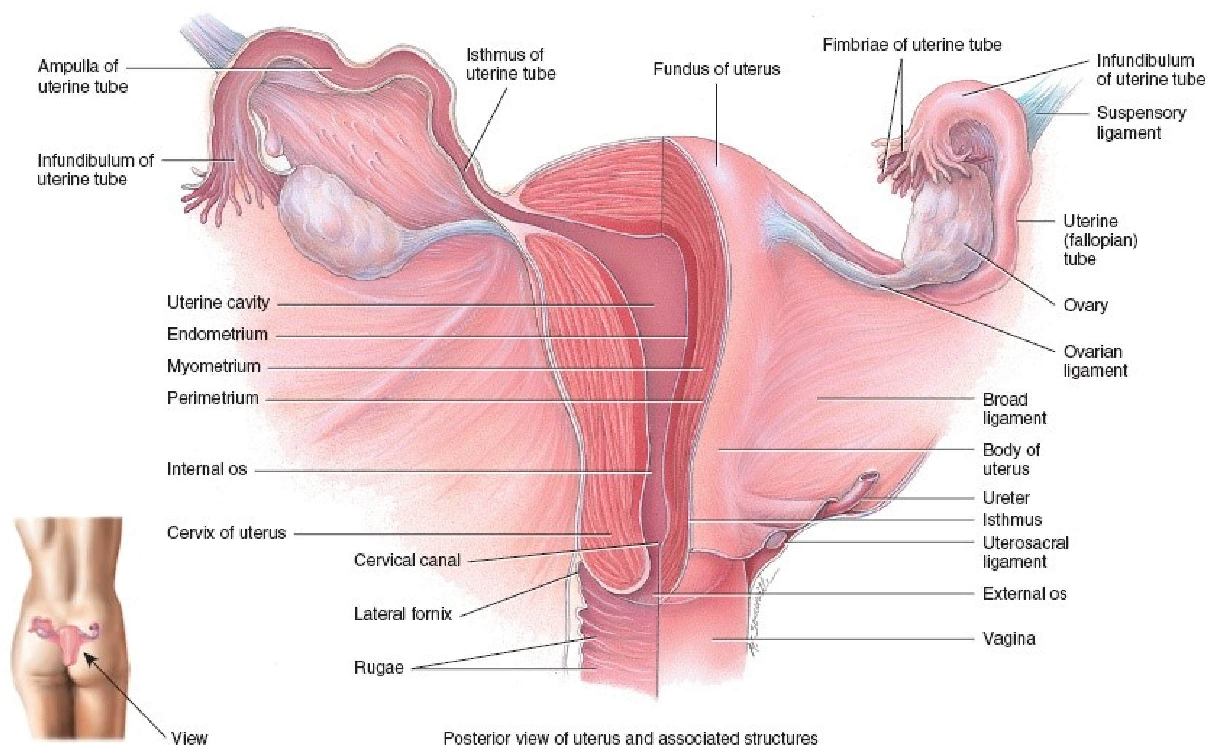

Fig 1 Anatomy of the female reproductive tract. Used with permission of John Wiley & Sons - Books, from Derrickson and Tortora (2017); permission conveyed through Copyright Clearance Center, Inc. Drawing by Kevin A. Somerville – Medical Art Studio. Used with the kind permission of the artist.

2.2 Ovarian cancer

Ovarian cancers are malignant tumors that involve the ovaries. These can arise in germ cells, hormone producing cells or epithelial cells. The ovaries have an epithelium, but histologically, ovarian epithelial tumors seem to have arisen from epithelia of the fallopian tubes, the uterine cavity, or the inner cervix. Where the tumors arise is not firmly established (Berek, Kehoe et al. 2018; Prat and Mutch 2018). In epidemiological studies, 'ovarian cancer' can refer to tumors that are non-epithelial or epithelial, borderline or invasive. Ninety percent of ovarian neoplasms are of epithelial origin (Prat and Mutch 2018), and 20% of these are borderline (low malignant potential) tumors (Trope, Kaern et al. 2012; Gynecologic Cancer Registry of Norway 2019). The present thesis is concerned with malignant (invasive) epithelial tumors, i.e. EOC. Referenced epidemiological studies may include non-epithelial cancers or borderline tumors. The descriptive epidemiology of ovarian cancer in Norway excludes borderline tumors (Cancer Registry of Norway 2021).

2.2.1 Epidemiology

In 2020 there were 487 new cases of ovarian cancer in Norway (all ages included), with a cumulative risk by age 80 of 1.6%. Median age at diagnosis was 68 years. The age-standardized incidence of ovarian cancer is currently 17.9 per 100,000 and declining. The trend over the past six decades shows a convex curve with a peak incidence rate of 22 per 100,000 in 1990, while the current incidence rate is similar to the 1960s (Cancer Registry of Norway 2021) (Fig 2). Age-specific incidence rates reveal that the diagnosis is becoming more frequent among older women, while among younger women the incidence is decreasing (Fig 2) (Gynecologic Cancer Registry of Norway 2021). Stage-specific incidence rates show a recent shift toward more cases being diagnosed with regional, rather than distant metastases (Cancer Registry of Norway 2021).

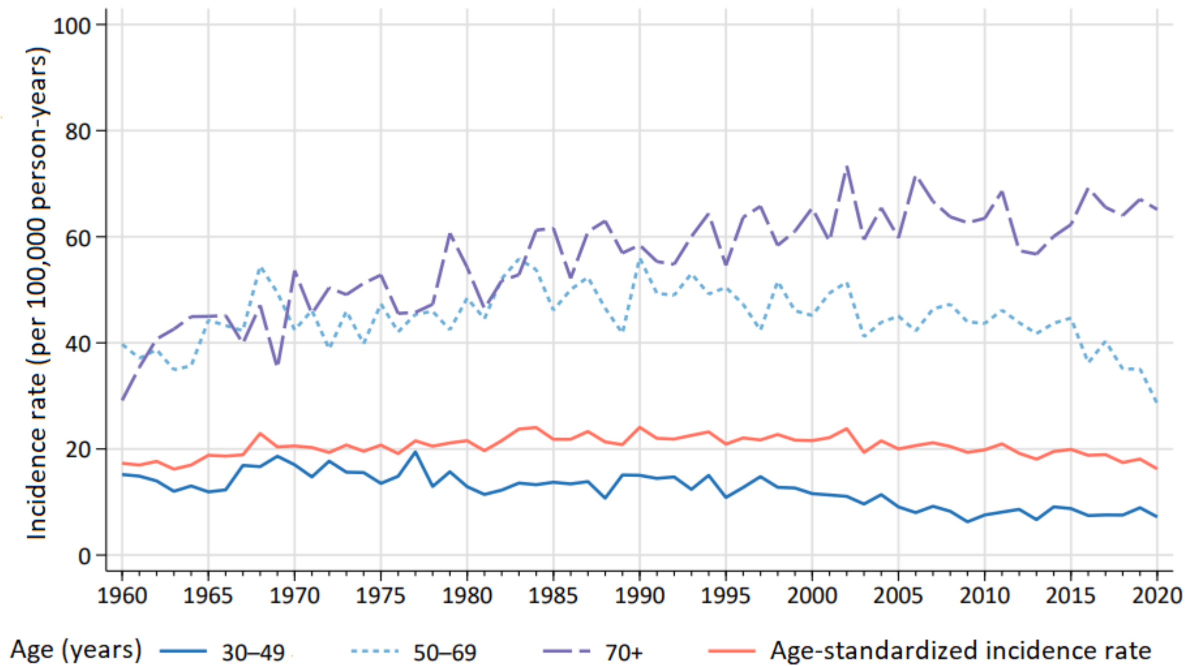

Fig 2 Ovarian cancer incidence rates in Norway 1960-2020 by age group and age-standardized (Norwegian standard). From: Gynecologic Cancer Registry of Norway (2021) Annual Report: 2020. Adapted with permission.

There were 275 deaths from ovarian cancer in 2020, with a five-year relative survival of 51% (Cancer Registry of Norway 2021). An important reason for the poor survival is that the main predictor of survival is complete surgical tumor resection (Elattar, Bryant et al. 2011), which is difficult to achieve since only 20% of ovarian cancers are diagnosed at the localized stage (Cancer Registry of Norway 2021). Advanced cancers are treated with surgery and chemotherapy, but 80% will recur (Berek, Kehoe et al. 2018). Despite an increase in five-year survival from 30% in 1965 to the current 51%, the mortality rate has remained largely unchanged until recently. The mortality and incidence curves have a similar shape, and presently, both are falling steeply, below the rates in 1965 (Fig 3) (Cancer Registry of Norway 2021). Clinical contributors to epidemiological trends include better diagnostics among older women from 1985 and prophylactic salpingo-oophorectomy (cancer prevention by surgical removal of ovaries and/or fallopian tubes) among younger women (Gynecologic Cancer Registry of Norway 2021).

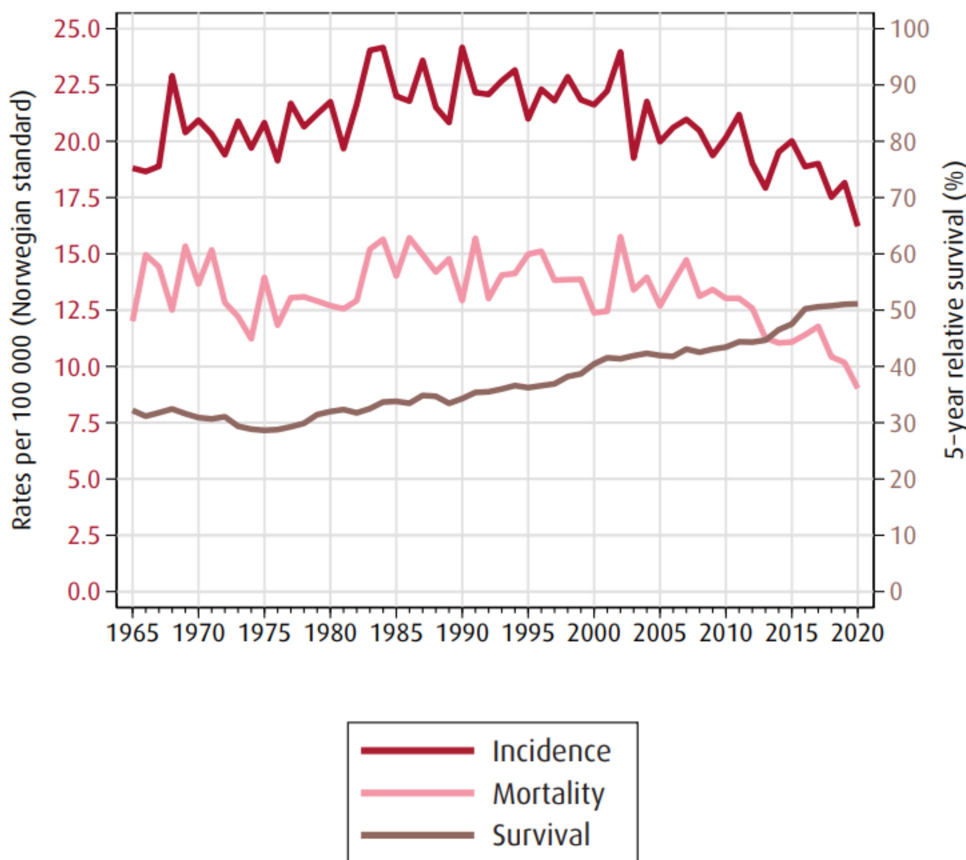

Fig 3 Ovarian cancer incidence, mortality and survival in Norway 1965-2020. Trends in incidence and mortality rates and 5-year relative survival proportions. Includes Ovary etc. (ICD-10 C56, C57.0-4), C48.2) The recent sharp decline may be partially attributable to a delay in diagnoses in 2020 From: Cancer Registry of Norway (2021): Cancer in Norway 2020 (free use).

2.2.2 Diagnosis

If diagnosed at the localized stage, the 5-year relative survival of ovarian cancer is 99.7% (Cancer Registry of Norway 2021). A study of Norwegian medical records concluded that survival could be improved by paying more attention to symptoms, because this would facilitate early diagnosis (Paulsen, Kærn et al. 2005). One obstacle to early diagnosis is that the most common type of EOC, high-grade serous carcinoma, often spreads before the tumor grows in volume (Brown and Palmer 2009). Observations consistent with this were made in an American case-control study, where patients diagnosed with advanced high-grade serous carcinoma did not recollect having the symptoms described by patients diagnosed with early-stage disease. Those diagnosed with early stage disease mainly suffered from low-grade serous or non-serous subtypes (Vine, Calingaert et al. 2003).

Screening for premalignant EOC is difficult because an ovarian biopsy is invasive, and because it is not clear what the premalignant lesion is (Karnezis, Cho et al. 2016). The most common imaging tool in the gynecologic setting, transvaginal ultrasound, sensitively detects ovarian masses and can detect early-stage EOC; however, 80% of tumors are benign (Prat and Mutch 2018), so that the positive predictive value of ultrasound for EOC is 2.8% (Menon, Gentry-Maharaj et al. 2009). The most widely used blood-based biomarker for EOC, CA125 (Bast, Feeney et al. 1981), is a serous membrane protein with low specificity, best suited for post-treatment monitoring of cancer recurrence (Cramer, Bast et al. 2011; Sikaris 2011). A combination of individualized tracking of CA125 and ultrasound has been tried in screening, and does lead to earlier diagnosis, but fails to reduce mortality (Menon, Gentry-Maharaj et al. 2021). Countries differ with regard to proportions of EOC subtypes, and the approach might work in a population with more non-serous EOC (Koshiyama, Matsumura et al. 2016). For serous EOC, new strategies include novel imaging techniques and methods for detection of EOC in cervical cancer screening samples (Bast, Lu et al. 2020).

Researchers are also investigating other blood-based analytes for early or non-invasive detection of EOC. Multi-protein blood marker panels improve the proportion of cases detected, but perform poorly on samples from clinically detected early-stage EOC or collected a longer interval prior to diagnosis of late-stage EOC (Nebgen, Lu et al. 2019). Other promising biomarkers include tumor autoantibodies, miRNA, and circulating tumor DNA. These are detectable farther from diagnosis, but require evaluation in prospective trials (ibid). Autoantibodies, which result from an autoimmune response to the tumor, are of interest because EOC tumors must be 2.5 cm to produce a diagnostic level of CA125, while a 50% mortality reduction requires detection at 0.5 cm (Brown and Palmer 2009; Bast, Lu et al. 2020). One study has investigated blood-derived mRNA (from circulating leukocytes) with the aim of identifying biomarkers for screening for early-stage EOC. The result was a panel of five mRNA transcripts plus CA-125 (Mok, Kim et al. 2017).

2.2.3 Prevention

The limited success in treatment and secondary prevention of ovarian cancer has led to a call for more focus on primary prevention (Long Roche, Abu-Rustum et al. 2017). Current strategies are prophylactic salpingo-oophorectomy for women at high risk due to germline *BRCA1*/2 mutations (Eleje, Eke et al. 2018), and opportunistic salpingectomy for women in the general population (Yoon, Kim et al. 2016). There are few easily modifiable risk factors for EOC (Wild, Weiderpass et al. 2020); The only intervention for EOC with evidence of a

net benefit is avoidance of excess body fatness (IARC 2019). It is likely that OC use prevents a substantial number of EOC cases, but a net reduction in all-cause mortality has not been proven (Havrilesky, Gierisch et al. 2013). The World Health Organization mentions aspirin as potential chemoprevention (Wild, Weiderpass et al. 2020). Still, most women use contraceptives, and as new modalities are introduced, providing information on long-term effects is a core task for population-based epidemiology (Doherty, Jensen et al. 2017).

2.3 Relationships between the ovaries and reproductive tract

2.3.1 Functional relationships between the ovaries and reproductive tract

The activity of the reproductive system is mainly regulated by the hypothalamic-pituitary-gonadal axis, which integrates signals on environmental and physiological state into a decision of whether to attempt to reproduce. The gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) act on the ovary (Hawkins and Matzuk 2008). Follicles continuously begin to develop, but depend on stimulation from FSH to fully mature. Maturing follicles recruit theca cells (Young and McNeilly 2010), which cooperate with granulosa cells to produce estrogen (estradiol), which feeds back to the pituitary to inhibit FSH production. This inhibits the maturation of other follicles. When estrogen levels are very high, there is a surge in LH followed by ovulation. The ovarian surface ruptures and the ovum is presented to the fallopian tube, which collects it and transports it to the uterine cavity, where the endometrium has grown thick in response to the high estrogen. The LH surge luteinizes the postovulatory follicle, which shifts hormone synthesis from estrogen to progesterone. Progesterone counteracts the proliferative effect of estrogen on the endometrium and induces a secretory phase, suited for embryonal implantation (Hawkins and Matzuk 2008). If implantation occurs, the conceptus sustains progesterone production and the endometrium remains quiescent (Soloff, Jeng et al. 2011). Otherwise the corpus luteum degenerates, and the endometrium is shed. The postovulatory loss of estrogen production causes a rise in FSH, which stimulates maturation of a new set of follicles and regeneration of the endometrium (Hawkins and Matzuk 2008). During this process, the ovarian surface epithelium undergoes replication to accommodate the large size of the ovulatory follicle, proteolytic breakdown to facilitate rupture, and epithelial-to-mesenchymal transition to migrate and repair the ovulatory wound (Carter, Cook et al. 2019).

2.3.2 Developmental relationships between the ovarian surface epithelium and reproductive tract epithelia

Organisms develop through cell division, where specialized tissues form through cellular lineage commitment over generations until terminal differentiation. The ovaries and the müllerian ducts (the precursors of the reproductive tract) are formed next to one another in the posterior wall of the primitive embryonal body cavity (coelom) (Fig 4). The coelomic mesothelium differentiates into the ovarian surface epithelium (Hummitzsch, Irving-Rodgers et al. 2013) and folds in on itself to create the müllerian ducts (Prat and Mutch 2018). This makes the coelomic mesothelium (which is the predecessor of the peritoneum) the cellular antecedent of the ovarian surface epithelium and of the epithelial linings inside the fallopian tubes and uterus (Auersperg, Wong et al. 2001; Auersperg, Woo et al. 2008; Robboy, Kurita et al. 2017) (Fig 4). The fallopian tubes, uterine body and endocervix develop from the müllerian (mesonephric) ducts, and their epithelial linings (fallopian tube epithelium, endometrium and endocervical epithelium) are collectively referred to as müllerian epithelia (Cunha, Robboy et al. 2018).

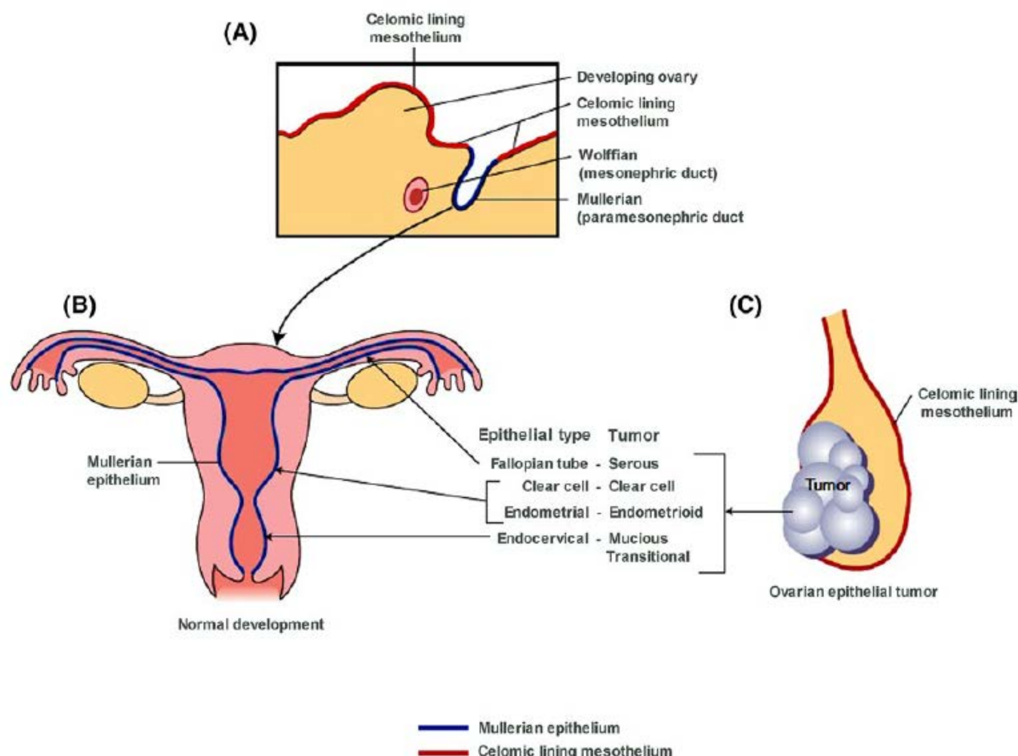

Fig 4 The embryologic origin of the ovarian surface epithelium and the müllerian epithelia in relation to the histogenesis of ovarian epithelial tumors. Used with permission of John Wiley & Sons - Books, from Prat and Mutch (2018); permission conveyed through Copyright Clearance Center, Inc.

The differentiated epithelia of these structures and of the pelvic peritoneum retain the ability to re-differentiate into other phenotypes. This metaplastic potential may explain the existence of benign lesions such as endometriosis (endometrium occurring outside the uterine cavity) or endosalpingiosis (cysts lined with tubal epithelium; on the ovary, endosalpingiosis is surface associated inclusions of tubal-lined epithelium, but if these have lost contact with the surface, they are called cortical inclusion cysts and may or may not be lined with tubal-like epithelium) (Irving and Clement 2019). Müllerian metaplasia can be triggered by chemical signaling, while mechanical irritation can trigger squamous metaplasia (ibid.). When found in lesions, fallopian tube-like epithelium is referred to as serous, endometrial-like as endometrioid, and endocervical-like as mucinous. Urothelial differentiation also occurs. Due to its müllerian metaplastic potential, the pelvic peritoneum is referred to as the 'secondary müllerian system' (Lauchlan (1972) in: Irving and Clement (2019)). Of note, some authors refer to the secondary müllerian system as vestigial müllerian cells (Dubeau 2008; Berretta, Patrelli et al. 2013). A different explanatory model suggests that epithelial cells from the reproductive tract can relocate and retain their differentiation, so that endometriosis stems from retrograde menstruation while endosalpingiosis and tubal-lined cortical inclusion cysts contain sloughed tubal fimbrial epithelium (Irving and Clement 2019).

2.4 Pathogenesis of ovarian carcinomas

2.4.1 Histopathology of carcinomas that involve the ovaries and reproductive tract

Ovarian carcinomas display different histological subtypes, mainly serous (frequency 75%), endometrioid (10%), clear cell (10%) and mucinous (3%). Serous carcinomas are further subdivided into high-grade (70%) and low-grade (5%) (Prat, D'Angelo et al. 2018) (Table 1). Serous and endometrioid carcinomas display müllerian differentiation, as described in the previous section (Kurman, Ellenson et al. 2019). Müllerian (endocervical) differentiation is common for borderline mucinous tumors, while mucinous carcinomas are usually more similar to the intestine (ibid.). Clear cell carcinomas are similar to carcinomas of the kidney (Ji, Wang et al. 2018). Carcinomas of the reproductive tract display the same subtypes. Among uterine carcinomas, 75-80 % are endometrioid, 10 % are serous, and < 5 % clear cell (Huvila and McAlpine 2021). Fallopian tube carcinomas are 90% high-grade serous or high-grade endometrioid (Berek, Kehoe et al. 2018). The different subtypes have distinct molecular characteristics, different epidemiological risk profiles and different clinical behaviors (Huvila and McAlpine 2021; Rendi 2021).

Table 1 Main types of ovarian carcinoma. Reproduced from Prat, D'Angelo et al. (2018) with permission from Elsevier.

	High-grade serous	Low-grade serous	Mucinous	Endometrioid	Clear cell
Usual stage at diagnosis	Advanced	Early or advanced	Early	Early	Early
Presumed tissue of origin/ precursor lesion	Fallopian tube or tubal neometaplasia in inclusions of ovarian surface epithelium	Serous borderline tumor	Adenoma–borderline–carcinoma sequence; teratoma	Endometriosis, adenofibroma	Endometriosis, adenofibroma
Genetic risk	*BRCA1/2*	?	?	HNPCC	?
Significant molecular Abnormalities	TP53 and BRCA	B-RAF or KRAS	K-RAS and ERBB2	PTEN, CTNNB1, ARID1A, PIK3CA K-RAS, MI	HNF-1β ARID1A PTEN, PIK3CA
Proliferation	High	Low	Intermedia te	Low	Low
Response to primary Chemotherapy	80%	26%–28%	15%	?	15%
Prognosis	Poor	Favorable	Favorable	Favorable	Intermediate

It is generally assumed that the morphology of a carcinoma is an indicator of the tissue in which it arose (Pecorino 2012). The müllerian differentiation of histologic subtypes EOC is therefore strange, but consistent with the metaplastic model of benign pelvic epithelial lesions (Scully (1995) in**:** Sun and Auersperg (2019)). However, histopathological evidence of the progressive stages from normal to malignant ovarian surface epithelium is rarely found (Kuhn, Kurman et al. 2012). This has led to an emphasis on models that correspond to the metastatic theory for benign pelvic epithelial lesions, with the fallopian tube as the origin of serous carcinomas (Crum, Drapkin et al. 2007). An 'intermediate' model suggests that the secondary müllerian system represents remnants (endometriosis, endosalpingiosis, endocervicosis) of the müllerian ducts, not a metaplastic potential (Dubeau 1999). The current 'unifying' view is that the *majority* of EOCs originate in their benign counterpart tissue, but grow preferentially on the ovary. Hence, EOC is defined as 'malignancies that involve the ovary and reproductive tract' (Vaughan, Coward et al. 2011; National Academies of Sciences 2016).

2.4.2 Cellular lineage of ovarian carcinomas

The postulate that carcinoma subtypes on the ovary are different diseases that share anatomical location (Vaughan, Coward et al. 2011) raises the question of whether similar subtypes of different anatomical locations are the same disease (Nik, Vang et al. 2014). This has been investigated for serous carcinomas of the ovary, fallopian tube and peritoneum (Sørensen, Schnack et al. 2015; Fortner, Rice et al. 2020). Molecular, clinicopathologic and epidemiologic parameters suggest that primary peritoneal serous carcinoma arises through a different etiologic pathway than fallopian and ovarian serous carcinoma, while principal difference between the latter two is the proportion of serous tubal intraepithelial carcinoma (Sørensen, Schnack et al. 2015).

The question on similarity between subtypes can be further expanded to intrauterine and extrauterine müllerian tumors of similar subtype. For example, extrauterine endometrioid and clear cell carcinomas are thought to arise in atypical endometriosis (Kuhn, Kurman et al. 2012; Vercellini, Somigliana et al. 2012; Kurman, Ellenson et al. 2019). The possibility of the uterine endometrium as a source of serous carcinoma has been investigated in terms of co-occurrence and genetic similarity of precursor lesions (Massuger, Roelofsen et al. 2010; Reitsma, Mourits et al. 2013; Mingels, van Ham et al. 2014). When the present work was initiated, a comparison of the risk factor profiles of intrauterine (endometrial) and extrauterine (ovarian/fallopian) carcinomas was lacking.

2.5 Risk factors for ovarian carcinomas

For the individual woman, the most important determinant of EOC risk is germline mutations in the *BRCA1* gene, leading to an almost 50% absolute lifetime risk of high-grade serous EOC (Lakhani, Manek et al. 2004; Kuchenbaecker, Hopper et al. 2017). Cancers caused by this and other inherited high-risk mutations constitute approximately 10% of EOC cases (Pearce, Stram et al. 2015). From a public health perspective, high parity is a good predictor of low risk of EOC, and likely preventive (La Vecchia 2017). In the population, the current decline in incidence of EOC in countries such as Norway (Fig 2) is attributed to use of OC (ibid.).

2.5.1 Anthropometric and lifestyle factors

Body mass index (BMI) is associated with EOC in a J-shaped fashion. The risk is statistically significantly elevated for BMI 28 or above, and lowest in the BMI range 20–23 (Aune, Navarro Rosenblatt et al. 2015). The risk may be limited to non-serous subtypes (Dixon, Nagle et al. 2016) and may be limited to premenopausal women (Qian, Rookus et al. 2019).

Smoking is not a risk factor for EOC overall, but is classified as an ovarian carcinogen (IARC 2019) due to a consistent association with mucinous EOC. This is most pronounced for borderline tumors (Santucci, Bosetti et al. 2019; Zhou, Minlikeeva et al. 2019). Ever smoking is associated with a 27% increased risk of invasive mucinous EOC (Wentzensen, Poole et al. 2016). A decreased risk of clear cell (and possibly endometrioid) EOC, and a null association with serous EOC, renders the total effect equivocal (Wentzensen, Poole et al. 2016; Zhou, Minlikeeva et al. 2019).

Incremental changes in physical activity and lifestyle have little impact on risk of EOC (Arthur, Brasky et al. 2019; Chen, Braaten et al. 2021). However, recent studies of sedentary behavior suggest a surprisingly strong association, with a risk increase of about 30% (Cannioto, LaMonte et al. 2016; Hermelink, Leitzmann et al. 2022). The strength of this evidence is moderate.

2.5.2 Reproductive factors

Age at menarche is negatively associated with EOC: women whose menarche occurred at age 15 are at 12% lower risk than those with menarche at age 11. Among subtypes, the risk of clear cell EOC decreases by 8% for each 1-year increase in age at menarche. Other subtypes are not statistically significantly associated, but subtype risks are not significantly different (Wentzensen, Poole et al. 2016).

Age at menopause is positively associated with EOC, with a 6% increase in risk for each 5-year increase in age at menopause. Among subtypes, the same interval increases risk of clear cell EOC by 37%, endometrioid EOC by 19% and serous EOC by 5% for serous carcinoma per 5-year increase, and these are significantly different (Wentzensen, Poole et al. 2016).

A full-term pregnancy is associated with an almost 20% lower risk of EOC compared to being nulliparous. Each subsequent child confers a further 8% reduction in risk of EOC overall (Wentzensen, Poole et al. 2016). Associations differ significantly between subtypes,

with a per-child risk reduction of approximately 30% for clear cell EOC, 20% for endometrioid EOC, and 10% for serous EOC (ibid.). The partially linear association suggests that nullipara have a higher risk of EOC, while parity has a protective effect (Gaitskell, Green et al. 2018).

Breastfeeding reduces the risk of EOC, independent of pregnancy. The risk reduction (ever vs. never) is estimated to 24% and is limited to (and is of similar magnitude for) serous, endometrioid and clear cell carcinomas. Associations are not heterogeneous. There is a significant trend for mean duration of breastfeeding per child (Babic, Sasamoto et al. 2020).

The time between menarche and menopause constitutes a woman's reproductive lifespan. Subtracting from this interval the total duration of events that interrupt ovulation produces the 'lifetime number of ovulatory cycles' by or 'total menstrual lifespan' (TML) (Yang, Murphy et al. 2016). The association with TML is cumulative, where each 5-year increase in ovulatory cycles is associated with a 14% increased risk of EOC. Subtype estimates range from 13% for serous to 37% for clear cell, while there is no association with mucinous carcinoma (Trabert, Tworoger et al. 2020).

2.5.3 Gynecologic surgery and pathology

Hysterectomy (surgical removal of the uterus) is associated with a decreased risk of clear cell EOC (Wentzensen, Poole et al. 2016). Tubal ligation (sterilization) decreases the risk of clear cell carcinoma, endometrioid carcinoma, and serous carcinoma where grade is unknown. Risk reductions are around 50% and strongest for clear cell carcinoma (ibid.). Self-reported endometriosis is associated with a 35% increased risk of EOC overall; for clinically verified ovarian endometriosis, the relative risk is ten times that of women without this condition (Kobayashi, Sumimoto et al. 2007; Wentzensen, Poole et al. 2016). The association is limited to endometrioid, clear cell, and low-grade serous carcinoma (Wentzensen, Poole et al. 2016).

2.5.4 Hormone use and endogenous hormone levels

Oral contraceptive pills reduce the risk of EOC by roughly 20% for each 5 years of use (22% with 1–4 years use and 58% with >15 years use). Associations with serous, endometrioid and clear cell carcinomas are not heterogeneous, and there is no association with mucinous carcinoma (Beral, Doll et al. 2008). Approximately 95% of OC users have used combined OC (estrogen-progestin) at some point. Therefore, it is difficult to assess whether the association with EOC is valid also for progestin-only pills, or is limited to the

combined type (ibid.). A previous analysis in the NOWAC cohort found a strong negative association between progestin-only pills and EOC (Kumle, Weiderpass et al. 2004), but a recent summary of available literature found evidence neither for, nor against, primarily due to few exclusive users (Phung, Lee et al. 2021).

The most used progestin-only contraceptive in Norway is the LNG-IUS (Skjeldestad 2007; Lindh, Skjeldestad et al. 2017; Sommerschild 2021). The 20 µg/24h LNG-IUS is licensed as a contraceptive device and as a treatment for menorrhagia (heavy menstrual bleeding) (Bayer Inc. 2021), and is an accepted treatment for low-risk endometrial hyperplasia (Norwegian Directorate of Health 2021). Based on the Norwegian prescription database and an assumed mean duration of use of four years, the prevalence of use was estimated to 10% in 2013 (Lindh, Skjeldestad et al. 2017). From 2013-2018 the number of devices sold annually increased by 60% (Sommerschild 2021). Questionnaire-based epidemiological studies tend to classify LNG-IUS together with non-hormonal intrauterine devices (IUDs) (Balayla, Gil et al. 2021). When the present work was initiated, the available information on the association between LNG-IUS and risk of EOC derived from a Finnish cohort of women reimbursed for LNG-IUS prescribed for menorrhagia. Follow-up ended at age 55, and showed a 41% lower risk of EOC with ever use of LNG-IUS (Soini, Hurskainen, Grenman et al. 2016).

Estrogen menopausal hormone therapy (or hormone replacement therapy, HRT) is classified as an ovarian carcinogen (IARC 2019). Ever use of HRT is associated with a 36% increase in EOC overall. Subtype risks are increased 41% for serous, and 67% for endometrioid carcinoma; there is a non-significant 10% decreased risk of clear cell carcinoma, and no association with mucinous carcinoma (Wentzensen, Poole et al. 2016).

The role of endogenous hormones in the etiology of EOC is unclear. Conditions and contraceptives that inhibit ovulation do this by lowering gonadotropins, which have been long-standing suspects (Stadel (1975), Cramer and Welch (1983) in: (Risch 1998)). However, current views emphasize ways that ovulation promotes cellular transformation in the fallopian tube and facilitates transfer of premalignant cells to the ovary (Kurman and Shih Ie 2010; Emori and Drapkin 2014). Insulin-like growth factor I (IGF-I) has been suspected because it promotes growth and angiogenesis (Lukanova and Kaaks 2005), and a prospective study confirmed an association between elevated IGF-I and risk of EOC (Ose, Fortner et al. 2014). Steroid sex hormones (Cramer and Welch (1983) in: Risch (1998)) and their metabolites are associated with risk of EOC subtypes, but whether it is the hormones, their metabolites, or

other sources of variation in metabolic pathways that mediate the risk, is unknown (Schock, Surcel et al. 2014; Trabert, Brinton et al. 2016; Ose, Poole et al. 2017). It is possible that an effect of steroid hormones is mediated by the immune system (Ness and Cottreau 1999; Brinton and Trabert 2018; Peres, Mallen et al. 2019).

2.5.5 Inflammatory and immunologic factors

Conditions of chronic pelvic inflammation (endometriosis, unresolved chlamydia infection) are associated with an increased risk of EOC (Ness 2003; Irving and Clement 2019; Trabert, Waterboer et al. 2019). Inflammation may also play a role in the link between ovulation and cancer (Duffy, Ko et al. 2019; Trabert, Tworoger et al. 2020). However, the causative feature of these risk factors in EOC may also be structural (Kurman and Shih Ie 2011; Trabert, Tworoger et al. 2020). It has been proposed that certain acute infections, injuries and exposures that are negatively associated with risk of EOC are protective because they induce systemic immunological surveillance (Cramer and Finn 2011; Jacqueline, Lee et al. 2020). Oppositely, immunological tolerance due to unresolved inflammation is thought to be permissive of cancer development (Rogovskii 2020). The types and degree of immune cell infiltration in tumor has prognostic impact on EOC (Zhang, Conejo-Garcia et al. 2003), but ovarian malignancies create an immunosuppressive milieu (Ness 2003; Coosemans, Decoene et al. 2016). With regard to the peripheral immune system, the proportion of circulating immune cells with immunosuppressive relative to cytotoxic (antitumor) functions has been associated with an increased risk of EOC (Cannioto, Sucheston-Campbell et al. 2017; Le Cornet, Schildknecht et al. 2020).

2.6 Ovarian cancer and gene expression in peripheral blood

The peripheral immune system is accessible through blood samples and can be investigated in a population-based epidemiological context. The infrastructure of large-scale cohorts allows collection of biological samples from persons without any clinical illness, including blood samples from persons with pre-clinical cancer. This is of obvious interest for discovering or testing potential early cancer markers (García-Closas, Vermeulen et al. 2011), but also for exploring the carcinogenic process (Lund, Plancade et al. 2015). Focusing on molecular biological methods, blood samples contain DNA (the genome), semi-permanent methylation patterns on DNA that regulate gene expression (the methylome), and RNA copies of expressed genes (the transcriptome). The transcriptome includes many RNA species. Relevant for the present investigation are protein-coding mRNA, which reflect the type and

current activity (phenotype) of a cell, and non-coding RNA species such as lncRNA and miRNA that regulate the transcription and translation of other genes.

Although the relationship between mRNA and protein is nonlinear, it has been shown that blood gene expression reflects immune status, and several acute illnesses are associated with characteristic blood gene expression patterns (Chaussabel 2015). There are few studies on gene expression in blood in relation to EOC. Two patient-only studies have found six RNA transcripts associated with presence of tumor mass and six with prognosis (Isaksson, Sorbe et al. 2012, 2014), but these did not indicate immune-specific processes. A case-control study (Mok, Kim et al. (2017); mentioned in section 2.2.2) found more than 9000 differentially expressed genes in whole blood from recently diagnosed EOC cases. These studies compared groups of 10–20 women. Leukocyte DNA methylation patterns are associated with EOC risk and with the presence of malignant tumor mass (Teschendorff, Menon et al. 2009). Methylation patterns may mediate genetic risk by influencing gene expression levels (Yang, Wu et al. 2018), although a methylation mark does not necessarily regulate the gene where the mark is located. Differences or changes in methylation may also be attributable to changes in leukocyte proportions, exposures, or changes in cellular phenotype (Fridley, Armasu et al. 2014).

2.7 Aims

The overall aim of this thesis was to investigate contemporary questions on EOC in a population-based, prospective cohort.

Specifically, the papers aimed to

I. Compare the risk factor profiles of extrauterine (ovarian/fallopian) and intrauterine (endometrial) carcinoma subtypes

II. Estimate the association between use of LNG-IUS and risk of ovarian, endometrial and breast cancer, with adjustment for potential confounding factors

III. Explore associations between gene expression in blood and a future diagnosis of EOC

3 Materials and Methods

Paper I and Paper II were based on self-reported (questionnaire-based) exposure information from the NOWAC Study. Paper III combined questionnaire and gene expression data from a subcohort of participants who provided a blood sample to the NOWAC Postgenome biobank. Follow-up information (cancer diagnoses, vital status, residence status) was obtained from national registries. Fig 6 (next page) shows an overview of participant enrollment, follow-up and blood sample collection in NOWAC.

3.1 Study population

3.1.1 The Norwegian Women and Cancer Study

The NOWAC Study is a prospective cohort study initiated in 1991 with the primary aim of estimating the impact of OC use on risk of breast cancer in the general population. Norway has a complete population registry and a near complete national cancer registry, which allows random sampling and follow-up of the whole population. Women born between 1927–1965 who held a Norwegian personal identification number (this applies to persons alive at the census in 1960 and thereafter) were eligible for the study (Lund, Kumle et al. 2003). The Central Bureau of Statistics sent to the selected women a letter explaining the study, a questionnaire (Appendix I) identified by a serial number, and a pre-paid envelope, which those wishing to participate returned to the study center (Lund, Kumle et al. 2003). The whole country was sampled. In the third wave (2003–2007) the sampling density was higher in North Norway, Rogaland and Oslo (Dumeaux, Borresen-Dale et al. 2008).

Participants were enrolled in waves in 1991–92 (response rate 57.5%, N=57585), 1995–97 (response rate 56.5%) and 2003–07 (response rate 48.4%, N=63232) (Lund, Dumeaux et al. 2008). The final cohort consists of approximately 172,000 participants, of which 86% were born 1943–1957. Follow-up questionnaires have been sent 5–7 year intervals (Fig 6 next page). In a 1998–2002 follow-up of the first wave, the response rate was 81% (Lund, Dumeaux et al. 2008).

3.1.2 The Postgenome biobank

A sub-cohort of the NOWAC participants contributed blood samples (some also donated tissue samples; these were not relevant for the present thesis) to build a biobank. This effort was designated ‘Transcriptomics in Cancer Epidemiology’ (TICE) (Dumeaux, Borresen-Dale et al. 2008). The TICE project was initiated in 2003, as epidemiology embraced the newly sequenced human genome; hence the designation ‘Postgenome cohort’.

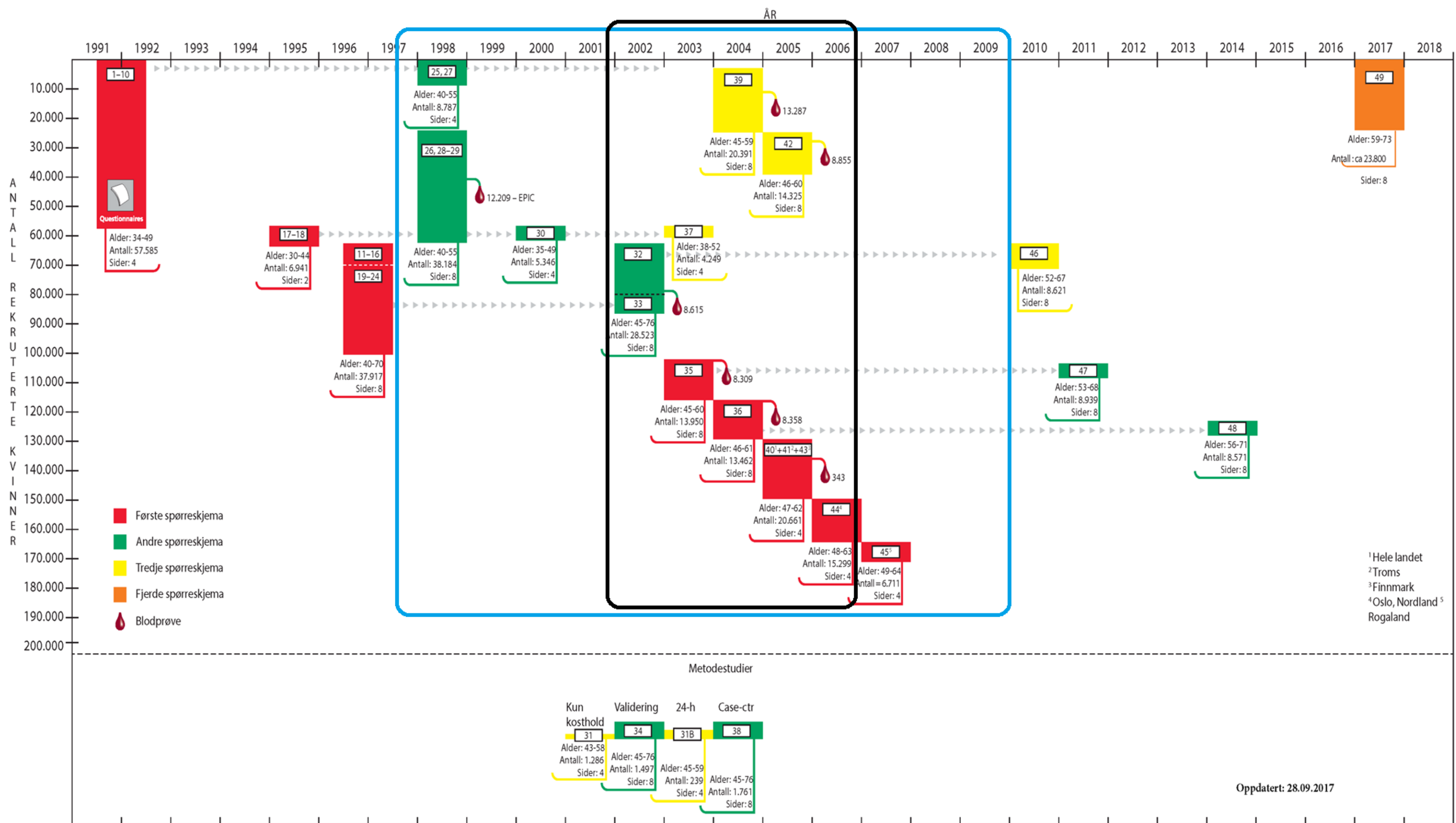

Fig 5 Inclusions and follow-up in the Norwegian Women and Cancer Study, 1991–2017. Inclusions (red), follow-up (green, yellow, orange).The full cohort was eligible for Paper II. Blue frame indicates eligible participants in Paper II. Blood drops within black square indicates participants in the Postgenome biobank.

On the enrollment questionnaires, NOWAC participants were asked to indicate whether they were willing to provide a blood sample; 95% indicated yes. Among these, a random selection of participants (inclusion criterion: birth year 1947–53) were sent a request, a questionnaire, and a blood sampling kit (Appendix II) (Dumeaux, Borresen-Dale et al. 2008). Between 2003–2006, approximately 48000 participants provided a blood sample (response rate 72%). Samples were requested/collected in mailing batches of 500 (ibid.). The participants provided one citrate buffered sample for plasma and buffy coat (leukocytes for DNA), and one PAXgene Blood RNA tube (PreAnalytiX [Qiagen/BD], obtained from BD Norway) for RNA. The RNA sample was used for Paper III of the present thesis. The PAXgene blood RNA tubes were stored at -70 °C. The PAXgene preservation method has been validated up to 11 years by the manufacturer (PreAnalytiX 2020), and up to 17 years for the purposes of the NOWAC Postgenome cohort (parameters: total RNA yield, purity, integrity, and performance in RT-PCR) (Olsen, KS; manuscript in preparation).

3.2 Follow-up

Cancer diagnoses, dates of emigration or death and cause of death were obtained from the Central Bureau of Statistics, which obtained the information using national identification numbers and conducted the linkage to NOWAC serial numbers. Verified or suspected cancer is subject to notification to the Norwegian Cancer Registry. In the period 1987–1996, the completeness of reporting of ovarian cancer was 99.6% and the accuracy 92%. The main reason for error was borderline tumors diagnosed as invasive (Tingulstad, Halvorsen et al. 2002). Today, 94% of ovarian cancers are morphologically verified (Cancer Registry of Norway 2021).

3.2.1 Case ascertainment

Cancer cases were identified by date of diagnosis, primary tumor location using International Classification of Diseases (ICD) 7, and tumor morphology using International Classification of Diseases for Oncology (ICD-O) 2 and 3.

Ovarian cancer was defined as ICD-175 and subsites, of which 175.0 is ovary and 175.2 fallopian tube (corresponding to ICD-10 locations C56 and C57.0–4). Uterine cancer was defined as location 172 (C54), and breast cancer as location 170 (C50). Tumor morphology was coded according to the International Classification of Diseases for Oncology (ICD-O-2 or ICD-O-3). Carcinomas were defined as epithelial tumors with invasiveness digit code 3 (malignant tumors); borderline tumors as code 1. Histologic subtypes were defined as

serous/papillary serous (ICD-O-2/3 code 8441, 8450, 8460, 8461), endometrioid (8380, 8382), or clear cell (8310).

3.3 Analytical samples

Paper I

The starting population was 172,478 participants in the NOWAC cohort who completed a baseline questionnaire (born 1927–1965, enrolled 1991–2008). Menopausal status was seen as an effect modifier that necessitated stratification. To maintain a two-group setup, the analysis was restricted to postmenopausal cancers. This led to exclusion of 2749 women who remained premenopausal during follow-up, including 69 ovarian and 50 uterine carcinoma cases. Further exclusions comprised prevalent cancer except basal cell carcinoma (n=6823), self-reported hysterectomy or oophorectomy at baseline or during follow-up (16,480), death or emigration prior to inclusion (n=70) or a negative TML value (n=40). The final study cohort N=146,316. End of follow-up was December 31, 2013.

Paper II

The starting population was 145,320 participants in the NOWAC cohort who completed a baseline or follow-up questionnaire in 1998 or later. From 1991–1997, NOWAC questionnaires addressed IUD use by the question "have you had an IUD? (Yes/No)". In 1998, this was replaced by "have you ever used a hormone IUD (Levonova)? (Yes/No)" Levonova changed brand name to Mirena in 2007 (Felleskatalogen 2017). Participants who indicated use prior to 1993 (one year before LNG-IUS was marketed in Norway) were excluded (n=2938). Further exclusion criteria were prevalent cancer except basal cell carcinoma, or death or emigration prior to inclusion (n=4813); did not answer the question on hormone intrauterine device (n=15,442), or self-reported hysterectomy or oophorectomy (n=17,740); technical reasons (n=7). The final study cohort N=104,380 (birth year 1927–57, 75% born between 1943–57), of which 9146 were ever users of LNG-IUS. End of follow-up was December 31, 2015.

Paper III

The starting population was participants in the NOWAC Study who provided prospective blood samples to the NOWAC Postgenome cohort. Participants were born 1943–1957; enrollment in the Postgenome cohort occurred 2003–2006. The initial study sample comprised cases of borderline or invasive ovarian cancer diagnosed between April 2004 and April 2011 (N=95). Controls were drawn from women of the same birth year in the same

blood collection batch of 500. After laboratory processing, 5 sample pairs were excluded for technical reasons. After computational preprocessing of the gene expression data, consideration of etiological differences led to exclusion of 20 borderline tumors and 4 non-epithelial ovarian cancers. The final study sample comprised 66 cases of EOC and their matched controls. One sample pair with negative case follow-up time was excluded from parts of the analysis.

3.4 Data

3.4.1 Variable selection

Paper I

As the purpose of the analysis was to use risk factor associations to discriminate between ovarian and uterine carcinoma subtype, there was no main exposure. Based on literature search, risk factors considered were age at menarche, OC use, age at first birth, parity, breastfeeding, use of copper-releasing IUD, use of LNG-IUS, age at menopause, use of HRT, TML, maternal history of breast cancer, diabetes, BMI and smoking. Because the aim of the analysis was to obtain precise regression estimates, a rule of thumb of ten cases per variable was applied (Peduzzi, Concato et al. 1995). Consequently, the selection was limited to five variables. Another consideration was to favor variables with few missing observations. The final selection comprised parity, OC use, TML, BMI and smoking.

Paper II

The main exposure was ever use of LNG-IUS. Potential confounders were identified among established risk factors for epithelial ovarian, endometrial, and breast cancer (Stewart and Wild 2014). The covariables considered were age at menarche, parity, OC use, menopausal status at start of follow-up, maternal history of breast cancer, BMI and physical activity level.

Paper III

Variables recorded on the blood questionnaire, plus parity from previous questionnaires, were available for assessment as confounders (Dumeaux, Borresen-Dale et al. 2008). For variables established as EOC risk factors (Stewart and Wild 2014), a literature search was conducted to assess any association with gene expression in blood. The variables current OC use parity, menopausal status, current HRT use, BMI, and current smoking were selected. There was no literature that documented an association between OC use and blood

gene expression, but it was considered worth including this variable since OC use alters DNA methylation and function of immune cells (Campesi, Sanna et al. 2012). Given the age of the participants, ever OC use would have been more relevant (if one considers DNA methylation as semi-permanent), but this would have complicated the variable coding. Possible confounding by cancer-associated differences in blood cell composition is a transfer of principle from methylation studies where it was realized that a proportion cancer-related DNA methylation markers were attributable to differences in blood cell composition (Houseman, Accomando et al. 2012; Teschendorff and Zheng 2017).

The microarray platform used in Paper III generates more than 48,000 observations per blood sample. Through preprocessing steps these were reduced to approximately 12000 expression values for approximately 9000 genes. The preprocessing steps are briefly described in Paper III; general information regarding the preprocessing of microarray data in NOWAC can be found in Günther, Holden et al. (2014).

For targeted tests of the microarray data, the PMC and Embase databases were searched for reports of gene expression in whole blood or peripheral blood lymphocytes in relation to EOC. This produced two gene expression studies that were hospital based and did not include controls (Isaksson, Sorbe et al. 2012, 2014). Gene sets from five DNA methylation studies were also included (Teschendorff, Menon et al. 2009; Fridley, Armasu et al. 2014; Koestler, Chalise et al. 2014; Li, Zheng et al. 2017; Yang, Wu et al. 2018). The previously mentioned blood gene expression study by Mok, Kim et al. (2017) was published in a journal that is not indexed in these databases, and was therefore not found during the search.

3.4.2 Exposure assessment

Age at menarche, Age at menopause

Age at menarche (used to calculate TML) was assessed by the question "how old were you when you had your first menstruation". Age at menopause asked the age at which menstruation stopped.

Menopausal status

In Paper I and Paper II, menopausal status was a composite variable based on questions on menstrual regularity, hormone use and reasons for cessation of menses. The variable was used as an inclusion criterion in Paper I and as a covariable in Paper II (categorical: pre, *peri*, post, unknown) and III (pre/*peri*, post). For Paper I, age at menopause was available for 41%

of the study cohort. For the remaining 59%, an imputed value (the cohort median, 50 years) was used. Validation of menopausal status (defined by menstrual regularity) was validated against serum hormone levels in a sample of women in the postgenome cohort (Waaseth, Bakken et al. 2008). Sensitivity of 92% and specificity of 73% on the day of blood sampling. Of 4 women who indicated unknown menopausal status, hormone levels classified 2 as pre- and 2 as postmenopausal (ibid.).

Oral contraceptives (OC use)

History of OC use was assessed by asking participants to report ever use, current use, age at first use and the total duration of use of combined or progestin-only contraceptive pills. Exposure was assessed as a continuous variable in Paper I (cumulative duration, years), and as a categorical variable in Paper II (ever/never use) and Paper III (current use; yes/no). The reason for including the variable in Paper III was to show that this potential confounder had been considered, though past use was not assessed.

Intraobserver reproducibility of the OC use variable was tested by sending the same questionnaire with a three month interval. The kappa coefficient was 0.97 for ever use and 0.87 for age at first use (Kumle 2003).

Parity, breastfeeding

The NOWAC questionnaires recorded the birth year of each child including stillbirths, and the duration of breastfeeding of each child. Parity was included as a continuous variable in Paper I and as a categorical variable in Paper II and III (0, 1–2, 3–4, ≥5 children). Parity was validated by comparison with the Birth Registry (Lund, Kumle et al. 2003).

Total menstrual lifespan (TML)

Total menstrual lifespan was calculated using an algorithm by Tsilidis, Allen et al. (2011), to which we added cumulative duration of breastfeeding. The algorithm used in Paper I was [years between menarche and menopause ÷ number of full-term pregnancies*0.75 ÷ total duration of combined OC use ÷ cumulative duration of breastfeeding]. Yang, Murphy et al. (2016) assessed differences between algorithms in capturing risk of ovarian and endometrial cancer. These were not considered in Paper I.

Levonorgestrel-releasing intrauterine system (LNG-IUS)

In Paper II, the main analysis assessed exposure to LNG-IUS as never/ever. The question on LNG-IUS asked for ever use, current use, age at first use and duration of use.

There was a sufficient number of breast cancer cases to assess risk of by duration of LNG-IUS use (<5 and >5 years).

The LNG-IUS was not validated, and use prior to 2004 could not have been objectively validated. Women indicated using LNG-IUS before it was on the market in Norway (1994); with one year margin (1993), these were excluded.

Hormone replacement therapy (HRT)

Current use of HRT (yes, no) was assessed in Paper III. The percentage of HRT users in NOWAC (14.75%) was identical to the Norwegian Prescription Database (Waaseth, Bakken et al. 2009).

Maternal history of breast cancer

The participants were asked whether their mother had been diagnosed with breast cancer (yes/no/unknown). Age at diagnosis was not asked on the 1991/92 questionnaires.

Physical activity level

Physical activity assessed global physical activity including leisure time and work, on an analog scale from 1 (very low) to 10 (very high). The scale was validated by objective measurements of activity and fitness 4-6 months apart. Overall, the scale ranks participants correctly. Intraclass correlation over time was 0.62 for activity and 0.87 for fitness (Borch, Ekelund et al. 2012).

Body mass index (BMI)

Body mass index was calculated by the formula weight (kg) / [height (m)]^2. This variable was assessed as a continuous variable in Paper I and as a categorical covariable (<25 kg/m2, ≥25 kg/m2) in Papers II and III.

The BMI variable was self-reported, and was validated in a sample of women who had been enrolled in the postgenome cohort within one year of enrollment in the NOWAC cohort. Height and weight were asked on both enrollment and blood questionnaire. In addition, the blood questionnaire recorded whether the values were self-reported or measured at the general practitioner's office (Skeie 2015). The weighted kappa for interobserver agreement was 0.73. Among women with BMI <18.5, 50% self-reported a BMI >18.5. Among women with BMI 18.5-25, 94% self-reported correct. Among women with BMI 25-30, 36% self-reported a BMI <25. Among women with a BMI>30, 20% self-reported a BMI <25 (Skeie, Mode et al. 2015).

Smoking

Paper I used a categorical smoking variable (never/ever) that was based on enrollment questionnaires asking “have you ever smoked” (questionnaires evolved to ask “have you smoked 100 cigarettes or more during your lifetime”) or “do you currently smoke”. In Paper III, the categorical variable ‘current smoker’ (yes/no) was based on whether the participant reported having smoked during the past week.

The smoking variable was not validated. Lukic (2018) compared smoking status on baseline and follow-up questionnaires. Approximately 1.8% reported that they were ever smokers at baseline and never smokers at follow-up.

Gene expression measurements

To measure gene expression, total RNA was extracted from whole blood. Polyadenylated RNA was amplified, labeled and hybridized to Illumina HT-12v4 microarrays (steps described in Paper III). Poly(A) primers are primarily intended to select protein-coding mRNA, but long, non-coding RNA with poly(A) tails will also be included. The laboratory procedures were conducted using kits according to the protocols provided by (and available from) the kit manufacturers referenced in Paper III. The protocols are publicly available and include validation data. Quality control and documentation was conducted on the isolated RNA and amplification products and on the generated gene expression data set. The processing steps were such that technical variation could be introduced on groups of 8 (one multipipette row), 12 (one microarray chip), 24 (one RNA extraction batch) or 48 (one microarray hybridization chamber). As there were less than 96 samples (corresponds to one PCR plate), batch effects on plate level are unlikely. Case-control pairs were processed next to each other (case status blinded).

Blood cell composition

Paper III included case-control differences in blood cell composition as a potential confounder. Cell type composition could not be measured directly (this requires flow cytometry on fresh blood samples, an algorithm that estimates relative proportions of different leukocyte types based on expression levels of cell-type specific genes (Newman, Liu et al. 2015). The CIBERSORT algorithm and LM22 matrix for blood deconvolution (ibid.) were chosen in consultation with the Norwegian University of Science and Technology (NTNU) Genomics Core Facility.

3.4.3 Study design

The purpose of Paper I was to the epidemiological similarity of similar histological subtypes of extrauterine and intrauterine carcinoma. Ovarian endometrioid and clear cell carcinomas are thought to originate in endometriosis (Karnezis, Cho et al. 2016), so these histotypes were grouped together to increase sample size, with a sensitivity analysis of endometrioid carcinomas alone. High-grade and low-grade serous tumors were analyzed in one group because the hypothesis of a uterine origin of serous carcinomas is not specific to grade (Massuger, Roelofsen et al. 2010).

In Paper II, endpoints were invasive epithelial carcinomas of the ovaries (including fallopian tube) and uterine body, and cancer of the breast. Breast cancer was analyzed by LNG-IUS user status at baseline (current/former user) and duration of use (<5 and >5 years).

Paper III explored associations between blood gene expression EOCs by clinical behavior (metastatic), tumor histology (serous). Serous carcinomas included high-grade and low-grade tumors. Case-control gene expression differences were assessed in all blood samples (all EOC), in subgroups of metastatic EOC and serous EOC, and EOC cases diagnosed ≤ 3 years and >3 years after blood sample collection.

3.5 Statistical analysis

Analyses for paper I were done using the software RStudio (RStudio, Inc., Boston, MA, USA) running R version 3.1.3 (Vienna, Austria; www.r-project.org); for Paper II, SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA); for Paper III, R versions 3.1.2 and 3.2.1 and the software Bioconductor (www.bioconductor.org) were used.

In papers I and II, a two-sided p-value <0.05 was considered statistically significant. In Paper III, a false discovery rate (FDR) <0.05 was considered statistically significant in explorative analyses of single genes. In a targeted analysis of 42 genes, p-values <0.05 were reported as nominally significant.

Paper I

To produce comparable risk profiles, Cox proportional hazards regression models were used to calculate hazard ratios (HR) with 95% CIs for the association between risk factors (parity, OC use, TML, BMI, smoking) and ovarian or uterine carcinoma (overall and subtypes). Age was adjusted for by using attained age as time-scale. This was at the cost of taking calendar period into account, but age was seen as a more important determinant of

cancer risk. Start of follow-up was given as age at menopause or age at enrollment, whichever was highest. Follow-up ended at age at event (ovarian or uterine carcinoma) or age at censoring (other cancer diagnosis except non-melanoma skin cancer, death, emigration) or end of the study period (31 December 2013), whichever occurred first. In the subtype-specific risk calculations, subtypes not under study were censored at age of diagnosis.

The proportional hazards assumption was checked using Schoenfeld residuals, and showed no deviation from proportionality. Missing information was handled by list-wise deletion of participants in the multivariable analysis. There was no main exposure, and in the multivariable regression model, parity, OC use, BMI and smoking were mutually adjusted, while TML was adjusted for BMI and smoking.

To compare risk factor profiles, the HR estimates for ovarian and uterine carcinomas and subtypes were compared using Wald test for heterogeneity. Subtypes with no heterogeneity between ovary and uterus were regrouped from ovary to uterus, risk associations were then recalculated and heterogeneity retested. In the main analysis, endometrioid and clear cell carcinomas were analyzed as one group (ECC) in order to increase sample size. Risk estimates for endometrioid carcinomas alone were calculated in an additional analysis.

Variables were selected *a priori* and included parity, OC use, TML, BMI and smoking. A sensitivity analysis showed no difference when TML was analyzed as 3 months breastfeeding per child instead of the cumulative duration. The formula with cumulative duration was used. Exposure information was taken from enrollment questionnaires, except for information on weight, where weight at follow-up was used as baseline weight if the weight had decreased by 50 kg or more. Follow-up information of menopause status was used to set start of follow-up and calculate TML.

Paper II

The objective of the analysis was to estimate the effect of use of LNG-IUS on risk of ovarian, uterine and breast cancer. As there is a paucity of data on LNG-IUS and cancer risk, descriptive epidemiology was of interest, and as the NOWAC cohort is population-representative, the NOWAC incidence rates are valid estimates for the Norwegian female population. Therefore, Poisson regression, which uses the incidence rate ratio as an estimate for relative risk, was used. Crude incidence rates were calculated, and Poisson regression was used to estimate age-adjusted relative risks (incidence rate ratios) with 95% CIs for ovarian,

uterine and breast cancer among ever users of LNG-IUS with never users as the reference group. A tradeoff was that the age adjustment was less precise than in Paper I, and was done by including, age at start of follow-up was included as a categorical variable (41–76 years, 5-year increments [the paper reads 4]). Follow-up time was calculated from the date of entrance into, until the date of exit from the study. Exit date was defined as the date of cancer diagnosis, death, emigration, or end of the study period, whichever occurred first.

Multivariable regression models were built by removing nonsignificant covariates from the full model, with list-wise deletion of participants with missing information. A regression model with robust error estimates was used. Model fit was assessed by testing the deviance against its assumed chi-squared distribution.

Additional analyses included a sensitivity analysis of the association between LNG-IUS and uterine carcinoma by ever/never use of OC use. Further, risk of breast cancer was calculated by duration of LNG-IUS use (≤ 5 or >5). Characteristics of NOWAC participants who did and did not answer the question on LNG-IUS use were compared using chi-squared tests of independence.

Paper III

To explore overall case-control differences in gene expression, a dissimilarity matrix with Euclidean distances was computed, and a dendrogram created by applying Ward's method for hierarchal clustering. Distances between samples were displayed in a multidimensional scaling plot using the 500 probes with lowest p-values in single-gene linear models (these could be different genes per pair). In all samples and metastatic and serous subgroups, the global test (Goeman, Geer et al. 2004), which uses all genes as predictor and EOC case status as a binary outcome, was applied. The global test was also used to test associations with questionnaire variables and estimated leukocyte proportions. Expression differences of single genes ($\log_2$ fold change [FC] values) between cases and matched controls in all EOC and subgroups was assessed using linear models in the R package *limma* (Ritchie, Phipson et al. 2015).

To test differential expression of gene sets, curated gene sets were obtained from the Broad Institute collections (Subramanian, Tamayo et al. 2005). These included manually curated gene sets (collection C2; gene constellations by humans according to literature or expert knowledge) related to chemical perturbations and canonical pathways, gene sets defined by gene ontology (collection C5; machine readable terms manually annotated to

genes in a hierarchical level of specificity according to cellular component, biological process or molecular function) (The Gene Ontology Consortium 2019), or immunologic signature gene sets (collection C7; manually curated gene sets from published studies of cell types, states, and perturbations within the human or mouse immune system) (Godec, Tan et al. 2016), cancer-related gene sets and KEGG pathways (Kanehisa and Goto 2000). The validity of these gene sets as measurement instruments for ovarian cancer was not assessed.

The R package clusterProfiler (Yu, Wang et al. 2012) was used to assess and visualize overrepresented gene ontology terms (Ashburner, Ball et al. 2000; The Gene Ontology Consortium 2017) among the 100 probes with lowest p-value in single-gene linear models. This analysis was limited to terms in the Biological Process category.

3.6 Ethics

The NOWAC cohort

The NOWAC Study was approved by the Regional Ethics Committee, REK nord, and by the Norwegian Data Inspectorate. Participants were informed that they were not obligated to answer all questions in the questionnaire, and that they could withdraw from the study and have their data deleted. Participants were informed that by returning a questionnaire they consented to follow-up via linkage to the Norwegian Cancer Registry and the Norwegian Cause of Death Registry. The questionnaires asked participants for consent to future contact, which included permission to update the participant's address from the National Population Register. Reminder postcards included an option to decline further contact. The exposure information used in Papers I and II did not include information that might identify participants, such as municipality and occupation.

The Postgenome biobank

Participants consented to analyses of genetic markers that might dispose for cancer and to testing of future hypotheses, and to no results being provided to them individually. Participants who provided blood samples consented to this on the condition that their sample would be de-identified. The data have been stored and analyzed according to contemporary laws and regulations for sensitive data, currently on a computer infrastructure (HUNT Cloud) in accordance with the EU General Data Protection Regulation. The study in Paper III was evaluated by the Regional Ethics Committee according to the requirements for ethics in health research on human subjects, and was in line with the consent given by the participants (2013/964/REK nord).

4 Results – summary of papers

4.1 Paper I

In this prospective study, 146,316 women in the NOWAC cohort were followed from the age of menopause or age 50 (mean age at inclusion 52.8 years) for a total of 1.6 million person-years (PY; median follow-up time 9.8 years). Mean age at uterine carcinoma diagnosis was 61.7 years. Mean age at EOC diagnosis was 60.0 years. Of 1006 uterine carcinomas, 768 were endometrioid/clear cell and 52 were serous. Of 601 EOCs, 68 were endometrioid/clear cell and 386 were serous. Twenty-seven ovarian carcinomas (22 serous and 5 clear cell) were sub-located to the fallopian tube.

In the multivariable analysis, ovarian and uterine carcinomas were differentially associated with parity, TML, BMI and smoking, but not OC use. None of these risk factors could differentiate ovarian and uterine endometrioid/clear cell carcinomas. This supported, or more strictly, did not contradict, a common cellular lineage of ovarian and uterine endometrioid/clear cell carcinomas. Smoking differentiated ovarian and uterine serous carcinomas, so a shared cellular lineage of ovarian and uterine serous carcinomas was not supported. Regrouping endometrioid/clear cell carcinomas from ovary to uterus decreased heterogeneity (TML not significant), showing that ovarian endometrioid and clear cell carcinomas are more similar to uterine carcinomas than to other ovarian carcinomas.

4.2 Paper II

This prospective study included 104,380 women in the NOWAC cohort, of which 9144 (9%) were ever users of LNG-IUS. Median age at inclusion was 52 years, mean follow-up time 12.5 years. Ever users of LNG-IUS contributed 107,701 PY, never users 1,197,734 PY. Median duration of LNG-IUS use was 4 years; 50% reported a duration of use between 2 and 6 years.

Among ever users of LNG-IUS there were 18 cases of EOC, 15 cases of endometrial cancer, and 297 cases of breast cancer. Compared to never users, the age-adjusted RR of EOC among ever users was 0.49 (95% CI: 0.30 – 0.82). Adjusted for age at start of follow-up, menopausal status at start of follow-up, ever use of OC, and parity, the RR was 0.53 (95% CI: 0.32 – 0.88). Parity was not significant in the model building, but qualified as a confounder and was included in the model.

The age-adjusted RR of endometrial cancer was 0.19 (95% CI: 0.11 – 0.40). Adjusted for age at start of follow-up, menopausal status at start of follow-up, OC use, parity, BMI, and physical activity level, the RR was 0.22 (95% CI: 0.13 – 0.40). Among never users of OC, the RR associated with ever versus never use of LNG-IUS RR 0.08; in ever users of OC, the RR was 0.34. These estimates were not significantly different ($p_{heterogeneity} = 0.18$).

Ever use of LNG-IUS was not associated with risk of breast cancer (RR 1.03; 95% CI: 0.91 – 1.17). Stratified by duration of use of LNG-IUS, neither <5 years of use (RR 1.06; 95% CI: 0.91 – 1.24) nor >5 years of use (RR of 0.88; 95% CI: 0.68 – 1.16) was associated with breast cancer. Stratified by current and former use, current use of LNG-IUS did not change risk (RR 0.97; 95% CI: 0.80 – 1.19) compared to never use, while former users were at lower risk of breast cancer (RR 0.79; 95% CI: 0.64 – 0.98) compared to never users.

Combining breast, ovarian and endometrial cancers, ever users of LNG-IUS were at decreased risk of these cancers overall (RR 0.86; 95% CI: 0.77 – 0.97).

4.3 Paper III

This molecular epidemiological study explored whole blood gene expression in the general population up to 7 years before a diagnosis of epithelial ovarian cancer. Case-control pairs were matched on age. Mean age at blood sample collection was 56.5 years; mean age at EOC diagnosis was 59.3 years.

On the group level, cases and controls did not differ significantly with regard to questionnaire variables. Based on gene expression, cases had slightly larger proportions of circulating CD8+ T cells and plasma cells, and slightly smaller proportions of monocytes and neutrophils ($p<0.1$).

There were no statistically significant associations between EOC case status and blood gene expression. Global tests of all EOC (66 case-control pairs) and subgroups of metastatic EOC (56 pairs) and serous EOC (45 pairs) resulted in p-values of 0.87, 0.72, and 0.67, respectively. The lower p-values in the metastatic and serous subgroups indicated less variation in gene expression between blood samples from women with similar tumor characteristics.

In single-gene linear models, the lowest p-value (non-FDR adjusted $p<0.0002$) was observed in metastatic EOC. The majority of expression differences were in the range $\log_2$FC ±0.2. In samples collected ≤ 3 years before diagnosis, larger $\log_2$FC values and higher p-values higher indicated more variability and a general upregulation of gene transcription,

possibly indicative of disease-related dysregulation. There was no common transcriptional profile in samples collected ≤ 3 and >3 years before diagnosis.

Among the transcripts with highest log_2FC values in all EOC, serum GZMH (protein), *APOBEC3G* mRNA of leukocyte origin in tumor, and circulating lncRNAs *SNHG5* and *MIAT* have been associated with EOC in literature. The present study indicates these as of potential interest in future studies of circulating markers of EOC, although high FDR q-values attach large uncertainty to the observation. In the targeted analysis of 42 genes previously associated with EOC, four transcripts were nominally differentially expressed (non-FDR-adjusted $p<0.05$). These genes (*LIME1*, *GPR162*, *STAB1*, and *SKAP1*) encode receptor proteins and adaptor proteins involved in Src pathways.

5 Discussion

The present studies used data from the prospective, nationally representative NOWAC Study and Postgenome Biobank to explore contemporary questions in population-based ovarian cancer epidemiology.

The presented papers found:

i) Based on a comparison of risk factor profiles, it is plausible that ovarian ECC originates in cell types that also occur in the endometrium. This is less likely for serous carcinomas.

ii) Ever users of LNG-IUS had a strongly reduced risk of ovarian and endometrial cancer compared to never users, with no increased risk of breast cancer.

iii) Gene expression in whole blood collected up to 7 years prior to EOC diagnosis revealed no statistically significant global or gene-wise associations with EOC case status.

5.1 Methodological considerations

5.1.1 Study design

Paper I and Paper II were prospective cohort studies based on questionnaire data from the NOWAC Study. The NOWAC Study was designed as a prospective cohort in order to avoid the selection and recall biases that threaten the validity of case-control studies. Longitudinal follow-up enables estimation of risk, and allows interpretation of explanatory variables as risk factors. Paper III was a nested case-control study of blood gene expression in prospective samples from the Postgenome biobank. Nesting a case-control study in a prospective cohort retains the cohort's advantage of obtaining controls from the same risk set as the cases. Population-based samples from persons without clinical cancer is a rare resource in cancer epidemiology in general, and peripheral blood mRNA collected in this manner is unique for the TICE project.

5.1.2 Impact of selection bias

The initial participant selection for NOWAC used the National Population Register to select a representative sample of the general population. The response rate was 62% in the age group 30–34, and decreased to 52% in the age group 60–64. To compare with the source population, registry information was obtained for an invitation batch of 15,000 women (Lund, Kumle et al. 2003). Response rate was positively associated with number of children, age at first birth, and years of education. The difference was greatest among nullipara (8.6% among

responders vs 10.4% in the whole invitation batch) and women with 9 years education or less (29.4% vs. 34.5%) (ibid.).

A response rate of 50–60% raised concerns of non-response bias. A higher educational level among responders suggested that the choice to participate was associated with socioeconomic status (Lund, Kumle et al. 2003). Batches with 70% response had the same distribution of variables as those with 50% response (ibid.). A small survey was carried out among non-responders. Those who did not participate, but responded to the non-response survey, represented the same segment as participants with regard to nulliparity and education. Compared to participants, a larger proportion of the 'responding non-participants' indicated having three children or more, and to never have used OCs, but these differences were not statistically significant. The main reasons given for non-participation were lack of time, privacy concerns, and forgetting the questionnaire (Lund, Kumle et al. 2003). The response rate and underrepresentation of persons of lower socioeconomic status are typical for studies with written questionnaires (Bhopal 2008).

The observed selection bias would lead to confounded risk estimates if persons from different socioeconomic groups were overrepresented in certain exposure categories and if other contributing causes of the cancers under study differ by socioeconomic status. This was not assed in the studies presented in this thesis. Several other studies have found a negative association between education and risk of EOC (Lund (1992), Lahmann, Cust et al. (2010) in: Alberg, Moorman et al. (2016)), but a previous study in NOWAC with follow-up until 2001 did not (Braaten, Weiderpass et al. 2005). In current Norwegian national statistics, higher income, but not education, is negatively associated with EOC (unadjusted associations) (Larsen and Myklebust 2019). For breast cancer, a strong positive trend has been found in NOWAC, with a 50% higher risk in the highest educated group (Braaten, Weiderpass et al. 2005). There was no trend for corpus uteri cancer (ibid). In Paper II, responders and non-responders to the LNG-IUS question corroborated the trend of more response from parous women (Paper II, supplementary table).

Lund and colleagues (Lund, Kumle et al. 2003; Lund, Dumeaux et al. 2008) assessed the impact of any selection bias by comparing cancer incidence rates (breast and cancer overall) in NOWAC to national rates. The rates were similar, which supports that the NOWAC cohort is population representative, although it was speculated whether this was because several risk factors canceled each other (Lund, Kumle et al. 2003). For EOC, incidence rates in the NOWAC cohort have been lower than in Norway. In Paper II, a

comparison of age-specific rates of EOC among ever and never users of LNG-IUS in the study cohort in Paper II was conducted to assess whether the effect of LNG-IUS was transitory. The data (Fig 7; not shown in the paper) show rates of EOC among never users of LNG-IUS that equal to the Norwegian background population (also shown in Fig 7). This seems to support that risk modulators with opposite effects on breast cancer and protective effects on EOC are at work in the NOWAC cohort. With appropriate adjustments, relative risks for LNG-IUS can still be representative for the population, but the incidence rates presented in the paper appear to be underestimated by about 10%.

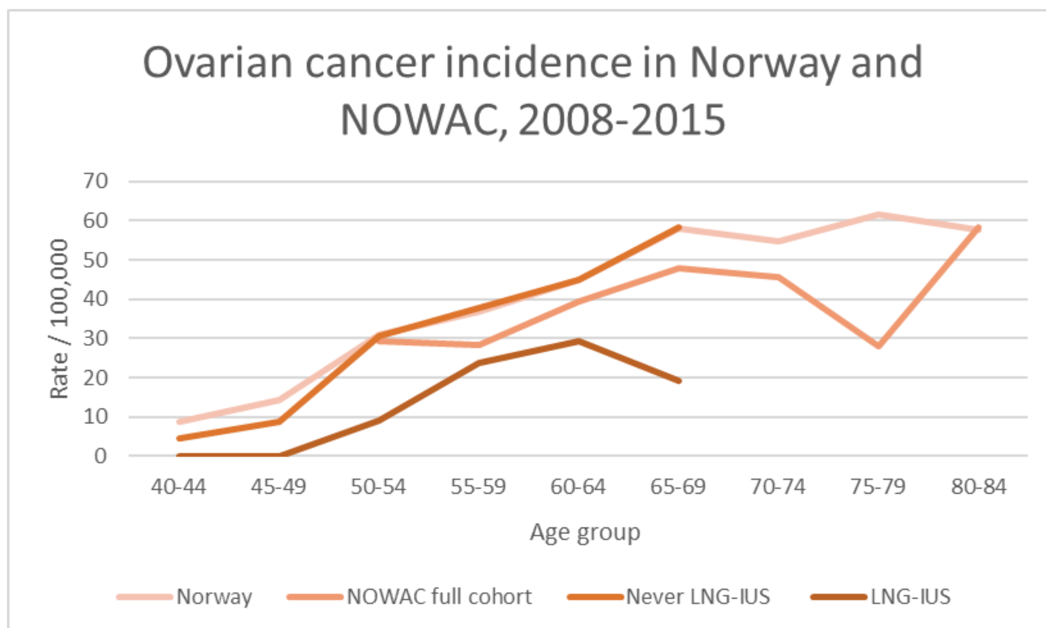

Fig 6 Ovarian cancer incidence in Norway and in the Norwegian Women and Cancer Study, 2008-2015. Illustration of the selection bias in the cohort versus the population. Note that the graph must be viewed in color, and that the follow-up periods differ (1998-2015 in Paper II, 2008-2015 in Norway and in the full cohort). Based on data from the Cancer Registry of Norway, in part provided to NOWAC and in part obtained via the NORDCAN 2.0 database (https://nordcan.iarc.fr/en/database).

Participants who did not respond to follow-up questionnaires (Lund, Dumeaux et al. 2008) had similar characteristics as initial non-responders (Lund, Kumle et al. 2003). The impact on variable distribution was small (Lund, Dumeaux et al. 2008). Women who did not respond to follow-up questionnaires would be overrepresented among those with missing duration of ongoing exposures and would not be represented in the results of complete-case analyses. This would impact the OC use variable in Paper I, but for a limited number of participants (in the 1991–97 wave of enrollments, 7% were current users of OCs (Kumle, Alsaker et al. 2003). In Paper II, incomplete information on duration of use would overestimate the effect of duration of use of LNG-IUS on breast cancer, and any resulting

cases would be missing from the category of longer duration, but the degree of misclassification is unlikely to have led to missed increased risks.

Participants were kept in the cohort unless they withdrew. By 2003, seven of 102,540 women enrolled in the first wave had withdrawn (Kumle, Alsaker et al. 2003). The completeness of reporting of ovarian cancer to the Cancer Registry was >99.5% (corpus uteri 99.9%, breast 100%) (Tingulstad, Halvorsen et al. 2002; Cancer Registry of Norway 2021), loss to follow-up of endpoints was low.

In Paper III, quantile normalization was applied before $\log_2$-transformation, and transcripts were called present if they were expressed in at least 70% of the case-control data set ($p<0.05$). Different combinations of normalization method, detection limit and percent present limit has been thoroughly assessed on NOWAC blood gene expression data (breast cancer) (Günther, Holden et al. 2014). This report showed that stricter cutoffs than those applied in Paper III (which were already quite strict) revealed significant case-control gene expression differences, but this drastically reduced the number of probes in the dataset. The report was based on larger datasets (100-300 sample pairs). Paper III did not include a sensitivity analysis of cutoff parameters and normalization method.

To conclude, the identified selection biases could not explain the presented results.

5.1.3 Impact of information bias

The exposure variable validation studies (referred under Methods) showed that validity and reproducibility of exposure measurements were category dependent. For OC use, the reproducibility of ever/never use was higher than age at first use (Kumle 2003). Given the age of the participants, some error in recall is to be expected. In prospective cohort studies, it is assumed that misclassification due to error in recall is nondifferential (Szklo and Nieto 2014).

For BMI, the validation study (Skeie, Mode et al. 2015) showed that errors in self-reported weight were systematic towards weight that produced a BMI within the normal range. In the present thesis, each paper constructed the BMI variable differently. Misclassification of women with higher BMI into lower categories will have led to overestimation of the effect of BMI in Paper I and underestimation of the effect of BMI in Papers II and III. More participants likely stopped than started smoking, which will have underestimated the effect of this exposure. If the reason for smoking cessation was perceived health, the misclassification is nondifferential with regards to cancer risk (Killie, IL. unpublished results).

Use of LNG-IUS may be difficult to objectively validate. In Paper II, the percentage of users in the study cohort was 8.8%, similar to the 8% observed by Graff-Iversen and Tonstad (2002) in the Cardiovascular Disease Study in Norwegian Counties in 1997. Based on general statistics from the Norwegian Prescription Registry, in Norway in 2004 there were 21834 prescriptions of LNG-IUS (NorPD 2022), while the Norwegian Medicines Agency reported that a total of 30494 devices were sold that year (Sakshaug, Strøm et al. 2009). This means that 71% of users fetched the device at the pharmacy prior to their appointment, while the remaining 29% had their LNG-IUS inserted by a health care provider who kept the device in stock. If these providers were specialists, it is possible that the consultation is registered in the Patient Registry. However, the participants' responses may be an indicator of reliability. Among participants enrolled in 1991–92 when only the copper-releasing IUD was available, the item response rate was 96%. In 1998–2007 when there were two available types, yet only one option, the response was 88%. (The question variants are shown in section 3.3 'Analytical samples'.) This could suggest that women who were in doubt either as to the type they were using, or whether to indicate "No" when they were using the copper-releasing type, refrained from completing the question. Comparing this to the intraobserver reproducibility of OC use (section 3.4.2 exposure assessment), it seems likely that participants who reported having used LNG-IUS prior to 1994 made an error of timing, rather than an error of type.

Whereas the IUDs available in Norway were either copper-releasing or levonorgestrel-releasing, a wider array of non-hormonal types have been in use internationally. Therefore, there is a paucity of data on use of the copper-releasing IUD specifically and risk of EOC. A recent analysis from the New England Case-Control study found no association (OR 1.04; 95% CI: 0.78 – 1.38) (Yang, Sasamoto et al. 2021). Studies that mix hormonal and non-hormonal IUD types (Wheeler, Desanto et al. 2019; Balayla, Gil et al. 2021) show a negative association with EOC, but this association is weaker than studies of LNG-IUS specifically. Therefore, misclassification of users of copper-releasing IUD as users of LNG-IUS is unlikely to overestimate protective effects of the LNG-IUS, but might underestimate harmful effects such as an increased risk of breast cancer.

In Paper I and II, missing values were handled by list-wise deletion in multivariable analyses. In Paper I, missing age at menopause was imputed by replacing missing values with the cohort median value (50 years). Age at menopause is thought to be the most important component of TML (Yang, Murphy et al. 2016), and a detailed investigation of TML and risk of endometrial cancer in the NOWAC cohort showed that the imputation method was more

impactful than the TML model or adjustment for BMI, smoking and physical activity (Gavrilyuk, Braaten et al. 2018). Paper II used menopausal status at start of follow-up as adjustment variable, which had few missing values. The misclassification would depend on age at enrollment.

The information on cancer diagnoses were based on registry information. These diagnoses were made according to contemporary pathology practice, which has changed over time (Köbel, Kalloger et al. 2010; Gilks, Oliva et al. 2013). In particular, the diagnosis of ovarian high-grade endometrioid carcinomas has changed (Doherty, Peres et al. 2017). This fit with our data in Paper I, where the percentage of high-grade endometrioid carcinomas decreased over time. Inaccurate diagnosis of histologic subtypes is a situation of nondifferential misclassification (Kelemen, Goodman et al. 2010), which would further weaken our ability to detect risk differences, but not lead to biased risk estimates. For Paper I, the problem could have been solved by reclassifying ovarian grade 3 endometrioid carcinomas as serous (Kelemen, Goodman et al. 2010; Doherty, Jensen et al. 2017). The emphasis given to this issue (Doherty, Jensen et al. 2017) is based on a study where 28% of endometrioid carcinomas were misclassified (Kelemen, Goodman et al. 2010). In Paper I, in 17 of the 22 years of follow-up, less than 10% of ovarian endometrioid carcinomas were high-grade. The grouping of tumor histologies for subtype analyses were based on literature and consultation with an experienced pathologist, but the final decisions were made by one researcher (MJ). No sensitivity analyses were conducted to investigate the impact of these group definitions.

Prior to inserting an IUD, the practitioner must establish the position of the uterus. The guideline in Norway instructs that this should be done by a bimanual pelvic exam (Johansen and Gamnes 2022). However, practitioners with ultrasound apparatus readily available may choose transvaginal ultrasound for this, or for checking proper placement of the device. A bimanual pelvic exam has a sensitivity of 5.1% for ovarian cancer (Doroudi, Kramer et al. 2017), while the sensitivity of transvaginal ultrasound for EOC is 75% (Menon, Gentry-Maharaj et al. 2009). Norway has a triennial screening program for cervical cancer for women aged 25–69 (initiated 1995; participation 70%) (Nygård, Skare et al. 2002; Braaten, Weiderpass et al. 2005). The cytology sampling guideline instructs the practitioner to perform a bimanual pelvic exam (Johansen and Gamnes 2022). Hence, depending on the type of pelvic examination at insertion of LNG-IUS, the EOC estimates in Paper II may suffer from either minimal or substantial surveillance bias. Further, women are tested for sexually transmitted

infections, minimum Chlamydia, prior to insertion (Johansen and Gamnes 2022). If the positive association between Chlamydia infection and EOC (Trabert, Waterboer et al. 2019) is causal, this may have introduced some additional surveillance bias. If these biases explain the association between LNG-IUS and risk of EOC, a similar association should be observed in users of copper-releasing IUD in the same region and period.

Considering EOC, one could imagine a situation where cases discovered at LNG-IUS insertion were diagnosed earlier than if their diagnosis was symptom based. Non-users at the corresponding stage at the same point in time would be diagnosed later. If the time since use in the EOC analysis is sufficient to say that cancers among non-users would have been detected clinically, the proportion of prevalent cancers would be equal among ever and never users, and the bias may be minimal.

The registry-based study by Iversen, Fielding et al. (2020) saw an increased risk of endometrial cancer in short-term users of LNG-IUS. This was attributed to protopathic bias (ibid.). A smaller proportion of the participants in Paper II than in the study of Iversen et al. will have contributed person-time from shortly after having the LNG-IUS inserted.

In Paper III, the influence of technical variables on the results of the analysis was not investigated because the paired sample processing served as technical matching. As the scope of the TICE project was explorative, purely computational investigation, gene expression levels were neither validated by PCR nor linked to the translated gene product by protein expression measurements.

Paper III tested differential expression of gene ontology, immune related gene sets, cancer-related gene sets and KEGG pathways. Their validity as measurement instruments for ovarian cancer-related processes in blood was not assessed. The same was the case for the gene ontology enrichment analysis of the 100 probes with lowest p-value. Gene expression measurements do not contain information on the location and activity of gene products, which can be highly specific.

The LM22 signature matrix is constructed by correlation with flow cytometry cell counts, and is considered a good choice for whole blood (Teschendorff and Zheng 2017). As noted in Paper III, other studies from NOWAC (Baiju, Sandanger et al. 2021; Nøst, Holden et al. 2021) display a similar pattern of divergence from typical cell counts. The same pattern appears in a study on healthy adult males <50 years that transported the samples on dry ice (Eftedal, Flatberg et al. 2016). This study also used the PAXgene system, Illumina HT12

microarrays and the same laboratory facility. Observing the same pattern in these studies rules out ovarian disease, cancer, the study size, the age and sex of participants, and the mail-based sample collection as the source of this divergent pattern. Cases and controls were affected equally, and results were as such not biased in the sense of differential misclassification by case status. A study of the correlation between cell type estimates based on methylation and gene expression versus cell counts in the NOWAC biobank samples is ongoing, and may provide a basis for a reanalysis or reinterpretation of the estimated leukocyte proportions presented in Paper III.

To conclude, the estimated blood cell proportions in Paper III were heavily influenced by the computational algorithm or matrix used, and are not comparable to results obtained by other methods. The other identified information biases could not explain the remaining results.

5.1.4 Impact of confounding

In Paper I, parity, OC use, BMI and smoking were mutually adjusted. These adjustments neither isolated the independent effects nor presented the total effect of each risk factor, but avoided basing an argument on risk associations that contained the effect of each other. The mechanism of OC use in EOC and in uterine carcinomas could be related to TML or directly through hormonal action (Yang, Murphy et al. 2016), and for the comparison of EOC and uterine carcinomas, adjustment for TML could have been of interest. The estimates for BMI and smoking were adjusted for parity and OC use, rather than TML. Yang, Brinton et al. (2010) mentioned that the negative association between smoking and endometrial cancer might be mediated by an earlier age at menopause. Indeed, this appears to be true in the NOWAC cohort (Gavrilyuk, Braaten et al. 2018); therefore, adjusting for TML would not have been appropriate.

In Paper II, the adjustment variables had little impact on the RR estimates. The LNG-IUS users were younger than never users, and this was dealt with by age adjustment, with age as a categorical variable with 5-year increments. Including age as a continuous variable would have forced a linear relationship between age and cancer, and would have led to loss of precision on risk estimates (larger confidence intervals). In the breast cancer analysis, education, alcohol use and smoking were not considered as adjustment variables in the model building. Braaten, Weiderpass et al. (2005) found that the positive association between education and breast cancer in NOWAC was mainly attributable to lower parity (25%), higher

alcohol consumption (25%), and ever use of OC (7%). Education was not considered in Paper II, but population data from Finland and Norway suggest that women with higher socioeconomic status (more than 9 years education) were overrepresented among users of LNG-IUS at the time. Other slightly overrepresented characteristics were being a non-smoker, non-teetotaler, highly active, BMI < 25, and excellent self-reported health (not all were assessed in both countries) (Graff-Iversen and Tonstad 2002; Soini, Hurskainen et al. 2014).

Menopausal status was included as an adjustment variable for all three cancers in Paper II. This was despite being identified as an effect modifier that required stratification in Paper I, despite Heikkinen, Koskenvuo et al. (2016) confirming effect modification of the association between LNG-IUS and breast cancer with risks in opposite directions, and despite being a potential mediator of the effect of LNG-IUS on EOC (self-reported "unknown" menopausal status). The low prevalence of LNG-IUS use among nullipara in NOWAC is likely because previous recommendations favored parous women for IUD use. This, as well as the two-component effect of parity, suggests that parous status was a confounder and should have been assessed as an adjustment variable in addition to parity.

In Paper III, none of the questionnaire variables (parity, current OC use, menopausal status, current HRT use, BMI, and current smoking) were associated with overall gene expression. Apart from OC use, the variables had been associated with gene expression in other studies. Baiju, Sandanger et al. (2021) did find an association between smoking and gene expression in NOWAC biobank samples (1700 cancer-free women), but did not find any association between number of children and gene expression. Notwithstanding, as no case/control differences could be detected in the variable distributions, the exposures did not qualify as confounders.

To conclude, confounding variables in the papers were identified and considered according to knowledge and practices at the time. Some possible adjustment variables were not considered, and some possible confounders were not measured. The present discussion could not show that the presented results are attributable to confounding.

5.2 Discussion - Paper I

Cellular lineage of ovarian carcinomas

Paper I was more in support of a common lineage of differentiation for ovarian and uterine endometrioid/cell carcinomas than for serous carcinomas, even though for four of the five risk factors, the point estimates for serous carcinomas were more similar than for endometrioid/clear cell carcinomas. It can be argued that the reasoning in Paper I rests on a heterogeneity test that was likely vulnerable to number of observations, and that the conclusion might have been different if the groups had been of equal size.

Because previous studies had found only a partial overlap in risk factors for endometrioid and clear cell carcinomas (Nagle, Olsen et al. 2008; Setiawan, Yang et al. 2013), Paper I included an analysis of endometrioid carcinomas without clear cell carcinomas. For ovarian endometrioid carcinomas the association with BMI was attenuated compared to ECC, and the $p_{heterogeneity}$ value dropped from 0.056 to 0.048. If endometrioid and clear cell carcinomas had not been combined, the conclusion of the paper might have been similar to the intra-location studies of EOC and uterine carcinoma subtypes that concluded that some risk factors are shared and some differ (Setiawan, Yang et al. 2013; Wentzensen, Poole et al. 2016). Some studies of EOC subtype etiology have applied hierarchal clustering to histological subtypes and risk factors (Wentzensen, Poole et al. 2016; Fortner, Poole et al. 2019). Such a presentation is less vulnerable to cutoff values used in significance tests. Clustering of the HR estimates in Paper I supports the conclusion that ovarian ECC have more in common with uterine ECC than with other ovarian carcinomas (Fig 8). If the HR estimate for ECC with respect to BMI is replaced by endometrioid alone, the outcome of the clustering does not change (not shown).

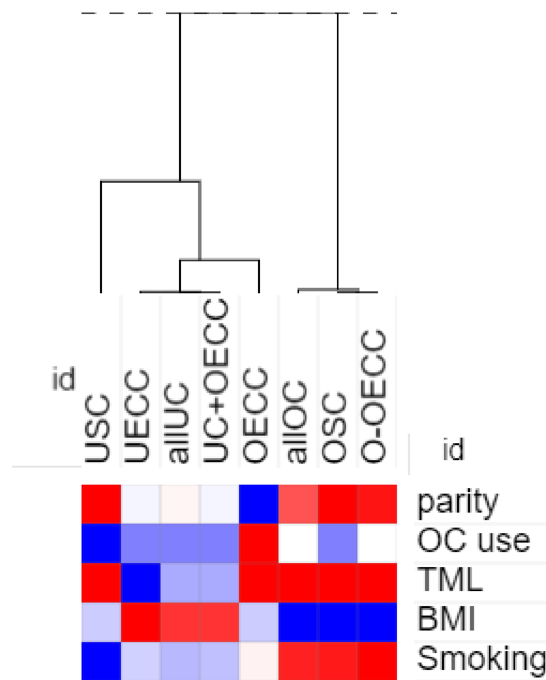

Fig 7 Ovarian endometrioid and clear cell carcinomas have more in common with uterine carcinomas than with other ovarian carcinomas. U, uterine; O, ovarian; SC, serous carcinomas; ECC, endometrioid and clear cell carcinomas. Unsupervised hierarchical clustering of uterine and ovarian canrcinoma subtypes with complete linkage and one minus Pearson coefficient. Based on multivariable HR estimates presented in Paper I (Table 3 in Paper I). Clustering performed using Morpheus https://software.broadinstitute.org/morpheus/

It is difficult to clearly define whether the results of Paper I contribute to characterize the properties or rather the distribution of cell types in the ovaries and reproductive tract. The epidemiological motivation for comparing uterine and ovarian carcinomas was the article by Kuhn, Kurman et al. (2012), which suggested that all EOC originate in cells from the reproductive tract. This hypothesis could be found in the 2011 edition of Blaustein's Pathology of the Female Genital Tract (Seidman, Cho et al. 2011), which emphasized that if true, only non-epithelial carcinomas are real ovarian cancers, in parallel to the male gonadal cancers. A literal language was used, for example about endometrioid and clear cell carcinoma, which were referred to as “endometrial cancers in the wrong place” (Karnezis, Cho et al. 2016). From an epidemiological point of view this constitutes endpoint misclassification. However, the clinical implications of a literal interpretation are not acceptable, and considering that non-epithelial ovarian cancers were excluded from the analysis in Paper I, this question was not answered.

The results can be taken as support of the view that the secondary müllerian system represents embryologic remnants, such that neither relocation nor metaplasia is required in order to produce ovarian carcinomas (Dubeau 2008; Bouquet de Jolinière, Ayoubi et al. 2012). The research group of Massuger, who suggested a uterine origin of serous EOC (Massuger, Roelofsen et al. 2010; Roelofsen, van Kempen et al. 2012), appears to have shifted focus to serous carcinomas of the fallopian tube (van der Steen, Bulten et al. 2017). While epidemiological associations between tubal ligation and risks of intrauterine and extrauterine serous carcinomas support endometrial precursor cells as the source of some serous EOC (Felix, Brinton et al. 2015), clinicopathological findings are not supportive, and instead favor reverse transportation to the uterus (van Niekerk, van Dijck et al. 2018). In 2018, Garavaglia et al. presented a parallel to that of Massuger, where they suggested that endometriosis-associated EOC may have an intrauterine origin (Garavaglia, Sigismondi et al. 2018). Here, the nuance is the intrauterine malignant transformation. Of note, these hypotheses do not discuss Lynch syndrome or synchronous ovarian and endometrial carcinoma.

5.3 Discussion - Paper II

The NOWAC Study comprises the first generations who used hormonal contraceptives, and was initiated to assess the effects of these pharmaceuticals. The oldest participants were age 40 when OCs were introduced in Norway in 1967, and the youngest were age 31 when the LNG-IUS was introduced in 1994 (primarily as an option for women older than 35). Since the Norwegian Prescription Registry was established 2004 and the Mammography program did not differentiate between LNG-IUS and copper IUD (Ellingjord-Dale, Vos et al. 2017), the NOWAC Study is among few sources of information on exposure to LNG-IUS specifically.

Levonorgestrel-releasing intrauterine system and risk of ovarian carcinoma

Paper II presented age- and multivariable RRs of EOC of approximately 0.5 in ever versus never users of LNG-IUS. Among the 85% of users who reported duration, 75% used LNG-IUS for five years (the duration of one device) or less. Duration was not investigated. In the registry study of Soini, Hurskainen et al. (2014) of cancer risks among women reimbursed for LNG-IUS (as treatment for menorrhagia), the SIR with use of one LNG-IUS was 0.60, and 0.51 with two periods of use (purchases). A meta-analysis of Paper II (Jareid, Thalabard et al. 2018) and Soini, Hurskainen, Grenman et al. (2016) showed a pooled OR of 0.58 (95% CI: 0.47 – 0.71) of EOC with ever use of LNG-IUS (Balayla, Gil et al. 2021).

Paper II reported a median duration of use of 4 years among all users in the cohort (mean 4.4 (SD 2.9) years). Of the 18 users of LNG-IUS with EOC, 15 reported duration, and among these, median use was 5 years (mean 4.3 (SD 2.3) years). Thus, in contrast to endometrial cancer (discussed in the next section), it is not immediately apparent that the negative association with use is due to exposure to the LNG-IUS, and the limited effect of the adjustments gives reason to suspect that other variables than those considered may be confounding this association. On the other hand, support for a direct chemopreventive effect of levonorgestrel on EOC have come from recent experimental studies (Wu, Huang et al. 2017; Wu, Fang et al. 2020) and a randomized controlled trial in high-risk women (Rodriguez, Kauderer et al. 2019). These studies investigated serous EOC, and neither study investigated LNG-IUS specifically. A recent observational study in high-risk women found a non-significant negative association between use of LNG-IUS and risk of EOC in the main analysis (Xia, Gronwald et al. 2022). A strong negative association with LNG-IUS was found when stratified by previous OC use (similar to the tendency for endometrial cancer in Paper II) (ibid.).

Paper II did not investigate histologic subtypes. Compared to 64% carcinomas of serous subtype among never users of LNG-IUS, the distribution among ever users was 15 serous (83%), 2 non-specified and 1 endometrioid. This is in line with Soini, Hurskainen, Grenman et al. (2016), who observed the greatest risk reduction in non-serous subtypes.

Shortly after Paper II was published, a registry study of 1.9 million premenopausal women in the Danish general population showed a more moderate risk reduction of EOC of 28% in current or recent users of LNG-IUS compared to never users of any hormonal contraceptives (RR 0.72; 95% CI: 0.53 – 0.99) (Iversen, Fielding et al. 2018). The variable distribution in Denmark confirms the tendency of higher education and less smoking observed in Norway and Finland, and further shows more endometriosis, hysterectomy and tubal sterilization among users of LNG-IUS (ibid). Iversen et al. did not present unadjusted estimates, which prohibits discussion of the potential confounding by these variables in Paper II. The study of Iversen et al. also showed weaker associations with LNG-IUS in women who never used OC. Importantly, the confidence interval in Paper II (95% CI: 0.32 – 0.88 for the multivariable estimate), which indicates the range of 95% of the possible true population risks supported by the data, is not in conflict with the other studies. A meta-analysis of Soini, Hurskainen, Grenman et al. (2016), Iversen, Fielding et al. (2018) and Paper II (Jareid, Thalabard et al. 2018) conducted by D'Alessandro, Frigerio et al. (2022) shows a pooled OR 0.66; 95% CI: 0.41 – 1.08). This meta-analysis concluded that current evidence is not sufficient to support that the LNG-IUS reduces the risk of EOC (ibid.). This also underscores the utility of using the confidence interval when discussing the potential impact of LNG-IUS in the population.

Levonorgestrel-releasing intrauterine system and risk of uterine carcinoma

Paper II found a strongly reduced risk of endometrial cancer in ever users of LNG-IUS compared to never users. The risk estimate was (non-significantly) lower in never users of OC. This indicated that the association with LNG-IUS was moderated by OC use, but unlikely to be explained by residual confounding by previous OC use.

The number of cases was insufficient to investigate duration of use in order to assess any dose-response relationship. However, data for the 12 cases for which information on duration exists, shows a median duration of 3 years (mean 3.1; SD 1.5 years), compared to 4 among LNG-IUS users without cancer. A recent study by Iversen, Fielding et al. (2020) investigated the association between LNG-IUS and endometrial cancer in premenopausal women in Denmark. This study found a strong negative association with duration of use. The

estimate in Paper II was similar to what Iversen et al. observed in current users who had used LNG-IUS for 5 years or more (RR 0.24; 95% CI: 0.10 – 0.60). Iversen et al. saw a clear indication bias in women who had used LNG-IUS 1 year or less. In women with 1–4 years of use, statistically significant reduced risks were found in former but not current or recent users, suggesting an indication bias also in this group. Their results for this duration of use give the impression of a risk that decreases with time since use. The results from Paper II fit with this apparent trend, and is promising, as the women were older.

Levonorgestrel-releasing intrauterine system and risk of breast cancer

Paper II has been included in two meta-analyses of LNG-IUS and breast cancer (Conz, Mota et al. 2020; Silva, Grande, Lacerda Macedo et al. 2021), which arrived at different conclusions. Both studies included systematic reviews of Backman, Rauramo et al. (2005), Lyytinen, Dyba et al. (2010), Dinger, Bardenheuer et al. (2011), Soini, Hurskainen, Grénman et al. (2016), Heikkinen, Koskenvuo et al. (2016), Mørch, Skovlund et al. (2017), Siegelmann-Danieli, Katzir et al. (2018) and Jareid, Thalabard et al. (2018), but the meta-analyses were conducted according to different inclusion and analysis criteria.

Conz et al. stratified studies according to age structure of the participants and used the reported effect measures, including various adjustments. The meta-analysis, which included all the reviewed studies except Backman et al. (2005), showed an OR of breast cancer of 1.12 (95% CI: 1.02 – 1.22) for use of LNG-IUS in the reproductive period (until age 50 years), and an OR of 1.52 (95% CI: 1.34 – 1.72) for use after menopause. Paper II was in a separate stratum because the participant structure was mixed (7% started use at age 49 or older). With all studies in the analysis, ever users of LNG-IUS had a 16% higher risk of breast cancer compared to never users. The method of Conz et al. was criticized (Al Kiyumi, Al Battashi et al. 2021; Silva, Grande, and Da Rosa 2021), but this was rebutted (Conz, Mota et al. 2021).

Silva, Grande, Lacerda Macedo et al. (2021) conducted a meta-analysis of studies for which they could obtain numbers of exposed and unexposed cases and controls. These authors excluded studies for which only person-years were available, as well as studies with potential overlap between study participants. Further, while the main results presented by Dinger, Bardenheuer et al. (2011) and Heikkinen, Koskenvuo et al. (2016) were based on comparisons between users of LNG-IUS and users of copper-releasing IUD, Silva and colleagues included all never users of LNG-IUS from these studies to harmonize with the other studies in the analysis. For cohort studies the summary OR was 0.93 (95% CI: 0.84 – 1.03), and for case-control studies 1.07 (95% CI: 0.91 – 1.26). Without any adjustments, the

study of Silva and colleagues is an assessment of whether ever use of LNG-IUS is an indicator of breast cancer risk, and their results suggest that it is not.

Paper II adds to the literature that women who use one LNG-IUS when they are between age 35 and 50 do not have an increased risk of breast cancer ten years later. This was in line with the intermittent risk associated with OC use mentioned in the introduction of the paper. Mørch, Skovlund et al. (2017) concluded that among premenopausal women, the absolute number of additional BC cases attributable to use of hormonal contraceptives is most likely low. Paper II adds that this appears true for postmenopausal BC, which is reassuring, as the absolute risk increases with age. However, because the main interest of Paper II was the association with EOC, the title focused on the finding of an apparently effective yet safe manner of use. Paper II did not fully consider the potential of the title to directly influence clinical practice in light of current trends in use. Although the mix of current and former users (White, Hunt et al. 1998) and other characteristics of the observed use and of the observation period were communicated in the paper, it would have benefitted the reader if the title and abstract had been either more neutral or more specific regarding the limitations of the conclusions for each cancer.

5.4 Discussion - Paper III

Blood gene expression prior to ovarian cancer diagnosis

Paper III mentioned as possible reasons for the lack of statistically significant findings the number of samples in the study, heterogeneous analytic groups, and small mean differences in gene expression. The majority of expression differences were in the range of $log_2FC \pm 0.2$. Issues regarding the overall small log_2FC values observed in NOWAC blood gene expression studies have been discussed previously (Plancade, Rozenholc et al. 2012). To measure expression levels of many genes simultaneously, fluorescent cRNA transcripts are hybridized to an array of microscopic beads, each bead covered by more than 500,000 probes (one probe sequence per bead) (Kuhn, Baker et al. 2004). For each probe type there are several replicate beads, and the expression level of a transcript is the mean saturation of the beads. However, the beads can also be considered as technical replicates (Lin, Du et al. 2008). The original power calculation for Paper III was based on 60 replicate beads on the Illumina HT-6 microarray. Based on 95 sample pairs, it was found that with 44000 bead types on the array, a case/control expression difference of 5-10% would be detectable (corresponding to a RR 1.05-1.10). However, the HT-12 array used in the current investigation has only 30 beads per probe type. This impacted the variance of the measurements (Lund, E; personal communication). Indeed, the analysis in Paper III detected a RR 1.15 ($log_2FC \pm 0.2$) with p-values <0.001. The power calculation did not discuss adjustment for multiple testing.

The initial sample set of 95 borderline or invasive ovarian cancer case/control pairs was reduced to 66 EOC case/control pairs before analysis, and further to groups of 56, 45, and finally 31 samples. The sizes of the analytical groups are typical for human transcriptomics studies (90% are based on 3-84 samples) (Holsbø and Møllersen 2020), but they were smaller than NOWAC blood transcriptomics studies on breast and lung cancer (Lund, Holden et al. 2016; Holden 2017; Holsbø and Olsen 2020; Nøst, Holden et al. 2021). The high-dimensional data constitutes both the promise and the problem of transcriptomics, and Holsbø and Møllersen (2020) demonstrate how halving the number of samples doubles the mean difference in expression required to conclude that a gene is differentially expressed. This practically excludes any possibility of finding statistically significant differences in a gene-wise analysis of 9000 probes, and any probes with large expression values would most likely not have been representative of the population, even if statistically significant (ibid.).

Whereas the low number of blood samples in Paper III led to a choice of grouping them by either metastasis, histology or interval, the above studies found significant differences in

samples that were collected up to 2–3 years before diagnosis and were from metastatic cases. The methods in these studies compared case-control expression differences between strata of metastasized and non-metastasized cases. The decision to exclude borderline ovarian cancers precluded a similar approach in the present investigation. The sample size in Paper III was similar to a set of prediagnostic blood samples from women with endometrial cancer investigated by Gavrilyuk, Snapkov et al. (2018). Gavrilyuk et al. found no gene-wise differences between cases and controls, neither over the whole sampling interval nor in single years.

In a study of postdiagnostic blood samples from women with breast cancer, Olsen, Holden et al. (2021) found statistically significant expression differences in group sizes comparable to Paper III. The characteristics of samples with a sufficiently strong expression amplitude for small groups were: The sample was collected within one year after diagnosis, and the participant died during the eight-year follow-up of the study. Metastasis status was less important. Considering the high mortality of EOC, there was likely a considerable number of deaths among the study participants in Paper III. Survival was not included as a study variable because the samples were collected years before diagnosis, but might have been relevant if gene expression indicates a property of the participant as disease host, irrespective of tumor load.

The analytical approach in Paper III was similar to the approaches of Olsen, Lukic et al. (2020) and (Nøst, Holden et al. 2021). Some of the most common bioinformatics tools (global test, gene-wise linear models) were applied, and FDR q-values were used to assess statistical significance, except in targeted tests. For the global test, the decrease in p-values in the metastatic and serous subgroups were similar to the decrease Olsen, Lukic et al. (2020) observed from high and very high versus low physical activity in NOWAC. Whereas gene-wise linear models are well-suited for small experimental microarray assays, this method is not optimal for small observational studies. Holsbø (2019) describes issues and develops alternative approaches to small, noisy datasets. These methods focus on prediction of metastasis in samples collected the final 1–2 years before diagnosis, and were not relevant for the EOC dataset.

Based in part on sobering experiences from studies where leukocyte DNA methylation predicted risk of breast cancer, but associations disappeared as the number of samples increased from ~500 to >1600 case-control pairs (van Veldhoven, Polidoro et al. 2015; Bodelon, Ambatipudi et al. 2019), the main priority in Paper III was to avoid type I errors

(reporting group differences not present in the population). This came at the cost of risking type II errors (missing real group differences). One option is to accept a more relaxed FDR rate, for example 20%, but in Paper III the FDR was ~1. The method used to test gene sets from databases (ROAST) was conservative in that it tested differential expression in each gene set independently, rather than assessing whether some gene sets were enriched compared to others (Wu, Lim et al. 2010).

It is not certain that the analyses in Paper III would have yielded any significant expression differences if only the groups had been larger. A Chinese study (patients only) found no association between breast cancer subtypes and gene expression in peripheral blood mononuclear cells. Instead, by applying unsupervised clustering, they identified two types of immune response that separated the samples (Ming, Xie et al. 2019). They were able to validate their findings in NOWAC blood samples (previously used in Dumeaux, Fjukstad et al. (2017)). The unsupervised clustering in Paper III would have revealed any strong expression differences on a pair-by-pair basis, and was dependent on case status as a relevant grouping variable, but not cancer subtype.

The leukocyte proportions that were estimated based on the gene expression data in Paper III were opposite of what is observed at diagnosis (Prodromidou, Andreakos et al. 2017). The poor accuracy of the leukocyte estimates was discussed in section 5.1.4. However, it has been demonstrated that in women with germline BRCA1/2 mutations, high-grade serous EOC is associated with an increase in circulating CD8+ T cells after diagnosis (Lee, Botesteanu et al. 2019). These authors suggested that a high mutational burden in these tumors triggered a strong immune response. There were likely few women with germline mutations in the data of Paper III, but in principle, an increase in CD8+ T cells is in agreement with an immune response to serous ovarian cancer.

In lieu of statistically significant genes and gene sets, the genes indicated by p-value in Paper III were examined one by one through searches in literature databases and specialized databases for gene function analysis. The same approach was used to discuss the gene ontology enrichment analysis of the 100 probes with lowest p-values. Issues associated with manual investigation of gene lists have been described and discussed (Fjukstad, Standahl Olsen et al. 2015), and the suggested tools have been designed (Fjukstad 2018), but the data in the present investigation were not suited for use with these tools.

Paper III underlines that the analyses were explorative and descriptive. ‘Descriptive’ makes clear that while there is a need for markers, and while sampling for the NOWAC

Postgenome biobank was according to standards for biomarkers in cancer epidemiology, the analyses were not designed according to the rigorous standards for studies aimed at identifying biomarkers (García-Closas, Vermeulen et al. 2011). ‘Explorative’ means that the experiment was meant to generate, rather than test, hypotheses; however, the statistical tools were hypothesis testing. From a statistical perspective, a more appropriate way of conducting explorative transcriptomics analyses is to determine interesting effect sizes (log_2FC values) and select specific processes to be interrogated by gene sets before conducting the analyses (Holsbø and Møllersen 2020). After the work on Paper I and Paper II, histological subtype was perceived as an important grouping variable for the analyses in Paper III. The immune- and cancer-specific databases were considered the best available tools to discover differential expression in pathways.

6 Conclusion

In the present thesis, Paper I considered intrauterine and extrauterine serous, endometrioid and clear cell carcinomas. There was no epidemiological difference between intrauterine and extrauterine endometrioid and clear cell histologies. Based on risk profiles, ovarian ECC is more similar to uterine ECC than to other ovarian carcinomas. Ovarian and uterine serous carcinomas appear to have a different association with smoking, but this could be due to a misclassification of high-grade endometrioid carcinomas.

Paper II investigated the association between use of LNG-IUS and risk of ovarian, endometrial and breast cancer. Important strengths of this questionnaire-based were the ability to adjust for risk factors, and the possession of data on use prior to the implementation of the Prescription Registry. This allowed assessment of a possibly protective effect in women older than 50 years, in whom cancer risk increases rapidly. Ever use of LNG-IUS predicted a strongly reduced risk of endometrial cancer. The relative risk in ever users was estimated to 0.22, but a risk reduction of 0.13 – 0.40 is compatible with the presented data. The risk of ovarian cancer might be considerably reduced; the relative risk was estimated to 0.53, but a RR 0.32 – 0.88 is compatible with the presented data. The study population was not optimal for assessing the association with breast cancer, as many participants were former users of LNG-IUS.

The presented investigation did not find strong case-control differences in gene expression in peripheral blood in the years preceding ovarian cancer diagnosis. This could be because there is little association between ovarian cancer and prediagnostic gene expression in blood, but could also be due to a small sample size, or the analytic approach that was used.

The articles in this thesis fall under the EOC research areas of the origin, prevention and detection. The slow progress in early detection and treatment of EOC has led to special emphasis on preventive measures (The Lancet 2008; Long Roche, Abu-Rustum et al. 2017). Consistent with this, the clearest findings in this thesis were demonstrated in the area of prevention.

7 Further research

To study the etiology of less common EOC subtypes, precise estimates are necessary. The possibly misclassified high-grade endometrioid carcinomas could be reclassified, and the investigation in Paper I could be repeated using data from an ovarian cancer cohort consortium.

The analysis of LNG-IUS could be repeated with a revised strategy for selection of adjustment variables. As the putative effect of LNG-IUS on gynecologic cancer is long-lasting, updated risk estimates are also of interest. For breast cancer, follow-up from the time of initiation of use is important. This is achieved in registry studies. At present, a Norwegian registry study of LNG-IUS has not been published.

The main limitation to the study of blood gene expression in EOC appears to be the number of samples collected close to diagnosis. However, if tumor histology/topography is not the defining property of how the immune system sees cancer, constraining studies to one topographic location may not be necessary. To plan analyses by biological hypotheses, a framework for modeling of the immune system through gene expression that goes beyond testing of gene sets needs to be implemented. Frameworks developed by groups that study clinical and preclinical oncoimmunology (Hiam-Galvez, Allen et al. 2021) may be suitable.

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Paper I

Article

Supplementary table 1 and 2

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Does an epidemiological comparison support a common cellular lineage for similar subtypes of postmenopausal uterine and ovarian carcinoma? The Norwegian Women and Cancer Study

Mie Jareid, Idlir Licaj, Karina Standahl Olsen, Eiliv Lund and Hege M. Bøvelstad

Department of Community Medicine, UiT The Arctic University of Norway, Tromsø, Norway

Cancer Epidemiology

Uterine and ovarian carcinomas have the same major histological subtypes, but whether they originate from the same cell types is a matter of ongoing debate. Uterine and ovarian endometrioid and clear cell carcinoma (ECC) and uterine and ovarian serous carcinoma (SC) may originate in the same location, or share a common lineage of differentiation. Epidemiologically, a common cellular lineage should be reflected in similar risk associations, and we explored the similarity of uterine and ovarian ECC and uterine and ovarian SC. We included 146,316 postmenopausal participants from the Norwegian Women and Cancer Study. Exposure information was taken from self-administered questionnaires, and cancer cases were identified through linkage to the Cancer Registry of Norway. Hazard ratios with 95% confidence intervals for uterine and ovarian carcinoma and their subtypes were calculated using multivariable Cox regression models, and a Wald test was used to check for heterogeneity. During 1.6 million person-years, 1,006 uterine and 601 ovarian carcinomas were identified. Parity, total menstrual lifespan, body mass index and smoking were differentially associated with total uterine and total ovarian carcinoma ($p_{heterogeneity}$ = 0.041, 0.027, <0.001 and 0.001, respectively). The corresponding associations for uterine and ovarian ECC did not differ significantly ($p_{heterogeneity} > 0.05$). Smoking was differentially associated with uterine and ovarian SC ($p_{heterogeneity}$ = 0.021). Our epidemiological analyses do not contradict a common differentiation lineage for uterine and ovarian ECC. Uterine and ovarian SC are less likely to be of a common lineage of differentiation, based on their difference in risk associated with smoking.

Combined, uterine and ovarian carcinoma constitute 9.4% of cancer incidence and 7.8% of cancer mortality in women in developed countries.[1] Uterine and ovarian carcinomas have the same major histological subtypes: endometrioid, serous, clear cell and mucinous. Uterine carcinomas are often endometrioid and ovarian carcinomas are most commonly serous. Endometrioid carcinoma and clear cell carcinoma (ECC) resemble endometrial cell types, and serous carcinoma (SC) resembles cells covering the peritoneum and fallopian tubes.[2] While the uterus and fallopian tubes originate in the Müllerian duct, the ovaries are not of Müllerian origin, and the resemblance of ovarian carcinomas to these Müllerian tissues has been debated.[3–5] Research now suggests that ovarian ECC arises in endometriosis, whereas ovarian SC originates in the fallopian tube[6] or in the uterus.[7] Endometriosis and other Müllerian tissue remnants, known as the secondary Müllerian system, can potentially explain the different subtypes occurring in extrauterine locations without a relocation of cells taking place.[8] Additional hypotheses for the origin of ovarian cancers also exist.[3,9]

Shared protective factors of uterine and ovarian carcinoma include having children, using oral contraceptives (OCs) and lower age at menopause.[10,11] Adiposity increases the risk of uterine carcinoma[12] but is less associated with ovarian carcinoma.[13–15] Smoking is associated with lower risk of uterine carcinoma[16] but not with overall risk of ovarian carcinoma.[17–19] Several studies have compared risk estimates for

Key words: prospective cohort study, uterine neoplasms, ovarian neoplasms, histological subtypes, etiology

Abbreviations: BMI: body mass index; CI: confidence interval; ECC: endometrioid and clear cell carcinoma; EPIC: The European Prospective Investigation into Cancer and Nutrition; HR: hazard ratio; NOWAC: The Norwegian Women and Cancer Study; OC: oral contraceptive; phet: pheterogeneity; SC: serous carcinoma; TML: total menstrual lifespan

Additional Supporting Information may be found in the online version of this article.

Disclaimer: Some of the data in this article are from the Cancer Registry of Norway. The Cancer Registry of Norway is not responsible for the analysis or interpretation of the data presented.

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Correspondence to: Mie Jareid, Department of Community Medicine, UiT The Arctic University of Norway, Pb 6070 Langnes, NO-9037 Tromsø, Norway, Tel.: +47-7762-5209, E-mail: mie.jareid@uit.no

What's new?

Do uterine and ovarian cancers share a common lineage? Depends on the type, new results suggest. To investigate the cancers' cellular origins, these authors compared risk factors between uterine and ovarian endometrioid and clear cell tumors (ECC) and between uterine and ovarian serous carcinoma (SC). If the cancers originate in the same cell types, the authors reasoned, risk factors should pose the same danger for both locales. After evaluating various risk factors, including smoking, parity and obesity, they concluded that uterine and ovarian ECC appear to share a common lineage. However, smoking affects the risk of uterine and ovarian SC differently, suggesting they may arise separately.

subtypes of uterine or ovarian carcinoma to determine if they have different risk factors.[14,20–23] These authors argue that when different subtypes have different risk estimates, they likely have different etiologies.

Here we present hazard ratios for risk factors of uterine and ovarian ECC and SC, and compare the risk factors by location. The shared epidemiology of uterine and ovarian carcinoma has been discussed.[24] To our knowledge, however, no epidemiological studies directly compare the risk factors for uterine and ovarian tumors of the same subtype in one cohort. To further explore the similarities of these cancers, we combined ovarian ECC with uterine carcinomas and recalculated risk estimates.

Material and Methods

Study cohort

The Norwegian Women and Cancer (NOWAC) Study is a population-based prospective cohort.[25] Women born in 1927–1965 were selected at random from the Norwegian Population Registry. They were sent a letter that explained the study and a self-administered questionnaire. Those who returned a completed questionnaire were enrolled in the study. Recruitment took place in two waves: (1) 102,540 participants were enrolled in 1991–1997 (response rate 57%) and (2) 63,232 participants in 2003–2006 (response rate 48.4%).[25] Including delayed additions, the number of participants in this study was 172,478. Follow-up questionnaires were sent at intervals of 5–7 years. The external validity of the NOWAC Study is found to be acceptable.[26]

We excluded 6,823 participants with prevalent cancer, 2,749 who were premenopausal during follow-up, 16,480 with self-reported hysterectomy or oophorectomy at baseline or during follow-up, 70 who emigrated or died prior to inclusion and 40 with a negative total menstrual lifespan (TML; defined in the next section) value. The final study cohort included 146,316 postmenopausal women. Of these women, 77,412 (52.9%) had one or more follow-up questionnaires available. Information on age at menarche (and thus TML) was missing for 2,456 women (1.7%), 3,850 (2.6%) had missing information on body mass index (BMI) and 3,128 women (2.1%) had missing information on smoking status.

Study variables

We selected the established risk factors of endometrial and ovarian carcinoma subtypes.[16,27] All information except age at menopause and body weight was taken from the enrollment questionnaire. Follow-up information related to weight and age at menopause was used if available. Six continuous variables were used. These were (1) age at menarche, (2) age at menopause, (3) parity, (4) cumulative duration of breastfeeding, (5) cumulative duration of OC use and (6) BMI. We used self-reported height and weight[28] to calculate BMI (kg/m^2). In cases where weight at follow-up differed >50 kg from baseline, the lower weight was used. This applied to 18 women. Smoking status at baseline (never/ever) was included as a dichotomous variable.

Total menstrual lifespan was calculated by subtracting the following values from age at menopause: age at menarche, number of years of OC use, 9 months for each child and number of months of cumulative breastfeeding.[11] We studied TML as a continuous variable, and the resulting hazard ratio (HR) was inverted to produce an HR per 1-year decrease in TML. When age at menopause was missing, it was set to 50 years. This is the median age of menopause in the NOWAC Study, and time at risk started at self-reported age at menopause ($N = 59,927$), or from age 50 years ($N = 86,389$). If different ages at menopause were reported at baseline and follow-up, we used the highest reported age below 53 years. Emigration and death were determined through linkage to Statistics Norway and the Cause of Death Registry.

Pathology

Cancer cases were identified through linkage to the Cancer Registry of Norway. International Classification of Diseases, Revision 7 (ICD-7) codes were used for corpus uteri cancer (ICD-7 code 172) and cancer of the ovary including the fallopian tube (ICD-7 code 175). Tumor morphology and grade were coded according to the International Classification of Diseases for Oncology, Revision 2 and 3 (serous/papillary serous: 8441, 8450, 8460, 8461; endometrioid: 8380, 8382; clear cell: 8310). In our dataset, code 8382 occurred only in the uterus, and codes 8450 and 8461 only on the ovary. Cases with these histologies were, however, not excluded from the analyses. As our women were included in two different waves, and cases were diagnosed over a period of 22 years, we checked for changes in subtype fractions through the years of follow-up.

To increase statistical power, we studied ECC as one group. Nonepithelial tumors (5.8% of uterine and 4.1% of ovarian cancers) were excluded. Nonspecified adenocarcinomas,

Table 1. Selected characteristics of the 146,316 postmenopausal women in the study cohort, by cancer site and subtype, Norwegian Women and Cancer Study

		Uterus				Ovary			
	Cohort	All carcinomas	ECC	SC	Noncarcinomas	All carcinomas	ECC	SC	Noncarcinomas
N	146,316	1,006[2]	768[3]	52	62	601[4]	68[5]	386	26
Age at menarche (years), mean	13.3	13.2[1]	13.1	13.1	13.1	13.3	13.3	13.3	12.9
Age at menopause (years), mean	49.6	50.0[1]	50.1	49.8	49.6	49.4	49.4	49.6	48.9
Number of children (among parous women), mean	2.2	2.1[1]	2.0	2.3	2.4	2.1	1.8	2.2	1.9
Ever breastfed (among all women) (%)	83.7	77.6[1]	77.9	86.5	77.4	81.7	75.0	81.3	76.9
Cumulative duration of breastfeeding (months), mean	13.7	11.5[1]	11.0	11.5	15.5	12.4[1]	12.2	12.3	9.2
Ever users of OC (%)	55.2	38.4[1]	39.2	34.6	45.2	45.1[1]	50.0	45.6	38.5
Cumulative duration of OC use among users (years), mean	5.2	3.6[1]	3.5	3.5	2.7	3.8[1]	4.4	3.6	2.8
BMI, mean	24.2	26.3[1]	26.4	25.4	24.3	24.3	24.6	24.2	24.3
Total menstrual lifespan (years), mean	30.7	33.0[1]	33.2	32.7	32.4	31.9[1]	31.7	32.1	32.8
Ever smokers (%)	64.4	55.2[1]	55.6	44.0	59.0	64.7	60.3	64.8	65.4

[1]Significantly different from the cohort ($p < 0.002$).
[2]Includes 186 cases that were not included in the subtype analysis (unspecified adenocarcinoma, squamous carcinoma and infrequent subtypes, including mucinous carcinoma).
[3]Includes 755 cases of endometrioid and 13 clear cell carcinomas.
[4]Includes 147 cases that were not included in the subtype analysis (unspecified adenocarcinoma, mucinous carcinoma and infrequent subtypes).
[5]Includes 39 cases of endometrioid and 29 clear cell carcinomas.
Abbreviations: BMI: body mass index; ECC: endometrioid/clear cell carcinoma; OC: oral contraceptive; SC: serous carcinoma; TML: total menstrual lifespan.

Table 2. Age-adjusted HRs with 95% CIs by cancer site of all carcinomas, ECC and SC among postmenopausal women, the Norwegian Women and Cancer Study

	Uterus				Ovary			
	All carcinomas	ECC	SC	All plus ovarian ECC	All carcinomas	ECC	SC	All minus ECC
N	1,006	768	52	1,136	601	68	386	533
Parity, per child	0.84 (0.79–0.88)	0.82 (0.78–0.87)	0.96 (0.77–1.18)	0.84 (0.80–0.88)	0.90 (0.84–0.96)	0.71 (0.57–0.87)	0.93 (0.86–1.01)	0.92 (0.86–0.99)
OC use per 1-year increase	0.92 (0.90–0.94)	0.92 (0.90–0.94)	0.93 (0.84–1.03)	0.92 (0.91–0.94)	0.94 (0.92–0.97)	0.97 (0.91–1.03)	0.94 (0.91–0.97)	0.94 (0.91–0.96)
TML per 1-year decrease	0.92 (0.90–0.93)	0.91 (0.89–0.93)	0.95 (0.89–1.01)	0.92 (0.91–0.93)	0.95 (0.93–0.97)	0.95 (0.90–1.01)	0.95 (0.93–0.97)	0.95 (0.93–0.97)
BMI per unit increase	1.09 (1.08–1.11)	1.10 (1.08–1.11)	1.05 (0.98–1.11)	1.09 (1.08–1.1)	1.01 (0.98–1.03)	1.04 (0.98–1.10)	1.00 (0.98–1.03)	1.00 (0.98–1.02)
Never smoker	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ever smoker	0.76 (0.67–0.86)	0.78 (0.67–0.90)	0.53 (0.30–0.92)	0.77 (0.68–0.86)	1.08 (0.91–1.28)	0.83 (0.51–1.36)	1.09 (0.88–1.35)	1.12 (0.93–1.34)

The table also shows the risk estimates of ovarian and uterine carcinoma with ovarian cases of ECC carcinoma regrouped with uterine carcinoma.
Abbreviations: BMI = body mass index; CI = confidence interval; ECC = endometrioid/clear cell carcinoma; HR = hazard ratio; OC = oral contraceptive; SC = serous carcinoma; TML = total menstrual lifespan.

mucinous carcinoma and other subtypes were included in analyses of total uterine and ovarian carcinoma. Because only 5% of the ovarian carcinomas had the fallopian tube as the location of origin, the hypothesis of a tubal origin of ovarian SC[6] was not investigated.

Statistical analysis

We used Cox proportional hazards regression[29] to calculate HRs with 95% confidence intervals (CIs) for the risk of uterine or ovarian carcinoma and their subtypes. Age was used as follow-up time with a left truncated start of follow-up, given as age at menopause or age at enrollment in the NOWAC Study, whichever was highest. Attained age was the age at event (ovarian or uterine cancer) or the age at censoring, defined as age at emigration, death, other incident cancer except basal cell skin carcinoma or the end of study (31 December 2013), whichever came first. In the subtype analyses, cases with subtypes other than those under study were censored at time of diagnosis.

We calculated age-adjusted and multivariable estimates with regard to parity, OC use, TML, BMI and smoking. Because TML included OC use and parity, we carried out two multivariable analyses: (1) one that included parity, OC use, BMI and smoking; (2) one that included TML, BMI and smoking. Women with missing information were excluded in the multivariable analyses. There is evidence which suggests that some risk associations are different between endometrioid and clear cell carcinoma.[30] Therefore, we calculated HRs for endometrioid carcinoma in a separate analysis. This was not done for clear cell carcinoma due to the limited number of cases. The proportional hazards assumption was checked using Schoenfeld residuals, and there was no evidence of deviation from proportionality.

Regrouping analysis and heterogeneity tests

The HRs of uterine and ovarian carcinoma and their subtypes were tested for heterogeneity by the Wald test.[31] If no significant differences were found between the HRs for the corresponding subtypes, we grouped ovarian cases of that subtype together with the corresponding uterine subtype. After completing this regrouping, we recalculated HRs for total uterine and ovarian carcinoma, and the heterogeneity test was repeated. All statistical tests were two sided with a 5% significance level. To avoid nondetection of differences between uterine and ovarian ECC and uterine and ovarian SC, no adjustment in multiple testing was adopted for the comparison between uterine and ovarian subtypes.

All analyses were done in RStudio (RStudio, Inc., Boston, MA, USA) running R package version 3.1.3.[32]

Ethics

The Regional Ethics Committee, REK Nord, approved the NOWAC Study. Written information was provided to the participants. Return of a completed questionnaire was considered consent to participate. Registry linkages were done by

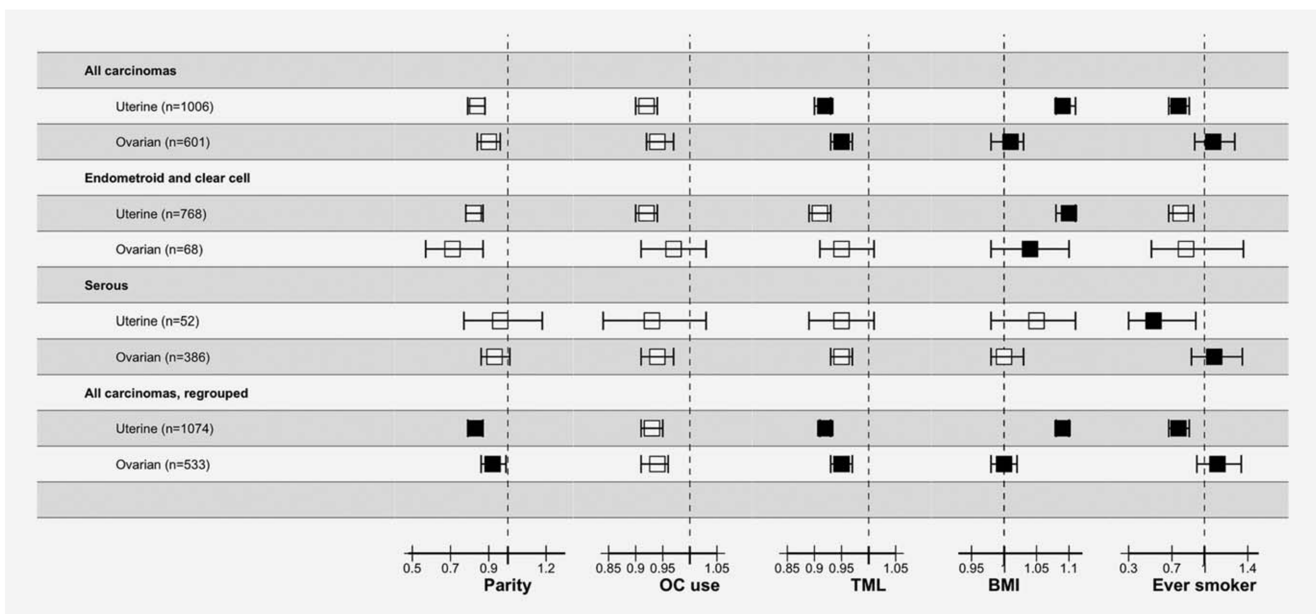

Figure 1. Forest plot of univariable HRs with 95% CI of all carcinomas, ECC and SC of the uterus and ovary in postmenopausal women in the Norwegian Women and Cancer Study. Where HRs of two corresponding histological subtypes are significantly different (pheterogeneity <0.05), the pairs are marked with black boxes. Abbreviations: BMI: body mass index; CI: confidence interval; ECC: endometrioid/clear cell carcinoma; HR: hazard ratio; OC: oral contraceptive; SC: serous carcinoma; TML: total menstrual lifespan.

Cancer Epidemiology

Statistics Norway, and participants' identities were concealed from researchers.

Results

Baseline characteristics

Mean age at inclusion was 52.8 years. During 1,629,317 person-years, and a median follow-up of 9.8 years, there were 1,006 cases of uterine carcinoma (mean age at diagnosis 61.7 years) and 601 cases of ovarian carcinoma (mean age at diagnosis 60.0 years). Uterine carcinoma cases differed significantly from the study cohort with regard to all variables studied, while ovarian carcinoma cases only differed with regard to duration of breastfeeding, OC use and TML (Table 1). The percentage of ECC and SC, and of high grade subtypes, fluctuated from year to year and gradually stabilized. Over time, there was a decrease in the proportion of high-grade endometrioid ovarian carcinoma. Additionally, there was an increase in the proportion of ovarian high-grade serous, uterine high-grade endometrioid and uterine high-grade serous carcinoma. More than 50% of the cases occurred after 2006.

Age-adjusted analysis

In the age-adjusted analysis (Table 2), parity, OC use and TML were negatively associated with both uterine and ovarian carcinoma, whereas BMI and smoking were associated only with uterine carcinoma. Total menstrual lifespan, BMI and smoking were differentially associated with uterine and ovarian carcinoma ($p_{\text{heterogeneity}} \leq 0.001$) (Fig. 1 and Supporting Information, Table 1). In the ECC subtype analysis, parity was the only variable significantly associated with both uterine and ovarian ECC, whereas only the association with BMI was significantly different at the two sites. When ovarian ECC was combined with uterine ECC, the HRs for parity were significantly different, in addition to TML, BMI and smoking. Smoking was differentially associated with the SC subtype (Fig. 1 and Supporting Information, Table 1).

Multivariable analyses

In the multivariable analyses (Table 3), the direction and magnitude of the HRs were similar to those in the age-adjusted analysis. Both multivariable analyses produced similar results with regard to BMI. The negative association between smoking and uterine SC (Table 3) attained borderline significance after adjustment for TML and BMI (not shown in table).

Parity, TML, BMI and smoking were differentially associated with total uterine and total ovarian carcinoma (p_{het} = 0.041, 0.027, <0.001 and 0.001, respectively) (Fig. 2 and Supporting Information, Table 2). In contrast to the age-adjusted comparisons, in the multivariable comparison BMI was no longer differentially associated with uterine and ovarian ECC (p_{het} = 0.056). Smoking was the only factor that was differentially associated with uterine and ovarian SC (p_{het} = 0.021) (Fig. 2 and Supporting Information, Table 2; results adjusted for TML and BMI are not shown).

In the analysis of endometrioid carcinoma (not combined with clear cell carcinomas), results for uterine endometrioid carcinoma were consistent with those for ECC. For ovarian endometrioid carcinoma, there were minor differences in risk estimates, and the CIs were wider than for ECC. For BMI, the HR for uterine endometrioid carcinoma was 1.10 (95% CI 1.08–1.11) and the HR for ovarian endometrioid

Table 3. Multivariable (age- and mutually adjusted) HRs with 95% CIs by cancer site of all carcinomas, ECC and SC among postmenopausal women, the Norwegian Women and Cancer Study

	Uterus				Ovary			
	All carcinomas	ECC	SC	All plus ovarian ECC	All carcinomas	ECC	SC	All minus ECC
N	950	729	48	1,017	571	67	363	504
Parity, per child	0.81 (0.77–0.85)	0.80 (0.76–0.85)	0.93 (0.74–1.15)	0.80 (0.76–0.85)	0.89 (0.83–0.95)	0.68 (0.55–0.84)	0.93 (0.85–1.01)	0.92 (0.85–0.99)
OC use, per 1-year increase	0.93 (0.91–0.95)	0.93 (0.91–0.95)	0.92 (0.82–1.03)	0.93 (0.92–0.95)	0.94 (0.92–0.97)	0.96 (0.91–1.03)	0.93 (0.90–0.97)	0.94 (0.91–0.96)
TML[1] per year decrease	0.93 (0.92–0.94)	0.92 (0.91–0.93)	0.95 (0.89–1.01)	0.93 (0.92–0.94)	0.95 (0.93–0.97)	0.95 (0.91–1.01)	0.95 (0.93–0.97)	0.95 (0.93–0.97)
BMI per unit increase	1.09 (1.08–1.10)	1.10 (1.08–1.11)	1.04 (0.98–1.11)	1.09 (1.08–1.10)	1.00 (0.98–1.03)	1.04 (0.98–1.10)	1.00 (0.97–1.02)	1.00 (0.98–1.02)
Never smoker	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ever smoker	0.77 (0.68–0.88)	0.80 (0.69–0.93)	0.55 (0.31–0.97)	0.78 (0.69–0.88)	1.12 (0.94–1.33)	0.87 (0.53–1.42)	1.13 (0.91–1.40)	1.16 (0.96–1.39)

The table also shows the risk estimates of ovarian and uterine carcinoma with ovarian cases of ECC carcinoma regrouped with uterine carcinoma.
[1]TML estimate was adjusted for BMI and smoking. Parity, OC use, BMI and smoking were mutually adjusted.
Abbreviations: BMI: body mass index; CI: confidence interval; ECC: endometrioid/clear cell carcinoma; HR: hazard ratio; OC: oral contraceptive; SC: serous carcinoma; TML: total menstrual lifespan.

carcinoma was 1.01 (95% CI 0.93–1.10). The heterogeneity test attained borderline significance ($p_{\text{het}} = 0.048$).

Regrouping analysis and heterogeneity tests

When ovarian ECC were grouped together with uterine ECC, parity, BMI and smoking remained significantly different (Fig. 2 and Supporting Information, Table 2). For TML, before regrouping the uterine carcinoma HR was 0.93 (95% CI 0.92–0.94) and ovarian carcinoma HR was 0.95 (95% CI 0.93–0.97), with $p_{\text{het}} = 0.027$. With ECC regrouped, the HRs and 95% CIs for TML remained unchanged while the heterogeneity test attained borderline significance ($p_{\text{het}} = 0.051$).

Discussion

In this large prospective cohort study, the multivariable HRs of total carcinoma were significantly different with regard to the most important shared risk factors for uterine and ovarian carcinoma, parity and TML, but not OC use. The risk estimates of uterine and ovarian carcinomas were also significantly different with regard to BMI and smoking. We found no significant differences between the HRs of uterine and ovarian ECC. The HRs of uterine and ovarian SC were significantly different with regard to smoking. In the regrouping analysis, the age-adjusted HRs for parity were significantly different, which further differentiated uterine and ovarian carcinoma.

The percentage of ovarian endometrioid carcinoma (6.5%) was lower than that observed in other large studies, while the percentage of ovarian clear cell carcinoma (4.8%) was similar to other observations.[14,30] Our risk estimates for uterine carcinoma were similar to those from the European Prospective Investigation into Cancer and Nutrition (EPIC) study.[10,16,33] Consistent with the low number of uterine clear cell carcinomas relative to endometrioid, the HRs of uterine ECC in our study were more in agreement with those reported for endometrioid carcinoma in a recent meta-analysis.[21] Our results for uterine SC were similar to those from the meta-analysis with regard to smoking and OC use, but not parity and BMI. The meta-analysis includes case–control studies.[21]

For ovarian carcinoma and its subtypes, the variables we investigated influenced the risk in the same direction as was observed in studies from EPIC, with some differences in effect size.[14,34] The associations of ovarian ECC (of which 43% were clear cell) with parity and OC use were intermediate to those of endometrioid carcinoma and clear cell carcinoma reported in the EPIC study. For TML, our estimate was closer to the EPIC estimate for endometrioid ovarian carcinoma.[14] We observed a similar negative association between smoking and uterine SC as in the previously mentioned meta-analysis,[21] and our observations for total uterine carcinomas were similar to those for postmenopausal women in the EPIC study.[16] The nonassociation between smoking and invasive ovarian SC is known from larger studies.[17,19]

Overall, our results suggest that uterine and ovarian ECC are more similar than uterine and ovarian SC. The significant difference in the associations between smoking and SC

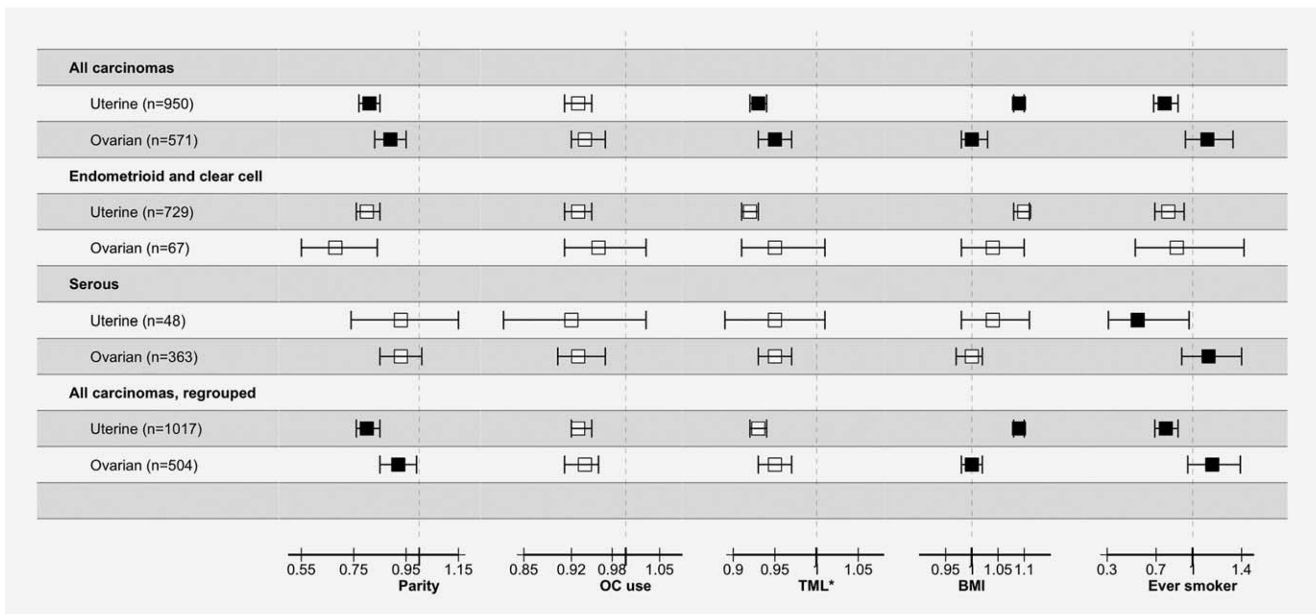

Figure 2. Forest plot of multivariable HRs with 95% CI of all carcinomas, ECC and SC of the uterus and ovary in postmenopausal women in the Norwegian Women and Cancer Study. Where HRs of two corresponding histological subtypes are significantly different (pheterogeneity <0.05) between HRs of corresponding histological subtypes, the pairs are marked with black boxes. *TML estimate was adjusted for BMI and smoking. Parity, OC use, BMI and smoking were mutually adjusted. Abbreviations: BMI: body mass index; CI: confidence interval; ECC: endometrioid/clear cell carcinoma; HR: hazard ratio; OC: oral contraceptive; SC: serous carcinoma; TML: total menstrual lifespan.

contradicts the hypothesis of Massuger *et al.*[7] They argued that precancerous cells could be transported from the uterus to the ovary. If this was the case, however, we would also expect smoking to be negatively associated with ovarian SC. Molecular evidence against a uterine origin of ovarian SC has already been presented.[35,36] In our data, cancers involving both fallopian tube and ovary were coded as ovarian, and we also included primary fallopian tube carcinoma in ovarian carcinoma. Thus, our epidemiological findings do not contradict a Müllerian lineage of ovarian SC, but the difference in risk factors speak against an intrauterine origin. Our findings do not contradict the hypothesis of a common cellular lineage for uterine and ovarian ECC.[6]

Based on the lack of risk differences, we grouped ovarian ECC together with uterine carcinomas and recalculated risk estimates, to further explore the potential similarities of these cancers. The 68 regrouped ovarian ECC comprised 11.3% (68/601) of the total ovarian carcinomas and 6.3% (68/1,074) of the group when combined with uterine carcinomas. For the age-adjusted estimates, the regrouping increased the number of significantly different risk factors between total ovarian and uterine carcinoma. However, with multivariable estimates, the heterogeneity test for TML attained borderline significance. This decrease in significantly different variables may be due to the reduction in number of cases, as the HR estimates for TML were unchanged after the regrouping. The results from both the initial and regrouped outcomes suggest that ovarian ECC has more in common with uterine ECC than with other ovarian carcinomas.

Strengths of our study include its prospective cohort design, national representativeness and near-complete follow-up of cancer diagnoses.[37,38] One weakness of registry information, however, is that if the cases were reevaluated today, a fraction of the histological subtypes would likely have been diagnosed differently.[39,40] Although more than half of our cases were diagnosed after 2006, at which time at least ovarian cancer diagnostics had improved,[40] this potential misclassification may have lowered the precision of our risk estimates, thus leading to wider confidence intervals and a failure to detect risk differences.

We used similar statistical methods to calculate risk of uterine and ovarian carcinoma, which ensured that any differences between risk estimates were not a result of differences in the exposure variables. However, we had small numbers of certain subtypes. To increase statistical power, we combined endometrioid and clear cell carcinoma into one category of ECC. Although both are thought to arise in endometriosis, they may arise through different molecular pathways,[41] and risk modifiers may act differently. However, in a recent study of ovarian carcinoma subtype etiology, which investigated many of the same risk factors that we included in this study, only age at menarche and smoking were differentially associated with endometrioid and clear cell carcinoma.[30]

We investigated shared risk modifiers for uterine and ovarian carcinoma (parity, OC use and TML), and BMI and smoking, which affect uterine carcinoma risk. These were well-established risk factors[16,27] for which we had complete information. We did not have information on endometriosis or tubal ligation.[42,43] Owing to a small number of cases of tubal carcinoma, we could not test the widely held hypothesis of a tubal origin of serous carcinoma.[6]

If most ovarian carcinomas are misclassified tumors of extraovarian origin, the misclassification bias in epidemiological studies of ovarian cancer is potentially very large.[6] Based on multivariable estimates, we found no significant differences between the risk estimates of uterine and ovarian ECC with regard to the most important shared risk factors. Our findings suggest that uterine and ovarian ECC have more in common than uterine and ovarian SC, and that ovarian ECC has more in common with uterine ECC than with other ovarian carcinomas. Regardless of cellular origin, however, the clinical parameters that guide the treatment of intrauterine and extrauterine carcinomas are different.[44] Our results contribute to the ongoing debate surrounding the lineages of differentiation for ovarian and uterine ECC and SC. More results are needed from genetic, molecular, histopathological and epidemiological studies to fully elucidate the origins of these cancers.

Competing Interests

We declare that we have no competing interests.

Author Contributions

EL and MJ designed the study and interpreted the results. MJ drafted the article. IL and KSO contributed with interpreting results and revising the article. EL is PI of NOWAC, conceived the study, oversaw the analyses and critically revised the article. HB designed and carried out the analyses, contributed to interpretation of results and drafting the manuscript. MJ had access to a partial dataset and HB to the full dataset. All authors read and approved the final manuscript.

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Supporting information

Jareid, Licaj et al. (2017) Does an epidemiological comparison support a common cellular lineage for similar subtypes of postmenopausal uterine and ovarian carcinoma?

Supplementary table 1 P-values for heterogeneity between age-adjusted HRs of all uterine and ovarian carcinomas, corresponding histological subtypes, and all carcinomas after regrouping.

Uterine vs ovarian	*Parity*	*OC use*	*TML*	*BMI*	*Ever smoker*
All carcinomas	0.091	0.211	0.001	<0.001	0.001
ECC	0.177	0.147	0.074	0.048	0.779
SC	0.811	0.903	0.995	0.209	0.018
All carcinomas, ECC regrouped	0.013	0.461	0.003	<0.001	0.001

Abbreviations: BMI=body mass index; ECC=endometrioid/clear cell carcinoma; OC=oral contraceptive; SC=serous carcinoma; TML=total menstrual lifespan.

Supplementary table 2 P-values for heterogeneity between multivariable-adjusted HRs of all uterine and ovarian carcinomas, corresponding histological subtypes, and all carcinomas after regrouping

Uterine vs. ovarian	*Parity*	*OC use*	*TML**	*BMI*	*Ever smoker*
All carcinomas	0.041	0.519	0.027	<0.001	0.001
ECC	0.149	0.294	0.189	0.056	0.760
SC	0.999	0.795	0.908	0.227	0.021
All carcinomas, ECC regrouped	0.003	0.844	0.051	<0.001	0.001

*TML estimate was adjusted for BMI and smoking. Parity, OC use, BMI and smoking were mutually adjusted.

Abbreviations: BMI=body mass index; ECC=endometrioid/clear cell carcinoma; OC=oral contraceptive; SC=serous carcinoma; TML=total menstrual lifespan.

Paper II

Article

Supplementary table 1

Gynecologic Oncology 149 (2018) 127–132

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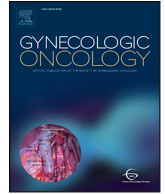
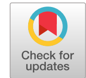


Levonorgestrel-releasing intrauterine system use is associated with a decreased risk of ovarian and endometrial cancer, without increased risk of breast cancer. Results from the NOWAC Study☆



Mie Jareid [a,*], Jean-Christophe Thalabard [b], Morten Aarflot [a], Hege M. Bøvelstad [a], Eiliv Lund [a], Tonje Braaten [a]

[a] Department of Community Medicine, Faculty of Health Sciences, UiT – The Arctic University of Norway, Tromsø, Norway
[b] Applied Mathematics Lab, UMR CNRS 8145, Paris Descartes University, USPC, Paris, France

HIGHLIGHTS

- We present a population-based prospective cohort study of LNG-IUS users.
- A mean of 4 years use was associated with 47% reduced ovarian cancer risk.
- Endometrial cancer risk was reduced by 78%.
- We found no association between LNG-IUS use and breast cancer.

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ABSTRACT

Objective. Women with ovarian cancer have poor survival rates, which have proven difficult to improve; therefore primary prevention is important. The levonorgestrel-releasing intrauterine system (LNG-IUS) prevents endometrial cancer, and recent studies suggested that it may also prevent ovarian cancer, but with a concurrent increased risk of breast cancer. We compared adjusted risks of ovarian, endometrial, and breast cancer in ever users and never users of LNG-IUS.

Methods. Our study cohort consisted of 104,318 women from the Norwegian Women and Cancer Study, 9144 of whom were ever users and 95,174 of whom were never users of LNG-IUS. Exposure information was taken from self-administered questionnaires, and cancer cases were identified through linkage to the Cancer Registry of Norway. Relative risks (RRs) with 95% confidence intervals (CIs) were estimated with Poisson regression using robust error estimates.

Results. Median age at inclusion was 52 years and mean follow-up time was 12.5 (standard deviation 3.7) years, for a total of 1,305,435 person-years. Among ever users of LNG-IUS there were 18 cases of epithelial ovarian cancer, 15 cases of endometrial cancer, and 297 cases of breast cancer. When ever users were compared to never users of LNG-IUS, the multivariable RR of ovarian, endometrial, and breast cancer was 0.53 (95% CI: 0.32, 0.88), 0.22 (0.13, 0.40), and 1.03 (0.91, 1.17), respectively.

Conclusion. In this population-based prospective cohort study, ever users of LNG-IUS had a strongly reduced risk of ovarian and endometrial cancer compared to never users, with no increased risk of breast cancer.

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Abbreviations: BMI, body mass index; CI, confidence interval; ICD, International Classification of Diseases; LNG-IUS, levonorgestrel-releasing intrauterine system; NOWAC, Norwegian Women and Cancer; OC, oral contraceptives; PY, person-years; RR, relative risk; SD, standard deviation; SIR, standardized incidence ratio.

☆ Disclaimer: Some of the data in this article are from the Cancer Registry of Norway. The Cancer Registry of Norway is not responsible for the analysis or interpretation of the data presented.

* Corresponding author at: Department of Community Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway, Pb 6070 Langnes, NO-9037 Tromsø, Norway.
E-mail addresses: mie.jareid@uit.no (M. Jareid), Jean-Christophe.Thalabard@parisdescartes.fr (J.-C. Thalabard), morten.aarflot@uit.no (M. Aarflot), hege.m.bovelstad@uit.no (H.M. Bøvelstad), eiliv.lund@uit.no (E. Lund), tonje.braaten@uit.no (T. Braaten).

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1. Introduction

In 2012, ovarian cancer caused an estimated 152,000 deaths worldwide [2]. The cumulative risk of ovarian cancer until age 75 is 1.3% in Norway and is similar in the United States [3,4]. The symptoms of ovarian cancer are vague, and there is no screening test. This has led to problems of late diagnosis and a 5-year survival of <50% [5]. Thus, ovarian cancer ranks eighth in cancer incidence, but fifth in cancer mortality among women [4]. Primary prevention therefore remains the best available measure against ovarian cancer [5].

Risk of ovarian cancer is reduced by 15–29% for every 5 years of oral contraceptive (OC) use, and globally, OC use prevents an estimated 30,000 cases of ovarian cancer each year [6]. Long-term OC use also reduces the risk of endometrial cancer, with 5–9 years of use reducing the risk by 34% [7]. However, OC use increases the risk of breast cancer by up to 38% with >10 years use, and for a minimum of 5 years after cessation [8,9], in addition to carrying other health risks. Prescribing OCs for ovarian cancer prevention to women who do not need contraception is not recommended [10].

The levonorgestrel-releasing intrauterine system (LNG-IUS) was introduced in Norway in 1994. In the Nordic countries, LNG-IUS is the second-most used form of contraception after OCs, and it is the most commonly used form of long-acting reversible contraception [11]. Recently, three Finnish studies have shown that, compared to the general population, LNG-IUS users have a standardized incidence ratio (SIR) of 0.59 for ovarian cancer and 0.46 for endometrial cancer [12,13], but also an increased risk of ductal and lobular breast cancer (SIR 1.20 and 1.33 respectively, increasing to SIR 1.37 and 1.73 with >5 years of use) [14]. However, these studies did not adjust for other hormonal risk factors.

Our study aim was to combine self-reported information on OC use and reproductive factors from the Norwegian Women and Cancer (NOWAC) Study, with registry-based follow-up of cancer cases to compare adjusted risks of ovarian, endometrial, and breast cancer in ever users and never users of LNG-IUS. We also included estimates of the reduction in the risk of endometrial cancer in this nationally representative cohort, given the well-known preventive effect of LNG-IUS use on this cancer [15].

2. Methods

2.1. Study cohort

The NOWAC Study is a population-based prospective cohort study designed to investigate the association between hormone use and hormone-dependent female cancers [16]. During 1991–2007, women born between 1927 and 1965 were randomly selected from the Norwegian Population Registry and were sent a questionnaire along with a letter that explained the study. Those who returned a completed questionnaire were enrolled. Statistics Norway replaced participants' names and personal identification numbers with serial numbers for use by researchers. Recruitment took place in two waves: 102,540 participants were enrolled in 1991–1997 (response rate 57%), and 63,232 participants in 2003–2006 (response rate 48.4%). The external validity of the NOWAC Study was found to be good [17]. Follow-up information has been collected up to two times after enrollment.

The NOWAC questionnaires targeted LNG-IUS use as from 1998 by the question: Have you ever used a hormone intrauterine device? A total of 145,320 women completed a questionnaire during 1998–2006, either at enrollment or as part of follow-up. From these, we excluded 33,182 that either did not answer the question on hormone intrauterine device or had a hysterectomy or oophorectomy; 4813 that either had prevalent cancer or died or emigrated before the start of follow-up; 2938 that indicated LNG-IUS use before the device was available in Norway, and seven for technical reasons. Thus the final study cohort consisted of 104,380 women, of which 9146 were ever users of LNG-IUS.

2.2. Exposure assessment

In addition to questions on LNG-IUS (ever use, duration of use, age at first use, current use), we identified eight exposure variables associated with ovarian, endometrial, or breast cancer [18], regardless of their association with LNG-IUS use: age at start of follow-up (41–76 years, in 4-year increments), body mass index at enrollment (BMI, <25 kg/m2, ≥25 kg/m2), physical activity level at enrollment (very low, low, intermediate, high, very high), maternal history of breast cancer (yes, no), age at menarche (<12, 12–14, ≥15), ever use of OCs (yes, no), parity (0, 1–2, 3–4, ≥5), and menopausal status at start of follow-up (pre, *peri*, post, unknown). Unknown menopausal status was given to those who used hormone replacement therapy, those who indicated that menses had stopped due to “medication, illness, exercise, or other” and to those who did not answer the question.

2.3. Outcomes

Primary cancers were identified through linkage to the Cancer Registry of Norway using the International Classification of Diseases, Revision 7 (ICD-7) codes. All citizens were identified by their personal identification number upon contact with health care providers, who are obliged to report all cancer cases to the Cancer Registry of Norway. Outcomes were defined as primary cancer of the ovary including the fallopian tube (ICD-7 code 175), cancer of the uterine corpus (ICD-7 code 172), and cancer of the breast (ICD-7 code 170). In order to restrict the analyses to epithelial ovarian cancer and endometrial cancer, non-carcinoma cancers of the ovary and uterine corpus were excluded from the analyses ($n = 62$). Deaths and emigrations were identified through the Cause of Death Registry and Statistics Norway. Follow-up ended on 31 December 2015.

2.4. Statistical analysis

We calculated person-years (PY) of follow-up from the date of entrance into, until the date of exit from the study. Exit date was defined as the date of cancer diagnosis, emigration from Norway, death, or end of follow-up, whichever occurred first. We used chi-squared tests of independence to compare the characteristics of ever users and never users of LNG-IUS, and to compare selected characteristics of those who did and did not answer the question on LNG-IUS use.

We calculated crude cancer incidence rates with 95% confidence intervals (CIs) assuming a Poisson distribution. Relative risks (RRs) and their 95% CIs were estimated with Poisson regression using a robust error estimate [1]. Adjusted RR models were built in a stepwise backward manner by removing nonsignificant covariates from the full model, with listwise deletion of participants with missing information. Model fit was assessed by testing the deviance versus its assumed chi-squared distribution. Statistical significance was defined as a test resulting in a p-value <0.05. We performed an additional analysis of the association between LNG-IUS use and endometrial cancer, stratified by ever OC use (yes, no), and did a Wald test of heterogeneity between the resulting RRs. We performed two additional analyses of the association between LNG-IUS use and breast cancer: one stratified by duration of use (≤5 and >5 years), and the other stratified by current and former users at the start of follow-up.

The analyses were performed in SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA).

2.5. Ethics

The Regional Ethics Committee, REK Nord, approved the NOWAC Study. Written information was provided to the participants, and return

of a completed questionnaire was considered as consent to participate. Data storage is in compliance with the rules of the Norwegian Data Inspectorate.

3. Results

Median age at inclusion was 52 years. Mean follow-up time was 12.5 (standard deviation [SD] 3.7) years for a total of 1,305,435 PY. Among all ovarian and uterine corpus cancers, 4% and 5%, respectively, were non-carcinoma cancers and were excluded. Of the women in the study cohort, 9144 (9%) reported LNG-IUS use during or prior to the data collection period (1998–2007). Among ever users of the LNG-IUS, 85% reported the duration of use. Median age at start of LNG-IUS use was 44 years, and median duration was 4 years, with 59% having used LNG-IUS for between 2 and 6 years. Compared to never users, ever users of LNG-IUS were younger at start of follow-up (Table 1).

The percentage of women that reported high or very high physical activity level was slightly higher among ever users of LNG-IUS (38% versus 30% of never users) (Table 1). Ever use of OCs was more common among ever users of LNG-IUS (71%) than never users (55%), and nulliparity was more common among never users of LNG-IUS (10% versus 3% among ever users). Menopausal status at start of follow-up was significantly different between the groups of LNG-IUS use, with 60% of never users reporting that they were postmenopausal, compared to 33% of ever users. Thirty percent ($n = 2753$) of ever users had unknown menopausal status, and of these, 85% were using LNG-IUS at the start of follow-up.

Participants who did not answer the question on LNG-IUS use ($n = 15{,}442$) differed significantly from the study cohort on all variables checked. Most notably there was a lower proportion of nulliparous women among non-responders (Supplementary Table S1).

Table 1
Characteristics of ever users ($N = 9144$) and never users ($N = 95{,}174$) of the levonorgestrel-releasing intrauterine system (LNG-IUS) in the Norwegian Women and Cancer Study, 1998–2015.

Characteristics		LNG-IUS				p-value*
		Ever users		Never users		
Age at start of follow-up (years)	41–45	1271	14%	11,177	12%	<0.01
	46–50	3855	42%	21,581	23%	
	51–55	3051	33%	30,526	32%	
	56–60	795	9%	18,589	20%	
	61–65	145	2%	7811	8%	
	66–70	20	<1%	3012	3%	
	71–76	7	<1%	2478	3%	
Body mass index (kg/m^2)	<25	5295	58%	54,133	57%	0.18
	≥25	3637	40%	38,306	40%	
	Missing	212		2735		
Physical activity level	Very low	248	3%	3506	4%	<0.01
	Low	1518	17%	18,200	19%	
	Intermediate	3479	38%	36,971	39%	
	High	2972	33%	23,871	25%	
	Very high	556	6%	4796	5%	
	Missing	371		7830		
Maternal history of breast cancer	Yes	478	5%	5032	5%	0.80
Age at menarche (years)	<12	811	9%	8428	9%	0.01
	12–14	6646	73%	67,897	71%	
	≥15	1543	17%	17,364	18%	
	Missing	144		1485		
Ever use of oral contraceptives	Yes	6476	71%	52,259	55%	<0.01
Parity	None	307	3%	9231	10%	<0.01
	1–2	5502	60%	49,935	52%	
	3–4	3173	35%	32,762	34%	
	≥5	162	2%	3246	3%	
Menopausal status at enrollment	Pre	2125	23%	24,323	26%	<0.01
	Peri	1206	13%	8533	9%	
	Post	3060	33%	57,128	60%	
	Unknown	2753	30%	5190	5%	

* P-value from a chi-square test of independence, excluding missing value.

3.1. Levonorgestrel-releasing intrauterine system and cancer incidence

Table 2 displays cancer incidences and risk estimates. The crude incidence rate of ovarian cancer among never users of LNG-IUS was 38.1 (95% CI: 34.7, 41.8). The crude incidence rate of ovarian cancer among ever users of LNG-IUS was 16.7 per 100,000 PY (95% CI: 9.9, 26.4), with an age-adjusted RR of 0.49 (95% CI: 0.30, 0.82) for ever versus never users. The final model for ovarian cancer included three significant covariates: age at start of follow-up, ever use of OCs, and menopausal status at start of follow-up. Parity was not significant in the model building, but qualified as a confounder and was included in the model. Adjustment for these covariates hardly changed the risk estimates (multivariable-adjusted RR 0.53 (95% CI: 0.32, 0.88)).

The reported duration of LNG-IUS use varied from <1 year to 14 years, with the latter value corresponding to the time difference between the introduction of the LNG-IUS in 1994 in Norway and the date of the last questionnaire (2008). There were 18 cases of ovarian cancer among ever users of LNG-IUS; 14 of these cases occurred in women who had been using LNG-IUS for <7 years, and 3 in women who did not report duration of use. Due to the low number of cases, duration-response estimates were not calculated.

The largest risk reduction was observed for endometrial cancer, with a multivariable RR of 0.22 (95% CI: 0.13, 0.40) among ever users compared to never users of LNG-IUS. The final model for endometrial cancer adjusted for age at start of follow-up, BMI, physical activity level, OC use, parity, and menopausal status at start of follow-up. The stratified analysis showed that among ever users of OCs, ever users of LNG-IUS had a RR of endometrial cancer of 0.34 (95% CI: 0.18, 0.65) compared to never users of LNG-IUS. Among never users of OC, ever users of LNG-IUS had a RR of 0.08 (95% CI: 0.02, 0.34 compared to never users of LNG-IUS. However, these estimates were not significantly different ($p_{heterogeneity} = 0.18$).

For breast cancer, both the age-adjusted and the final adjusted model, which included age at start of follow-up, BMI, physical activity level, maternal history of breast cancer, OC use, and menopausal status at start of follow-up, showed no association with LNG-IUS use. The incidence rate of breast cancer was 275.7 per 100,000 PY among ever users of LNG-IUS and 281.6 per 100,000 PY among never users. The multivariable-adjusted RR of breast cancer among ever users of LNG-IUS was 1.03 (95% CI: 0.91, 1.17).

Compared to never users, current users of LNG-IUS had a multivariable RR of breast cancer of 0.97 (95% CI: 0.80, 1.19), and former users had a RR of 0.79 (95% CI: 0.64, 0.98). When stratified by duration of LNG-IUS use, ever users with <5 years of use had a multivariable RR of 1.06 (95% CI: 0.91, 1.24) compared to never users. Those with >5 years of use had a RR of 0.88 (95% CI: 0.68, 1.16). Among ever users of LNG-IUS with breast cancer, mean time since LNG-IUS cessation was 7.5 (SD 4.4) years ($n = 237$). For ever users of LNG-IUS not diagnosed with cancer, mean time since cessation of use was 12.5 (SD 3.3) years.

When all cancers were added together to produce an estimate of the total effect of LNG-IUS use, in ever users the RR of any hormone-related cancer was 0.86 (95% CI: 0.77, 0.97) compared to never users.

4. Discussion

In this population-based prospective cohort study, women who reported ever use of LNG-IUS showed a strongly reduced risk of both ovarian and endometrial cancer compared to those who did not. LNG-IUS use was not associated with an increased risk of breast cancer.

4.1. Levonorgestrel and risk of ovarian cancer

Several studies have investigated the association between the use of intrauterine devices and ovarian cancer, but most did not include LNG-IUS users, save one American, population-based, case-control study, which consisted of 104 cases and 299 controls. This study included 14

Table 2
Site-specific cancer incidence rates and relative risks comparing ever users (person-years [PY] = 107,701) and never users (PY = 1,197,734) of the levonorgestrel-releasing intrauterine system (LNG-IUS) in the Norwegian Women and Cancer Study.

Cancer type	LNG-IUS user status	Cancer cases	Incidence rate per 100,000 PY (95% CI)	Age-adjusted RR (95% CI)	Multivariable-adjusted RR (95% CI)
Epithelial ovarian	Ever	18	16.7 (9.9, 26.4)	0.49 (0.30, 0.82)	0.53 (0.32, 0.88)[a]
	Never	457	38.1 (34.7, 41.8)		
Endometrial	Ever	15	13.9 (7.8, 23.0)	0.19 (0.11, 0.40)	0.22 (0.13, 0.40)[b]
	Never	839	70.0 (65.4, 74.9)		
Breast	Ever	297	275.7 (245.3, 309.0)	1.02 (0.90, 1.15)	1.03 (0.91, 1.17)[c]
	Never	3373	281.6 (272.2, 291.3)		
Combined (ovarian, breast, endometrial)	Ever	330	306.4 (274.2, 341.3)	0.84 (0.74, 0.94)	0.86 (0.77, 0.97)[d]
	Never	4669	389.7 (378.7, 401.2)		

RR = relative risk; CI = confidence interval; BMI = body mass index; OC = oral contraceptive.
[a] Adjusted for OC use, age at start of follow-up, menopausal status at start of follow-up, parity.
[b] Adjusted for OC use, age at start of follow-up, menopause status at start of follow-up, BMI, physical activity, parity.
[c] Adjusted for OC use, age at start of follow-up, maternal history of breast cancer, BMI, physical activity, menopause status at start of follow-up.
[d] Adjusted for OC use, age at start of follow-up, maternal history of breast cancer, BMI, physical activity, menopause status at start of follow-up, parity.

LNG-IUS users, and found a negative association between ever use of intrauterine device and ovarian cancer. When analyzed by duration, only 4 or fewer years of use was protective [19]. A Chinese prospective cohort study that may have included LNG-IUS users found no association [20].

Two prospective cohort studies by Soini et al. described the association between LNG-IUS use and ovarian cancer [12,13]. The most recent study [12] was based on 77 ovarian cancer cases occurring in a cohort of 93,843 women who had been prescribed LNG-IUS for menorrhagia. The study did not adjust for risk factors. When the entire follow-up period was taken into account, the age-adjusted SIR of ovarian cancer among women with one or more LNG-IUS purchases was 0.59 (95% CI: 0.47, 0.73). The SIR of histological subtypes was 0.49 (95% CI: 0.24, 0.87) for mucinous, 0.55 (95% CI: 0.28, 0.98) for endometrioid, and 0.75 (95% CI: 0.55, 0.99) for serous ovarian carcinoma. After adjusting for important risk factors, our findings confirm those of Soini et al., and although our sample size did not permit analyses on histological subtypes, our adjusted results strengthen the evidence of a causal association between LNG-IUS and decreased risk of ovarian cancer.

It is generally assumed that combined OCs prevent ovarian cancer by inhibiting ovulation [21] and possibly by reducing menstrual bleeding [22]. Sparse menstruations lead to less retrograde menstruation, which, by implanting as endometriosis, is thought to be a source of either endometrioid carcinoma, clear cell carcinoma, or possibly low-grade serous carcinoma [23]. By other mechanisms, retrograde menstruation and follicular fluid released during ovulation may induce serous tubal intraepithelial carcinoma [22], which potentially could enter the ruptured ovarian epithelium and, stimulated by the hormone-rich milieu of the ovary, cause high-grade serous carcinoma [24].

Levonorgestrel is a potent progestin. LNG-IUS used in Norway at the time questionnaires were completed initially release 20 μg LNG per day, decreasing to 11 μg/day for an average of 14 μg/day over a five-year period [25]. LNG-IUS exerts its contraceptive effect by suppressing the endometrium, thickening the cervical mucus, and, partly, by inhibiting ovulation through the hypothalamic-pituitary axis [26]. Most LNG-IUS users have light menstruations and 20–50% become amenorrheic [27]. In the present study, 30% of LNG-IUS users had unknown menopausal status, compared to 5% of non-users. In an ultrasound study of 22 women, of which one-third were amenorrheic after 7 or more years of LNG-IUS use, approximately 30% of amenorrheic women and 60% of still menstruating women had ovulatory cycles with follicular rupture [26].

Risch [28] argued that, since the protective effect of progestin-only contraceptives, which do not completely suppress ovulation, is comparable to the effect of combined OCs on ovarian cancer, progestogens likely have a direct anti-tumorigenic effect on ovarian cancer. Such a concept was supported by Merritt et al. [29] notably with regard to high natural progesterone levels during pregnancy, though the effects of natural progesterone and those of synthetic progestins are not superimposable. The LNG-IUS alleviates symptoms of endometriosis, and Lockhat et al. [30] showed that in addition to the vascular delivery of levonorgestrel to endometriotic implants, direct contact with levonorgestrel in peritoneal fluid (transferred to this fluid via blood, not by diffusion from the uterine cavity) likely plays a significant role. A similar direct effect on ovarian tumors or tumor precursor cells is also possible [31]. This hypothesis, however, does not correspond with a Danish population-based case-control study [21] nor with a previous study from the NOWAC cohort [32], both of which found that only use of combined OCs, not oral progestogens alone, prevents ovarian cancer. Faber et al. [21] concluded that OCs prevent ovarian cancer through the inhibition of ovulation. It is plausible that the preventive effect of LNG-IUS on ovarian cancer works through partial inhibition of both ovulation and menstruation.

4.2. Levonorgestrel and risk of endometrial cancer

Our adjusted results also confirm the observations of Soini et al. [13] for endometrial cancer. That study adjusted for smoking, diet and alcohol consumption, socioeconomic status, and physical activity, and reported a SIR of endometrial cancer of 0.46 (95% CI: 0.33, 0.64) in LNG-IUS users compared to the general population. In a pooled analysis of four cohort and 14 case-control studies, Felix et al. [33] calculated the association between use of different intrauterine devices and the risk of endometrial cancer and found no association with LNG-IUS. However, due to the low number of women in the LNG-IUS exposure group, they disregarded this result and called for further studies.

The anti-proliferative effect of LNG-IUS is superior to that of oral progestins in the treatment of endometrial hyperplasia [15], and a protective effect of this device on endometrial cancer in the general population is to be expected. Our results indicate the size of the risk reduction in a cohort representative of the general population. Since the proportion of ever users of OCs was significantly different among ever and never users of LNG-IUS, we performed an analysis stratified by ever OC use. The difference was non-significant, but suggestive of a stronger protective effect of LNG-IUS among never users of OCs.

4.3. Levonorgestrel and risk of breast cancer

Contrary to Soini et al. [14], we did not observe an increased risk of breast cancer among LNG-IUS users. Soini et al. [14] reported a clear increased risk of certain types of breast cancer, but did not present SIRs of total breast cancer. In the earlier study by Soini et al. [13], the SIR of total breast cancer was 1.19 (95% CI: 1.13, 1.25). In all three studies by Soini et al. [12–14] follow-up ended at age 55 years. The discrepancy between our findings and those of Soini et al. [14] could be due to their lack of adjustment, although adjustment had little effect on our estimates.

In a recent nested case-control study of women in the Norwegian breast cancer screening program (aged 50–69 years), Ellingjord-Dale et al. [34] did not find an association between duration of IUD use and overall risk of breast cancer by duration of use (in intervals), although there was a statistically significant trend. The results indicated increased and decreased risks of different breast cancer subtypes. This study did not differentiate between types of intrauterine devices, but assuming a population-representative sample and data collected from 2006 onwards, LNG-IUS users constituted a large fraction of intrauterine device users [11]. When we stratified analyses by duration of use (<5 and >5 years), we observed no association with breast cancer in either stratum. We did not study breast cancer subtypes, and we did not test for trend.

A recent prospective cohort study showed that current and recent users of LNG-IUS had an increased risk of breast cancer compared to never users of hormonal contraceptives (RR 1.21; 95% CI: 1.11, 1.33). This study included all women aged 15–49 in Denmark, and it adjusted for age, calendar year, education, polycystic ovary syndrome, endometriosis, parity, and family history of premenopausal cancer of the breast or ovary. Our null finding remained when we restricted the analyses to current users of LNG-IUS. However, our study had few participants aged younger than 46 years. Moreover, the mean duration of LNG-IUS use was 4 years, and average time since cessation of use was 7.5 years. When Mørch et al. stratified by duration of use and time since cessation, women in the corresponding category did not have increased and risk of breast cancer. Mørch et al. found that >5 years of use was associated with increased cancer risk, which lasted up to 10 years after cessation of use [9]. However our analysis stratified by duration of use did not reproduce this finding.

Among previous studies, a Finnish case-control study of 9537 breast cancer cases and 21,598 controls adjusted for age at menarche, smoking, alcohol use, BMI, and family history of breast cancer and found a positive association between ever use of LNG-IUS and breast cancer in postmenopausal women (aged 51–64 years), while for premenopausal women no association was observed [35]. The authors mentioned the possible presence of selection bias, as some practitioners, at least in Finland (this is also the case in Norway), have regarded the LNG-IUS as a preferable option for women with an increased risk of breast cancer.

4.4. Strengths and limitations

Strengths of this study include its prospective design, inclusion of lifestyle information, and a population-based study cohort with women who were likely using the LNG-IUS for both contraceptive and medical reasons. BMI and OCs were validated by test-retest in a subset of participants [16,36], and physical activity and menopausal status by measurements [37,38]. Parity was validated by Lund, Kumle [17]. The LNG-IUS variable has not been validated, nor has maternal history of breast cancer or age at menarche. Compared to non-responders, responders were at a disadvantage with regard to some risk factors for the cancers considered in this study (lower age at menarche and nulliparity), but also had favorable characteristics (proportion of ever OC users and maternal history of breast cancer). We included OC use as a dichotomous variable, as analyzing OC use by duration did not change the estimate of the main exposure. We did not adjust for time since OC use. Insufficient adjustment for this, and for use of other hormonal contraceptives, may have caused residual confounding in our estimates.

The use of cancer registry data ensured near complete follow-up of cancer cases. However, due to the strong protective effect of LNG-IUS, the study had a limited number of ovarian and endometrial cancer cases. We were not able to calculate specific rates by subtype, nor could we analyze duration effects on these cancer types.

The mean age at enrollment was lower among ever users of LNG-IUS than never users. The gynecological practice of removing or leaving LNG-IUS in place at the time of menopause, varies; nevertheless, even if left in place, its protective effect, if any, could be transitory, potentially delaying the "natural" appearance of ovarian cancer. We created a Lexis diagram of the distribution of ovarian cancer incidence in both ever users and never users of LNG-IUS, which showed a lower, but parallel incidence rate among all LNG-IUS users aged <65 years, and a decreased incidence rate among those aged >65 years, as compared to never users. However, among ever users of LNG-IUS, there was one case that occurred after age 64 years, which introduces uncertainty into the estimation.

This is one of the few epidemiological studies that presents data specifically on LNG-IUS use, with estimates generalizable to the general female population of Norway. However, we used self-reported exposure data, which introduces a risk of misclassification. Considering the prescription routines, it is likely that women were counselled by their physician and required to make a choice, and thus were aware of which type of intrauterine device they were using. Nevertheless, we excluded women who indicated using LNG-IUS before it was on the market.

5. Conclusion

This study shows that a relatively short period of LNG-IUS use is associated with an almost halved risk of ovarian cancer, while the risk of breast cancer remains unchanged. Our results are in agreement with existing data, and show a negative association in a cohort in which the majority of women were older than in previous studies. Although these findings suggest that, in addition to OCs, LNG-IUS should be considered for inclusion in the prevention strategy against ovarian cancer for normal-risk women [39], an updated meta-analysis of the effect of LNG-IUS on breast cancer is needed before firm conclusions can be drawn.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2018.02.006.

Author contributions

EL and HMB conceived the study. MJ contributed to designing the analyses, interpreted results and drafted the paper. EL and JCT oversaw the analyses, interpreted results and critically revised the paper. TB designed the analyses, carried out analyses, and interpreted the results. HMB carried out preliminary analyses, and MAA carried out final analyses. EL is the PI of the NOWAC Study. All authors read and approved the final manuscript.

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While employed at UiT, Nicolle Mode contributed to the design of, and scripts for, statistical analysis, as well as contributing text to this paper. The authors are supported by the Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway. JCT has a full-time position at the Medical Faculty, Paris Descartes University, and is the beneficiary of a part-time position at UiT. The funding bodies had no role in the design, collection, analysis, or interpretation of data; in the writing of the manuscript, or the decision to submit the manuscript for publication. We sincerely thank the women who participate in the NOWAC Study.

Conflict of interest statement

We declare that we have no conflicting interests.

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Supporting information

Jareid, Thalabard et al. (2018) Levonorgestrel-releasing intrauterine system use is associated with a decreased risk of ovarian and endometrial cancer, without increased risk of breast cancer.

Supplementary table 1 Selected characteristics of responders and non-responders to the question 'Have you ever used a hormone intrauterine device (IUD)?' in the Norwegian Women and Cancer Study, 1998-2015

Characteristics		Have you ever used a hormone IUD?				
		Responders 104 380		Non-responders 15 442		p-value*
Maternal history of breast cancer	Yes	5515	5 %	940	6 %	<.001
Age at menarche (years)	<12	9246	9 %	1318	8 %	<.001
	12-14	74584	71 %	10767	70 %	
	≥15	18920	18 %	3030	20 %	
	missing	1630	2 %	327	2 %	
Ever use of oral contraceptives	Yes	58761	56 %	8366	54 %	<.001
Parity	None	9547	9 %	939	6 %	<.001
	1-2	55466	53 %	8546	55 %	
	3-4	35957	35%	5535	36 %	
	≥5	3410	3 %	422	3 %	

* P-value from a chi-square test of independence, excluding missing value

Paper III

Article

Supplementary figs 1-3

Supplementary tables 1-9

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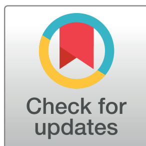

RESEARCH ARTICLE

The blood transcriptome prior to ovarian cancer diagnosis: A case-control study in the NOWAC postgenome cohort

Mie Jareid[1]*, Igor Snapkov[2], Marit Holden[3], Lill-Tove Rasmussen Busund[4], Eiliv Lund[1,5], Therese Haugdahl Nøst[1]

1 Faculty of Health Sciences, Department of Community Medicine, UiT – The Arctic University of Norway, Tromsø, Norway, **2** Department of Immunology, Oslo University Hospital Rikshospitalet and University of Oslo, Oslo, Norway, **3** Norwegian Computing Center, Oslo, Norway, **4** Faculty of Health Sciences, Department of Medical Biology, UiT – The Arctic University of Norway, Tromsø, Norway, **5** Cancer Registry of Norway, Oslo, Norway

* mie.jareid@uit.no

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Data Availability Statement: Data cannot be shared publicly because of national and institutional policies on research ethics and data security. Access to data requires approval from the Regional Committee for Medical and Health Research Ethics (REC North) and is conditional on adherence to the procedures of the Norwegian Women and Cancer Study and UiT – The Arctic University of Norway. Please contact the Norwegian Women and Cancer Study, att. Tonje Braaten <tonje.braaten@uit.no> or Arne Bastian Wiik <arne.b.wiik@uit.no>.

Abstract

Epithelial ovarian cancer (EOC) has a 5-year relative survival of 50%, partly because markers of early-stage disease are not available in current clinical diagnostics. The aim of the present study was to investigate whether EOC is associated with transcriptional profiles in blood collected up to 7 years before diagnosis. For this, we used RNA-stabilized whole blood, which contains circulating immune cells, from a sample of EOC cases from the population-based Norwegian Women and Cancer (NOWAC) postgenome cohort. We explored case-control differences in gene expression in all EOC (66 case-control pairs), as well as associations between gene expression and metastatic EOC (56 pairs), serous EOC (45 pairs, 44 of which were metastatic), and interval from blood sample collection to diagnosis ($\leq$3 or >3 years; 34 and 31 pairs, respectively). Lastly, we assessed differential expression of genes associated with EOC in published functional genomics studies that used blood samples collected from newly diagnosed women. After adjustment for multiple testing, this nested case-control study revealed no significant case-control differences in gene expression in all EOC (false discovery rate q>0.96). With the exception of a few probes, the $\log_2$ fold change values obtained in gene-wise linear models were below ±0.2. P-values were lowest in analyses of metastatic EOC (80% of which were serous EOC). No common transcriptional profile was indicated by interval to diagnosis; when comparing the 100 genes with the lowest p-values in gene-wise tests in samples collected $\leq$3 and >3 years before EOC diagnosis, no overlap in these genes was observed. Among 86 genes linked to ovarian cancer in previous publications, our data contained expression values for 42, and of these, tests of *LIME1*, *GPR162*, *STAB1*, and *SKAP1*, resulted in unadjusted p<0.05. Although limited by sample size, our findings indicated less variation in blood gene expression between women with similar tumor characteristics.

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Competing interests: The authors have declared that no competing interests exist.

Introduction

Epithelial ovarian cancer (EOC) is the eighth most common cancer among Norwegian women, who have a 1.3% risk of developing this cancer by the age of 75 years. Further, age-standardized rates show that EOC is the fifth most common cause of cancer death [1]. EOC is often diagnosed in late stages, with 70% of cases diagnosed with stage III or IV disease. This is partly because markers of early-stage disease are not available in current clinical diagnostics. The symptoms that could lead to EOC diagnosis tend to manifest only after metastasis has already occurred, at which point curative treatment is difficult to achieve. The most common EOC subtype, serous carcinoma, is associated with a particularly poor prognosis [2].

The origin and pathogenesis of EOC vary by subtype, and are still not completely understood. Models have suggested that serous tumors exist as *in-situ* or stage I or II invasive tumors for a median of 5.1 years (95% confidence interval [CI]: 3.2–8.1 years), and advancement to stage III or IV can occur up to 2 years (median 0.8, 95% CI: 0.4–1.9 years) before diagnosis [3].

Ovarian malignancies are associated with skewed proportions of circulating immune cell types, and immunologic studies suggest induction of tumor tolerance through local, and potentially also systemic, immunosuppression mechanisms [4]. Functional genomic studies of circulating immune cells collected at EOC diagnosis have identified markers of risk, presence of tumor in patients, or prognosis [5–13]. However, few have investigated the blood transcriptome [8,10–13].

Whereas blood collected postdiagnostically reflects clinical cancer, random sampling of the general population allows researchers to study persons at different prediagnostic stages of tumorigenesis [14]. The aim of the present study was to investigate whether EOC is associated with transcriptional profiles in blood collected up to 7 years before diagnosis. For this, we used RNA-stabilized whole blood, which contains circulating immune cells, from a sample of EOC cases from the population-based Norwegian Women and Cancer (NOWAC) postgenome cohort. We explored case-control differences in gene expression in all EOC, as well as associations between gene expression and metastatic EOC, serous EOC, and interval from blood sample collection to diagnosis ($\leq$3 or $>$3 years). Lastly, we assessed differential expression of genes associated with EOC in published functional genomics studies that used blood samples collected from newly diagnosed women.

Materials and methods

Study population and sample collection

The present case-control study was nested within the NOWAC postgenome cohort, a subcohort of the NOWAC Study [15]. The NOWAC postgenome cohort is a population-based, prospective study initiated with the purpose of exploring associations between blood gene expression and cancer, with the inclusion of questionnaire information on a variety of exposures and lifestyle factors. Participants were recruited to the NOWAC Study by mail; those who consented to donate blood received a sampling kit with PAXgene blood collection tubes with RNA-preserving buffer (Preanalytix GmbH, Hembrechtikon, Switzerland). Participants then took this kit to a general practitioner's office, where the blood sample was collected. Between 2003 and 2006, blood samples from close to 50,000 women born between 1943 and 1957 were collected [16] and shipped to the study center, where they were stored at -80 degrees Celsius between 24 hours and 3 days after their collection.

Case ascertainment and assignment of matched controls. Epithelial ovarian cancer cases were identified through linkage to the Cancer Registry of Norway using the personal identification number assigned to all Norwegian citizens and permanent residents. Norwegian

health care providers are obligated to report all cancer cases to the registry, which ensures near complete national follow-up [17]. Participants of the NOWAC postgenome cohort with registered cancer of the ovary or fallopian tube (International Classification of Diseases revision 7, location 175) diagnosed between April 2004 and April 2011 (n = 95) were eligible for inclusion in this analysis. Tumors were then categorized as borderline, non-epithelial, EOC, and serous EOC; metastasis status was categorized as none, any, or unknown. Controls were matched to cases by birth year and blood sample storage time.

Questionnaire variables. On the day of blood sample collection, participants completed a two-page questionnaire concerning recent exposures. Information on variables known to be associated with EOC risk [18] and with gene expression in leukocytes was extracted from this questionnaire, and from NOWAC Study questionnaires: body mass index (BMI) [19], current smoking [20] including number of cigarettes smoked, parity [21], menopausal status [22], and current hormone replacement therapy (HRT) use [23]. We also included current oral contraceptive (OC) use, which modulates EOC risk and could influence gene expression.

Sample processing

Blood samples were processed at the Genomics Core Facility at the Norwegian University of Science and Technology according to the protocols of kit manufacturers. Samples from case-control pairs were processed together, blinded for case/control status. Total RNA was extracted from whole blood using the PAXgene Blood miRNA Kit (Qiagen GmbH, Hombrechtikon, Switzerland), and cRNA was prepared with the Illumina TotalPrep-96 RNA Amplification Kit (Ambion Inc., Austin, TX, USA). RNA quantity and purity were assessed using a NanoDrop ND 8000 spectrophotometer (ThermoFisher Scientific, Wilmington, DE, USA), and RNA integrity was assessed using Bioanalyzer capillary electrophoresis (Agilent Technologies, Palo Alto, CA, USA). cRNA was hybridized to Illumina HumanHT-12 v4 Expression BeadChip microarrays (Illumina, Inc. San Diego, CA, USA). Illumina GenomeStudio software was used to extract the raw data.

Preprocessing of microarray data. Background correction was performed using negative control probes (limma package, nec function) [24]. Probes reported by Illumina to be of poor quality, that were not annotated, that had a detection p-value <0.05, or that were present in less than 70% of the samples, were filtered out. Quantile normalization (lumi, LumiN function) [25] and $\log_2$ transformation (lumi, LumiT) was performed on the expression values. Finally, probes were mapped and annotated (lumi, nuID2RefSeqID and illuminaHumanv4.db). If multiple probes mapped to the same gene, all were kept in the dataset as duplicates/triplicates.

Statistical analysis

Preliminary quality control of laboratory measurements resulted in the exclusion of five case-control pairs; therefore 90 case-control pairs were included in the preprocessing of microarray data. After preprocessing, the dataset included 12,153 probes for 9,633 genes across 90 cases and 90 controls. We then further excluded cases with borderline tumors (20 pairs) and non-epithelial tumors (4 pairs), leaving 66 case-control pairs in the final dataset. We assessed case-control differences in gene expression in all EOC (66 pairs), as well as associations between gene expression and metastatic EOC (56 pairs), serous EOC (45 pairs, 44 of which were metastatic), and interval from blood sample collection to diagnosis ($\leq$3 years and $>$3 years, 34 pairs and 31 pairs, respectively). Exclusions and analytical samples are shown in Fig 1. To protect the identity of participants, date of diagnosis was generalized to the month of diagnosis. This resulted in negative follow-up time for one case, and exclusion of this case-control pair from the analysis of blood samples collected $\leq$3 years before diagnosis. The questionnaire variables

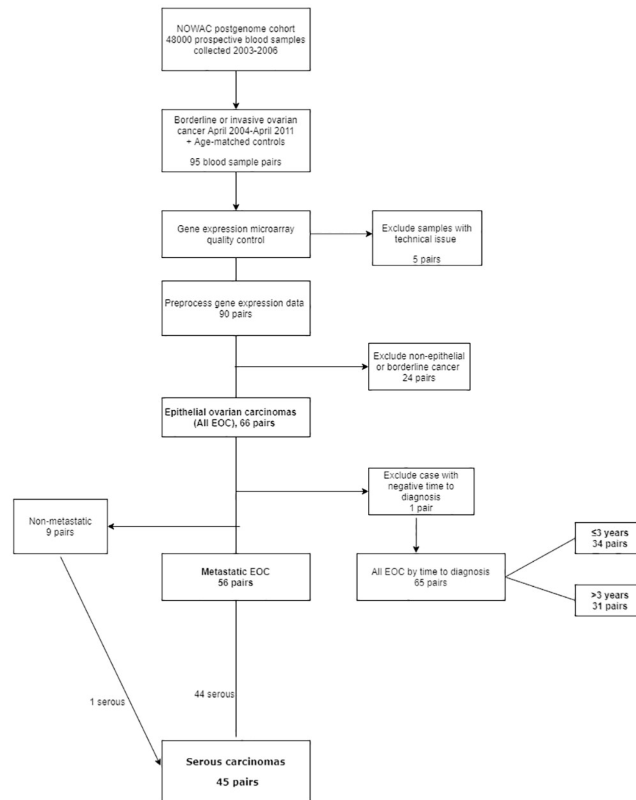

Fig 1. Flow chart of exclusions and analytic groups in gene expression analyses. Bold text indicates analyzed groups. The group "All EOC by time to diagnosis" was used in the analysis of all EOC adjusted for leukocyte populations.

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BMI (< median 25.8, ≥25.8), current smoking (yes/no), parity (0, 1–2, 3–4, ≥5), menopausal status (pre- or perimenopausal, postmenopausal), current HRT use (yes/no), current OC use (yes/no) were evaluated as potential confounders by testing their association with case status by two-sided t-tests or chi-square tests. Further, to facilitate the evaluation of confounding by differences in leukocyte populations between cases and controls, we estimated leukocyte fractions in individual samples based on gene expression values, using the cell deconvolution procedure Cibersort and the LM22 signature matrix [26]. Variables that were associated with both case/control status ($p<0.1$) and gene expression (global test [see below], family-wise error-rate adjusted $p<0.05$) were adjusted for in the analyses.

Assessment of case-control differences in gene expression

To explore case-control differences in gene expression in all EOC, we computed a dissimilarity matrix with Euclidean distances and applied hierarchal clustering using Ward's method to create a dendrogram. Further, we made a multidimensional scaling plot to display distances between samples. We used the global test [27] to assess case/control sample status as a function of difference in overall gene expression in all EOC, metastatic EOC, and serous EOC. Using linear models in the limma package [24], we assessed differences in expression of single genes ($\log_2$ fold change [FC] values) between cases and matched controls in all EOC, metastatic EOC, serous EOC, and EOC cases diagnosed ≤3 years and >3 years after blood sample collection.

We used the global test [27] to evaluate associations between potential confounding variables and gene expression overall, and created an adjusted gene-wise model of all EOC. To explore expression differences in sets of genes, we used the mroast method (using 10^5 rotations) [28] to test gene sets from the C2, C5, C7 [29,30] and KEGG [31] collections in the Broad Institute databases [32].

Genes were considered differentially expressed if the false discovery rate (FDR)-adjusted p-value (q value) was <0.05. We present non-FDR-adjusted p-values in the tables and text. The open source softwares R [3.1.2 and 3.2.1] (Vienna, Austria; www.r-project.org) and Bioconductor (bioconductor.org) were used for the analyses, with the exception of the chi-square test [33].

Gene Ontology enrichment. To explore the biological functions of the genes indicated according to case-control differences in expression, we used the R package clusterProfiler v.3.12.0 [34], which assesses potential overrepresentation of Gene Ontology (GO) terms [35,36]. We assessed the 100 probes with the lowest p-values in the limma models without covariate adjustments.

Differential expression of genes identified in published functional genomics studies. We used the metastatic EOC group to test case-control differences in the expression of seven sets of 5–33 genes reported to be associated with EOC in published functional genomics studies that used blood samples collected from newly diagnosed women. Of these, two gene sets were identified in whole blood gene expression studies comparing patients given a poor or better prognosis according to tumor characteristics [10,11]. Five gene sets were identified in case-control studies of DNA methylation in circulating leukocytes. We tested for differential expression of genes adjacent to CpG sites where differential methylation was reported indicative of EOC case status [6,9], CpGs indicative of EOC predisposition [5]; CpGs where methylation mediates genetic risk of EOC [7], and a set of genes where expression levels was suggested to mediate genotype-associated risk of EOC [8]. We tested a total 86 genes using a two-sided t-test for each gene, and did not adjust the p-values for multiple testing.

Ethics

The Regional Committee for Medical and Health Research Ethics (REC North) approved the NOWAC Study, the storage of blood samples, and the gene expression analyses in the present study. The Norwegian Data Inspectorate approved the linkages to the Cancer Registry of Norway. Participants received written information about the study and their right to withdraw. Signing the informed consent form, or completing a written questionnaire and donating a blood sample, was regarded as informed consent for cohort enrollment.

Results

Participant characteristics

Mean age at blood sample collection among cases and controls was 56.5 years; mean age at EOC diagnosis among cases was 59.3 years. Cases and controls did not differ significantly with regard to questionnaire variables. Both cases and controls tended toward being overweight, with a mean BMI of approximately 27, and 23% were current smokers. Fewer cases than controls were nullipara, and more cases than controls had 3–4 children (32% vs 24%), but parity distribution did not differ overall (p = 0.78). In both groups, approximately 90% were post-menopausal, 20% were current HRT users, and there were no current OC users (Table 1).

Of the 66 women with EOC, 56 (85%) had any metastasis, nine had no metastasis, and one had unknown metastasis status (Table 2). EOC subtype distribution included endometrioid (6%), clear cell (6%), mucinous (4.5%), other/non-specified histologies (15%), and serous EOC

Table 1. Participant characteristics on day of blood sample collection.

Variable	Cases (n = 66)	Controls (n = 66)	P-value[a]
	Mean (SD) or frequency (%)		
Age (years)	56.5 (3.7)	56.5 (3.7)	-
Time to epithelial ovarian cancer diagnosis (years)	2.8	-	
Body mass index (kg/m^2)	26.8 (6.5)	27.0 (4.5)	0.81
Current smoker	15 (23%)	15 (23%)	-
Number of cigarettes yesterday	11.8 (7.9)	10.1 (8.1)	0.57
Number of cigarettes today	1.9	2.9	0.25
Parity			0.78[b]
0	6 (9%)	7 (11%)	
1–2	37 (56%)	40 (60%)	
3–4	21 (32%)	16 (24%)	
≥5	2 (3%)	3 (5%)	
Menopausal status			
Pre- or perimenopausal	6 (9%)	9 (14%)	-
Postmenopausal	59 (91%)	56 (86%)	0.44
Current hormone replacement therapy use (%)	13 (20%)	14 (21%)	0.47
Current oral contraceptive use[c]	0	0	

[a] p-values obtained from a two-sided t-test.

[b] p-value comparing the distribution of number of children among cases and controls was obtained from a chi-square test.

[c]38% missing values.

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(n = 45), which constituted 68% of all EOC (24 with blood sample collected ≤3 years and 21 collected >3 years before diagnosis) (Table 2). Among serous EOC, the percentage of high, low, and unknown grade was 69%, 4%, and 27%, respectively. Of the high-grade serous EOC, 40% and 60% had blood samples collected ≤3 years and >3 years before diagnosis, respectively. Among those with low- and unknown-grade serous EOC, the corresponding distributions were 50% and 50%, and 60% and 40%, respectively. Compared to controls, cases had larger estimated mean fractions of CD8+ T cells and plasma cells (p = 0.08 and p = 0.07, respectively), and smaller fractions of neutrophils, monocytes, and resting mast cells (p = 0.06, p = 0.08, and p = 0.02, respectively; S1 Table).

Table 2. Distribution of epithelial ovarian cancer (EOC) cases in analytical groups (bold text) of case-control differences in gene expression.

Interval from blood sample collection to diagnosis	≤3 years	>3 Years	Sum
All EOC	**35[a]**	**31**	**66**
Metastatic EOC	30	26	**56**
Non-metastatic EOC	5	5	10[b]
Serous EOC	24	21	**45**
Non-serous EOC	11	10	21

[a]One case was diagnosed same month as sample collection and this case-control pair was excluded in the single-gene linear models of samples collected ≤3 years before diagnosis.

[b]One case with unknown metastasis status was categorized as non-metastatic.

https://doi.org/10.1371/journal.pone.0256442.t002

Case-control differences in gene expression

Hierarchal clustering of all EOC cases and controls (S1 Fig) and multidimensional scaling of pairwise distances between case-control pairs (S2 Fig) showed no tendency toward clustering of samples by case/control status. The global tests of all EOC, metastatic EOC, and serous EOC resulted in p-values of 0.87, 0.72, and 0.67, respectively. The single-gene linear models did not identify any genes differentially expressed between cases and controls (FDR q-values ranged from 0.96–0.99; S2–S6 Tables). The lowest p-value was observed in metastatic EOC (*FBLN5*; $\log_2$FC = 0.07, p = 0.0002) (S3 Table). In all EOC, the lowest p-value was observed for the probe *ENSA* ($\log_2$FC 0.06, p = 0.01) (S2 Table). The gene set analyses did not indicate any differentially expressed set of genes (lowest unadjusted p-value = 0.001).

S2–S6 Tables list the 100 probes with lowest unadjusted p-values in single-gene linear models of all EOC and investigated subgroups (Fig 1). We observed 36 overlapping probes in all EOC, metastatic EOC, and serous EOC (Fig 2). However, when separated into groups of blood samples collected ≤3 years and >3 years before diagnosis, the lists of probes with the 100 lowest p-values did not overlap (Fig 2).

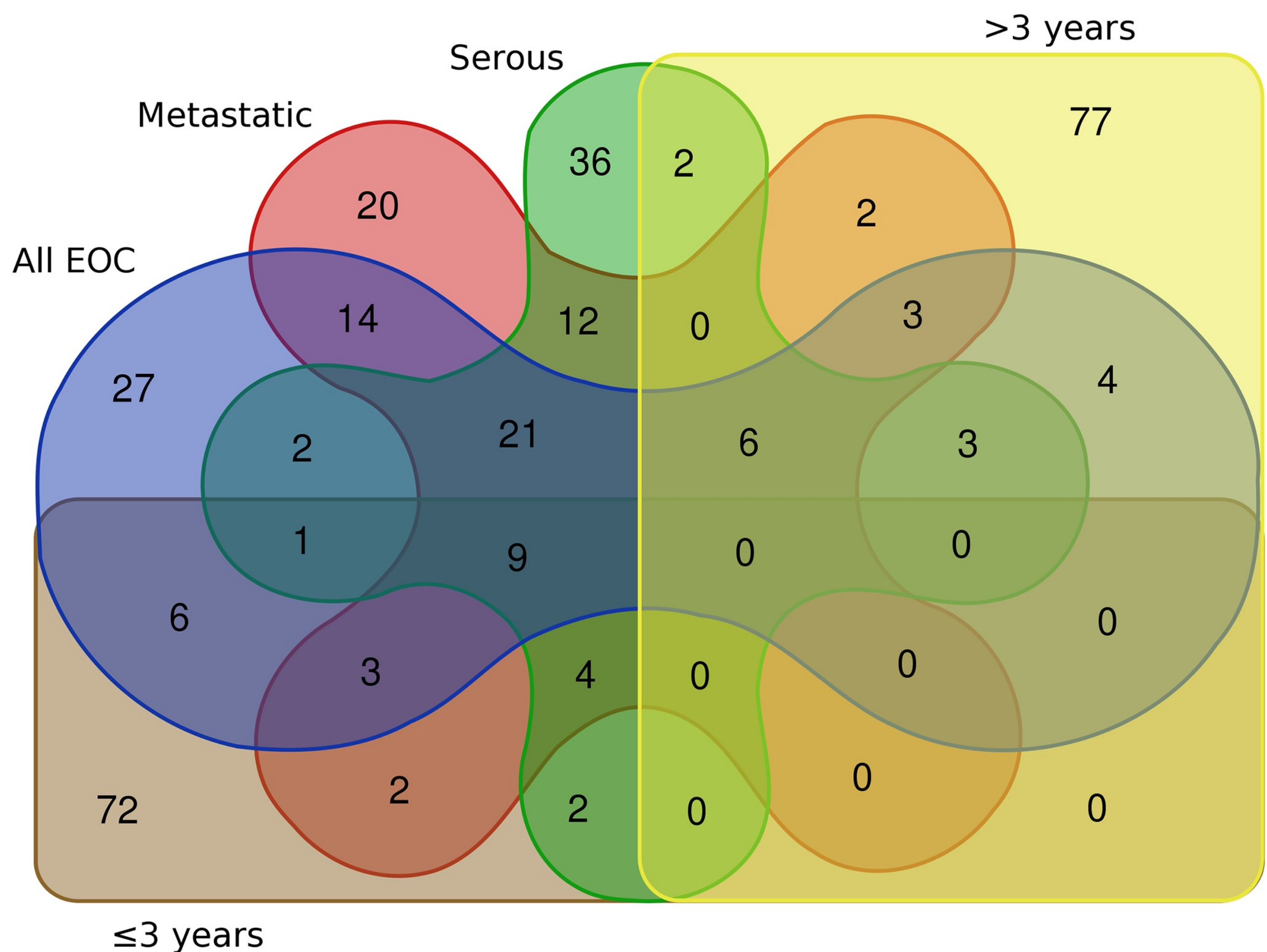

Fig 2. Overlap between the 100 probes with lowest p-values in single-gene linear models (case-control) of prospective blood samples from all cases of epithelial ovarian cancer (EOC; 66 pairs) and subgroups (metastatic at diagnosis (56 pairs), serous subtype (45 pairs), or interval to diagnosis (≤3 years or >3 years; 34 and 31 pairs, respectively)). The 100 probes are listed in S2–S6 Tables. (Created with BGE Venn diagram tool, Ghent University).

https://doi.org/10.1371/journal.pone.0256442.g002

Table 3. Probes with the 20 greatest absolute log_2FC values[a] among the 100[b] lowest p-values in single-gene linear models (case-control) of prospective blood samples from all cases of epithelial ovarian cancer (EOC) and subgroup analyses by clinicopathologic characteristics and interval to diagnosis.

All EOC N = 66	log_2FC	Metastatic EOC n = 56	log_2FC	Serous EOC n = 45	log_2FC	≤3 years n = 34	log_2FC	>3 years n = 31	log_2FC
Positive log_2FC									
GZMH	0.31	***LOC642161***	0.16	*BTN3A2*	*0.20*	*DEFA1B*	*0.64*	*LEF1*	*0.21*
SNHG5	0.25	*CD2*	0.15	***LOC642161***	*0.19*	*C21orf7*	*0.25*	*ETS1*	*0.20*
MIAT	0.15	*EEF1G*	0.13	*CD7*	*0.16*	*DEFA4*	*0.23*	*EEF1G*	*0.17*
LOC642161	0.14	*GIMAP5*	0.13	*GIMAP5*	*0.16*	***MIAT***	*0.20*	*GLO1*	*0.16*
CD8A	0.12	*CD3E*	0.13	***RPL8***	*0.13*	*MCOLN2*	*0.19*	*EEF1A1*	*0.14*
RPL8	0.11	***RPL8***	0.12	***LOC387882***	*0.10*	*ASCL2*	*0.16*	*EDG1*	*0.13*
LOC728855	0.11	***CD8A***	0.12	*KLHDC4*	*0.10*	*DGKQ*	*0.15*	*NUP88*	*0.12*
APOBEC3G	0.09	***APOBEC3G***	0.11	*TSEN54*	*0.09*	***LOC642161***	*0.15*	*C10orf32*	*0.11*
LOC387882	0.08	*CPT1B*	0.10	*HERC1*	*0.09*	*MT1X*	*0.13*	*CCT8*	*0.11*
RAB11FIP5	0.08	*NELF*	0.09	*SAMD3*	*0.09*	*MT1E*	*0.13*	*EXOSC8*	*0.11*
Negative log_2FC									
PPT1	-0.13	*RHOQ*	-0.15	***TMEM154***	-0.19	*C20orf111*	-0.14	*SCAP*	-0.17
NA (AL080095)	-0.13	***NA (AL080095)***	-0.15	***TAOK1***	-0.19	*LAT2*	-0.14	*TRPC4AP*	-0.18
MPPE1	-0.13	***FLJ22662***	-0.15	*FLJ22662*	-0.20	*TRIB1*	-0.15	*ELANE*	-0.19
TMEM154	-0.13	***MPPE1***	-0.17	*IGSF6*	-0.20	*OSBPL8*	-0.15	*ANXA11*	-0.19
TAOK1	-0.13	***TAOK1***	-0.18	*RYBP*	-0.21	*RRP7A*	-0.15	*VCL*	-0.19
USF1	-0.14	***TMEM154***	-0.18	***CD93***	-0.23	*CYBRD1*	-0.16	*TSPAN9*	-0.21
LAIR2	-0.16	*TMEM154-dupl*	-0.18	*FCGR3B*	-0.25	*CXCR5*	-0.17	***CD93***	-0.22
LAIR2-dupl	-0.17	***CD93***	-0.20	***KCTD12***	-0.25	*FLJ22662*	-0.18	***TAOK1***	-0.22
CD93	-0.17	*FCGR3B*	-0.21	*PI3*	-0.40	*SKAP2*	-0.18	***USF1***	-0.25
KCTD12	-0.20	***KCTD12***	-0.24	*LOC644936*	-0.53	***PPT1***	-0.21	*GP9*	-0.38

Bold text indicates probes that occurred among the 10 probes for all EOC as well as another group.

[a]Unadjusted p-values for the displayed probes ranged from 0.001 to 0.03, and were lowest in metastatic EOC.

[b]The 100 probes with lowest p-values are listed by p-value in S2–S6 Tables.

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Among the 100 probes with the lowest p-values, few log_2FC values exceeded ±0.2 (Table 3; S2 Fig shows the volcano plot for all EOC). The largest absolute log_2FC values observed were for *DEFA1B* (log_2FC = 0.64, p = 0.01) in blood samples collected ≤3 years before diagnosis, and *LOC644936* (log_2FC = -0.53, p = 0.02) in serous EOC. These probes did not occur among the 100 lowest p-values in any other group.

No questionnaire variables were significantly associated with case-control status (Table 1) or with gene expression overall (p>0.12). The estimated leukocyte fractions found to be associated with case-control status (neutrophils, CD8+ T cells, monocytes, resting mast cells, and plasma cells; S1 Table) were associated with gene expression overall (p = 0.02, 0.04, 1.75e-11, 3.00e-05, 5.00e-06, respectively). Therefore, the adjusted gene expression model included these five leukocyte types and no questionnaire variables. The lists of 100 probes with lowest p-values resulting from the unadjusted and adjusted models of all EOC (S2 and S7 Tables) overlapped by 29 probes.

Gene Ontology enrichment. S8 Table displays GO categories related to biological processes overrepresented among the 100 probes with the lowest p-values in all EOC, metastatic EOC, serous EOC, and in blood samples collected ≤3 years or >3 years before diagnosis. Fig 3 presents the GO categories with the lowest p-values in each group, as well as GO categories that overlapped between the groups. In all EOC, the main enriched categories were "execution phase of apoptosis" and "intrinsic apoptotic signaling pathway in response to oxidative stress"

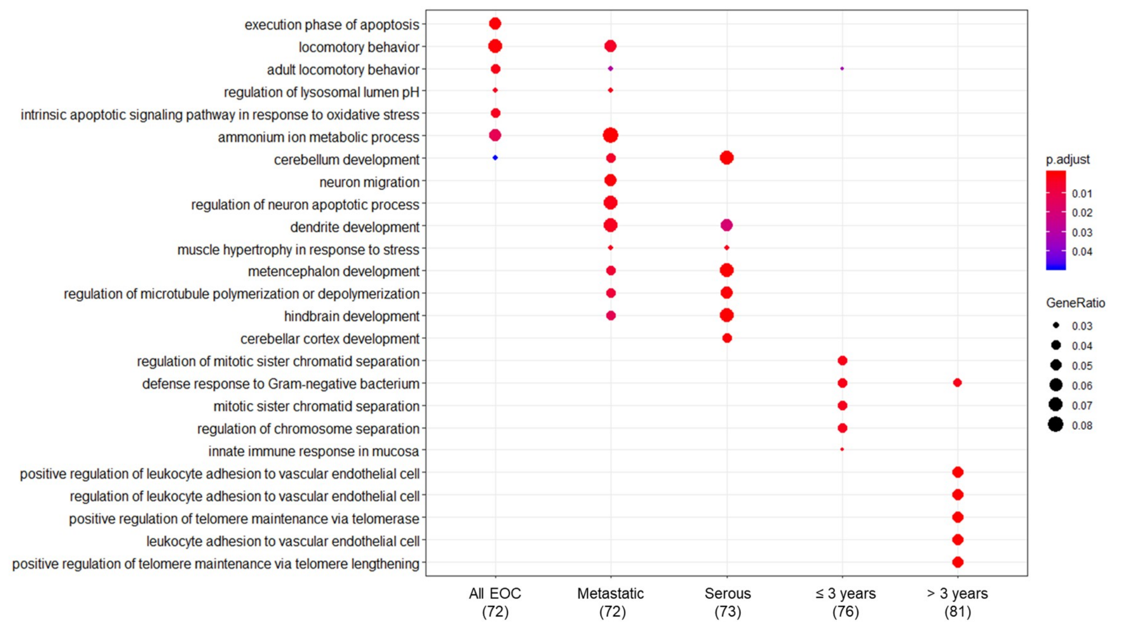

Fig 3. Gene Ontology (GO) enrichment of biological processes among the 100 probes with the lowest p-values in single-gene linear models (case-control) of blood samples from all cases of epithelial ovarian cancer (66 pairs) and according to metastasis status (56 pairs), serous subtype (45 pairs), and interval to diagnosis (≤3 years or >3 years; 34 and 31 pairs, respectively). Numbers below each column indicate the number of probes for which GO categories could be found. The figure presents the five GO categories with lowest p-values. Enriched GO categories ($p<0.05$) beyond the top five are included in addition if they are among the five most enriched of one of the other investigated groups. S8 Table presents the complete GO enrichment list.

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(contributing genes: *TAOK1*, *STK24*, *RFFL*, *HTRA2*, *DIABLO*), "locomotory behavior" (*CLN6*, *NR4A2*, *PPT1*, *PDE1B*, *HTRA2*), and "regulation of lysosomal lumen pH" (*CLN6*, *PPT1*). With the exception of *PDE1B*, these probes displayed negative $\log_2$FC values.

In metastatic EOC, the main enriched categories were "ammonium ion metabolic process" (*NR4A2*, *PLA2G7*, *PDE1B*, *PLBD1*, *CHKA*, *CPT1B*), "neuron migration" and "regulation of neuron apoptotic process" (*NR4A2*, *MEF2C*, *PPT1*, *CDK5R1*, *NSMF*), and "dendrite development" (*EZH2*, *MEF2C*, *CDK5R1*, *NSMF*, *CD3E*). Among these transcripts, *PDE1B*, *CD3E*, *EZH2*, and *NSMF* displayed positive $\log_2$ FC values.

In serous EOC, enriched GO categories were "cerebellum development", "metencephalon development", "hindbrain development", "cerebellar cortex development" (*CDK5R1*, *PAK1*, *EZH2*, *SERPINE2*, *HERC1*), and "regulation of microtubule polymerization or depolymerization" (*CDK5R1*, *PAK1*, *FES*, *TAOK1*). The four genes associated with microtubule polymerization displayed negative $\log_2$FC values, while the remaining $\log_2$FC values were positive. In blood samples collected ≤3 years before diagnosis, the main enriched GO categories were "regulation of mitotic sister chromatid separation" (*PTTG3P*, *CENPE*, *PTTG1*), "defense response to Gram-negative bacterium" (*DEFA1B*, *DEFA4*, *TNFRSF14*), and "innate immune response in mucosa" (*DEFA1B*, *DEFA4*). These probes displayed positive $\log_2$FC values, and none of the genes were included in the top five enriched categories of the other groups.

Finally, in samples collected >3 years before diagnosis, the enriched GO categories were "positive regulation of leukocyte adhesion to vascular endothelial cell" (*NFAT5*, *ICAM1*, *ELANE*, *ETS1*) and "positive regulation of telomere maintenance via telomerase and telomere lengthening" (*CCT2*, *CCT8*, *MAPKAPK5*, *HMBOX1*). In the first mentioned GO category, all

probes except *ETS1* displayed negative log_2FC values, whereas in the latter, all except *HMBOX1* were positive.

Differential expression of genes identified in published functional genomics studies. Our metastatic EOC group contained expression values for 42 of the 86 genes from relevant publications. S9 Table lists the genes, the log_2FC values we observed for these probes, and the difference in expression or methylation status in the original studies. From the two gene sets obtained from whole blood gene expression studies, our data contained expression values for two of six genes identified by qPCR [10] and five of six genes previously identified using gene expression microarrays [11]. The lowest p-values we observed from these gene sets were for the probes *CTNNA1* (log_2FC = -0.05, p = 0.09) and *NCALD* (log_2FC = 0.08, p = 0.08).

Our dataset contained expression values for genes adjacent to more than two-thirds of the methylation sites identified by Teschendorff et al. [5] and Koestler et al. [7]. Among these gene sets, three probes had p-values <0.05: *LIME1* (log_2FC = 0.11, p = 0.05) and *GPR162* (log_2FC = -0.17, p = 0.04) from Teschendorff et al. [5], and *STAB1* (log_2FC = -0.05, p = 0.01) from Koestler et al. [7]. Our dataset contained expression values for less than half of the genes adjacent to methylation sites identified by Fridley et al. [6], Li et al. [9], and Yang et al. [8]. Among these, the test of *SKAP1* identified by Yang et al. [8] resulted in a p-value <0.05 (log_2FC = 0.07, p = 0.04).

Discussion

This nested case-control study of gene expression in whole blood collected up to 7 years prior to EOC diagnosis revealed no statistically significant global or gene-wise associations with EOC case status. The data were high-dimensional, which hampered the statistical power, and the sample size limited the possibilities for analyses according to tumor characteristics or time intervals. Nevertheless, group differences in p-values indicated smaller variation in analyses restricted to metastatic EOC or serous EOC, and greater variation in blood samples collected $\leq$3 years before diagnosis. Compared to controls, cases had larger estimated mean fractions of CD8+ T cells and plasma cells and smaller fractions of neutrophils, monocytes, and resting mast cells. Adjusting for these differences altered the ranking of probes by p-value, but otherwise did not change the results. In targeted gene-wise tests of 42 genes associated with EOC in previous genetic, epigenetic, and transcriptomic studies in blood, four genes were nominally significant among the metastatic cases in the present study.

Case-control differences in gene expression

Neither unsupervised clustering methods, the global test, single-gene linear models, nor gene sets identified statistically significant case-control differences in blood gene expression. With the exception of a few probes, the log_2FC values obtained in gene-wise linear models were less than ±0.2. A log_2FC value of 0.2 equals a fold change of 1.15, which, if interpreted as an indicator of effect size in epidemiological terms, corresponds to a 15% increase in risk of disease.

As no genes were significantly differentially expressed in this study, the interpretation of single genes was kept to a minimum. The probe with lowest p-value in all EOC, *ENSA*, was also among the 100 probes with the lowest p-values in metastatic EOC and serous EOC, and displayed a larger log_2FC value in blood samples collected $>$3 years before diagnosis. *ENSA* encodes α-endosulfine, a cytoplasmic unstructured phosphoprotein with various binding partners depending on cellular context, and regulatory functions depending on its phosphorylation state [37]. Its functions include regulation of cell cycle and platelet activity [38]. In relation to EOC, a small study of serum autoantibodies detected in women with EOC has indicated ENSA as a potential autoantigen [39].

Among the probes with highest log_2FC values in all EOC were four genes (*GZMH*, *APOBEC3G*, *SNHG5*, *MIAT*) that have previously been indicated in studies targeting EOC. In a network analysis of serum proteins, EOC case status was associated with levels of granzyme H (GZMH) in blood samples collected >34.5 months prior to diagnosis [40]. A study of tumor transcriptome data associated quantities of the long, non-coding RNAs *SNHG5* and *MIAT* with EOC stage [41], while *APOBEC3G* expression in tumor infiltrating lymphocytes has been associated with EOC survival [42,43]. These transcripts could potentially be of interest in future studies of circulating markers of EOC, but could not be considered as associated with EOC in our whole transcriptome analysis.

Case-control differences by metastasis status, histological subtype, and interval to diagnosis. The majority of the cases in this study were metastatic at diagnosis, and the majority of the metastatic cases were of serous subtype. The lower p-values in these subgroup analyses compared to all EOC indicated less variation in gene expression between blood samples from women with similar tumor characteristics. Previous studies in the NOWAC postgenome cohort that investigated prospective blood samples from women diagnosed with breast cancer [44] and lung cancer [45] found significant case-control differences in gene expression when analyses were restricted to metastatic cancers. It is uncertain whether the lower p-values we observed for metastatic EOC compared to all EOC reflects a similar phenomenon that would have reached statistical significance with a larger sample size.

Our study was based on blood and would detect signals of cancer developing in the ovaries only by association with the composition of the blood transcriptome. Since serous EOC in particular tends to spread while at a low volume [3], early changes in peripheral immune cells could potentially be a more sensitive systemic indicator of malignant disease than substances of tumor origin, which are produced in proportion to tumor mass [9].

The interval from blood sampling to diagnosis in the present study covers the estimated duration of the development of serous EOC from *in-situ* to stage IV metastatic disease [3]. Inferring from the estimations of Brown and Palmer [3], the women in our study who were diagnosed with serous EOC and had blood samples collected ≤3 years before their diagnosis likely suffered from some degree of metastasis at the time of sample collection. Assuming a rapid development of the tumor in the final year before diagnosis [3], the higher p-values and larger log_2FC values we observed in samples collected ≤3 years before diagnosis could reflect larger transcriptional variation in this group, possibly as an indicator of disease-associated transcriptional dysregulation. The percentage of probes with positive log_2FC values was 70% in this group, compared to 50% in other groups except for all EOC adjusted for leukocyte populations, where this percentage was also 70%. This could suggest a general upregulation of gene transcription in samples collected ≤3 years before diagnosis, rather than a specific composition of leukocyte types.

In the samples collected >3 years before diagnosis, which could theoretically contain signals of stage I and II serous EOC [3], the case-control differences in gene expression were not as strong. When comparing the 100 probes with the lowest p-values in samples collected ≤3 years and >3 years before diagnosis, no overlap in probes was observed. These groups were similar with regard to the distribution of metastatic and serous EOC. Thus, we observed no common transcriptional profile associated with EOC across the postulated time frame for its development. A recent study used mouse models to confirm shifts in systemic immune status during cancer development [46], and it is possible that if our analyses were designed to capture the dynamics of the disease course, we would have been able to identify similar changes associated with EOC. However, due to the small number of samples, we chose not to perform analyses of shorter time intervals.

Gene Ontology enrichment. To explore whether metastasis status, EOC subtype, or time to diagnosis were reflected in biological processes in blood, we compared overrepresented GO categories from the 100 probes with the lowest p-values in single-gene linear models. The overlap of gene lists and shared GO categories (Figs 1 and 2) reflected that all EOC, metastatic EOC, and serous EOC were nested and largely contained the same samples, and that samples collected ≤3 years and >3 years before diagnosis simply represent subdivisions of all EOC.

Among the GO categories indicated in all, metastatic, or serous EOC, locomotory behavior, neuronal migration and central nervous system development have been designated as relevant for the immune system [47]. Migration is a feature of developing neural cells that immune cells share [48]. Overlapping functions of these genes in the immune and neural systems also include the cellular apparatuses related to signaling pathways and cell-to-cell communication [49,50]. Microtubule polymerization and depolymerization, which was enriched in serous EOC, is intrinsic to lymphocyte migration, but also to formation of the immunological synapses necessary for activation of T and B cells [51]. Thus, the main common feature of the overrepresented GO categories for all EOC, metastatic EOC, and serous EOC was their relation to locomotion. If this observation is related to case status, it could suggest that leukocyte migration is affected by EOC.

For blood samples collected ≤3 years before diagnosis, "innate immune response in mucosa" and "defense response to Gram-negative bacterium" were among the main enriched GO categories. Interestingly,"defense response to Gram-negative bacterium" was also overrepresented in blood samples collected >3 years before diagnosis, though neither samples nor probes overlapped. If linked to EOC, the $\log_2$FC values were suggestive of initial downregulation of this process, followed by upregulation closer to diagnosis.

In blood samples collected >3 years before diagnosis, the categories "positive regulation of leukocyte adhesion to vascular endothelial cell" and "positive regulation of telomere maintenance via telomerase and telomere lengthening" were overrepresented. Telomere maintenance is activated during proliferation of activated T and B cells [52]. While this observation is epidemiologically relevant [52], it could be related to the larger proportion of CD8+ T cells in cases overall. Adhesion to endothelial cells is a core mechanism of leukocyte migration, which adds to the above mentioned results for metastatic and serous EOC.

The RNA species investigated in this study included mRNA and polyadenylated long noncoding RNA, and comprised the transcriptome of all circulating immune cells as well as circulating extracellular RNA. Whole blood transcriptomics may thus offer insight into systemic disease processes or enable discovery of circulating markers of disease. Our study design and sample collection were aimed at performing such explorative analyses; however, our study sample was small, and small differences in expression between cases and controls resulted in gene lists that likely included noise. It has been emphasized that GO databases include certain genes that are annotated to many categories [53] and represent current knowledge of genes. Therefore, we have interpreted GO categories with caution.

Estimated leukocyte fractions. The estimated relative sizes of leukocyte populations varied considerably between individuals. On a 10% significance level, EOC cases had slightly larger fractions of CD8+ T cells and plasma cells (adaptive immune system), and smaller fractions of neutrophils, monocytes, and resting mast cells (innate immune system) compared to controls. Adjusting our gene expression models for these leukocyte proportions altered the probes with the lowest p-values, indicating that genes with expression differences according to case-control status were due to differences in these populations.

EOC has been associated with altered proportions of CD8+ T cells, monocytes, and granulocytes (neutrophils, eosinophils, basophils) at diagnosis [7,54], but these studies reported case-control differences opposite to our estimates. Our non-significant observation of higher

proportions of regulatory T cells and M2 macrophages in cases (S1 Table) is more in line with previous studies (summarized in [4]). It is possible that our mean estimates conceal a time-dependent shift during the prediagnostic interval, or that we did not estimate the cell types most relevant for EOC [55].

We estimated relative proportions of 22 leukocyte types. The estimates diverged from the normal physiological range [56] in a manner similar to a divergence observed in other recent studies in the NOWAC postgenome cohort [45,57], which indicates bias. The source might be the deconvolution matrix [58] or upstream laboratory or data processing.

Differential expression of genes identified in published functional genomics studies. Finally, we used the metastatic EOC group to assess signatures from previous studies of post-diagnostic blood samples from women with EOC. These genes of interest were identified in gene expression studies of patients grouped by tumor characteristics [10,11], or DNA methylation studies of EOC cases and controls [5–9]. Although study designs differed, we could assess how these genes associate with EOC on the transcriptional level in prediagnostic samples. Targeted analyses also let us overcome the problem of multiple testing that arises in explorative analyses.

Gene-wise tests of 42 genes resulted in four probes (*LIME1*, *GPR162*, *STAB1*, *SKAP1*) with p-values <0.05 (S9 Table). We observed the largest $\log_2$FC values for *LIME1* and *GPR162* from the study by Teschendorff et al. [5]. *LIME1* (Lck interacting transmembrane adaptor 1; $\log_2$FC = 0.11) is expressed in T cells and B cells, where it links T and B cell receptors to downstream signaling pathways via kinases in the Src family [59]. *GPR162* (G Protein-Coupled Receptor 162; $\log_2$FC = -0.17) encodes an orphan receptor with adrenaline and noradrenaline as putative ligands [60]. Its mRNA is enriched in neutrophils, monocytes and fallopian tube, but the protein is primarily expressed in the brain [59]. Teschendorff et al. [5] partially attributed the methylation differences they observed to tumor-associated changes in circulating leukocyte composition, and they reported hypermethylation of *LIME1* and *GPR162* in EOC cases. We observed divergent $\log_2$FC values for these probes, which, considering the cell type specificity of the transcripts, was in line with our estimated differences in leukocyte populations. However, if the expression difference we observed for *GPR162* is partially attributable to a global change in methylation, this could suggest an altered reception of adrenergic signaling [61–63].

STAB1 (Stabilin 1; $\log_2$FC = -0.05) from the study of Koestler et al. [7] encodes a scavenger receptor suggested to mark immunosuppressive monocytes and macrophages, where decreased expression appears to increase T cell antitumor cytotoxicity [64]. *SKAP1* (Src kinase-associated phosphoprotein 1; $\log_2$FC = 0.07) from the gene set of Yang et al. [8] encodes a T cell receptor adaptor protein and is a known EOC risk locus with a possible cell-autonomous role in EOC tumorigenesis [65]. Yang et al. [8] reported two methylation sites for this gene in leukocytes: one site was associated with higher *SKAP1* expression and higher EOC risk, and the other with lower *SKAP1* expression and lower EOC risk. Our observation supports a positive association between EOC and levels of *SKAP1* transcripts in blood, though this could simply reflect the proportion of T cells in our study.

In summary, the genes with nominally significant differential expression coded for receptor proteins and for adaptor proteins involved in Src pathways. These genes derived from methylation signatures of EOC predisposition or early disease [5] and methylation-mediated genetic risk [7,8].

Strengths and weaknesses

The main weakness of this study is its sample size, which hampered the power of the statistical analyses and limited the methodological possibilities for modeling continuous relationships

between gene expression and time to diagnosis. We excluded borderline epithelial tumors *a priori*, which further reduced the sample size. These tumors could have been included as non-metastatic EOC, but they represent a pathological entity separate from invasive carcinomas. We did not evaluate potential confounding by past exposure to exogenous hormones. Further, the NOWAC postgenome cohort has not contributed repeat blood samples at different time points during follow-up, a practice which has proven useful in linking proteomic data to EOC [40]. The present study was designed to be explorative and descriptive. Even though any findings might have been useful for biomarker development, the sample size in this study was insufficient to adopt a training, validation and test approach. There were no clear candidate transcripts to pursue in further analyses as potential biomarkers.

Strengths of this study include an epidemiological design aimed at avoiding sampling bias, and blood sample collection during a period that addresses the need for data on circulating molecular markers from women with early-stage EOC. Further, the case-control pairs were matched on age and sample storage time, and we evaluated potential confounding by leukocyte proportions and risk factors.

We chose an analytical approach commonly used in gene expression studies, and which was in line with another whole blood gene expression study related to EOC [11]. The small case-control differences implies that potential signals in the data are subtle against a noisy background; the data are high-dimensional and the results non-significant when adjusted for multiple testing.

Conclusion

This nested case-control study did not reveal statistically significant differences in the peripheral blood transcriptome prior to a diagnosis of EOC. The exploration of transcriptional profiles in blood indicated case-control differences that were small in magnitude and did not reach statistical significance when adjusted for multiple testing. The estimated leukocyte population distributions suggested larger proportions of adaptive immune cell types and smaller proportions of innate immune cell types in cases than in controls, and the functional enrichment suggested lower expression of genes involved in migration. Blood samples collected $\leq$3 years before diagnosis, a larger proportion of which likely represented cases who suffered from advanced EOC, displayed a somewhat larger variation and magnitude in expression, yet we did not observe statistically significant case-control differences in gene expression. Among genes previously linked to ovarian cancer, tests of *LIME1*, *GPR162*, *STAB1*, and *SKAP1* resulted in unadjusted p-values <0.05.

The prospective, population-based sampling was a major strength of this study, but the statistical power for explorative transcriptomics was limited. Including a greater number of samples or repeated measurements will allow closer investigation of whether transcript levels change during the course of EOC development.

Supporting information

S1 Fig. No separation of epithelial ovarian cancer cases and controls in hierarchical clustering of gene expression data. Cases shown in orange, controls in cyan. Dendrogram based on $\log_2$FC values of the 500 probes with lowest p-values in single-gene linear models of each case-control pair in all EOC.
(JPG)

S2 Fig. No separation of epithelial ovarian cancer cases and controls in multidimensional scaling of gene expression data. Cases shown in orange, controls in cyan. Plot based on

log_2FC values of the 500 probes with lowest p-values in single-gene linear models of each case-control pair in all EOC.
(PNG)

S3 Fig. Small differences in gene expression among the probes with the lowest p-values. Few log_2FC values exceeded ±0.2. Volcano plot of log_2FC values and p-values of the 100 probes with lowest p-values in single-gene linear models of all EOC.
(PNG)

S1 Table. Mean estimated fractions of leukocyte populations in blood samples from all cases of epithelial ovarian cancer and controls. Based on deconvolution of gene expression values. P-value from a two-sided t-test of the mean difference.
(XLSX)

S2 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of gene expression in blood samples from cases of epithelial ovarian cancer (66 pairs). The presented p-values are not adjusted for multiple testing. All FDR q-values >0.96.
(XLSX)

S3 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of gene expression in blood samples from cases of metastatic epithelial ovarian cancer (56 pairs). The presented p-values are not adjusted for multiple testing. All FDR q-values >0.96.
(XLSX)

S4 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of gene expression in blood samples from cases of serous epithelial ovarian cancer (45 pairs). Almost all serous cases were metastatic. The presented p-values are not adjusted for multiple testing. All FDR q-values >0.96.
(XLSX)

S5 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of gene expression in blood samples collected ≤3 years before diagnosis (34 pairs). The presented p-values are not adjusted for multiple testing. All FDR q-values >0.96.
(XLSX)

S6 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of gene expression in blood samples collected >3 years before diagnosis (31 pairs). The presented p-values are not adjusted for multiple testing. All FDR q-values >0.96.
(XLSX)

S7 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of blood samples from all cases of epithelial ovarian cancer (EOC) (66 pairs) in models adjusted for leukocyte populations. Adjusted for estimated fractions of resting mast cells, plasma cells, neutrophils, monocytes, and CD8+ T cells (S1 Table). The presented p-values are not adjusted for multiple testing; all FDR q-values >0.96.
(XLSX)

S8 Table. Background data for Fig 2. Enriched Gene Ontology (GO) categories for biological processes among the 100 probes with the lowest p-values in single-gene linear models (case-control) of blood samples from all cases of epithelial ovarian cancer (EOC), metastatic

EOC (56 pairs), serous EOC (45 pairs, almost all were metastatic), and from blood samples collected ≤3 years or >3 years before diagnosis (34 and 31 pairs, respectively).
(XLSX)

S9 Table. Summary of tests of genes identified in published functional genomics studies. Results from targeted tests of single genes identified in published studies investigating gene expression in peripheral whole blood or DNA methylation in circulating leukocytes from women with epithelial ovarian cancer.
(XLSX)

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Disclaimer

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Author Contributions

Conceptualization: Mie Jareid, Eiliv Lund.

Data curation: Therese Haugdahl Nøst.

Formal analysis: Therese Haugdahl Nøst.

Funding acquisition: Eiliv Lund.

Investigation: Mie Jareid, Eiliv Lund.

Methodology: Marit Holden.

Resources: Eiliv Lund.

Supervision: Lill-Tove Rasmussen Busund, Therese Haugdahl Nøst.

Writing – original draft: Mie Jareid, Therese Haugdahl Nøst.

Writing – review & editing: Mie Jareid, Igor Snapkov, Marit Holden, Lill-Tove Rasmussen Busund, Eiliv Lund, Therese Haugdahl Nøst.

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Supporting information

Jareid, Snapkov et al. (2021) The blood transcriptome prior to ovarian cancer diagnosis

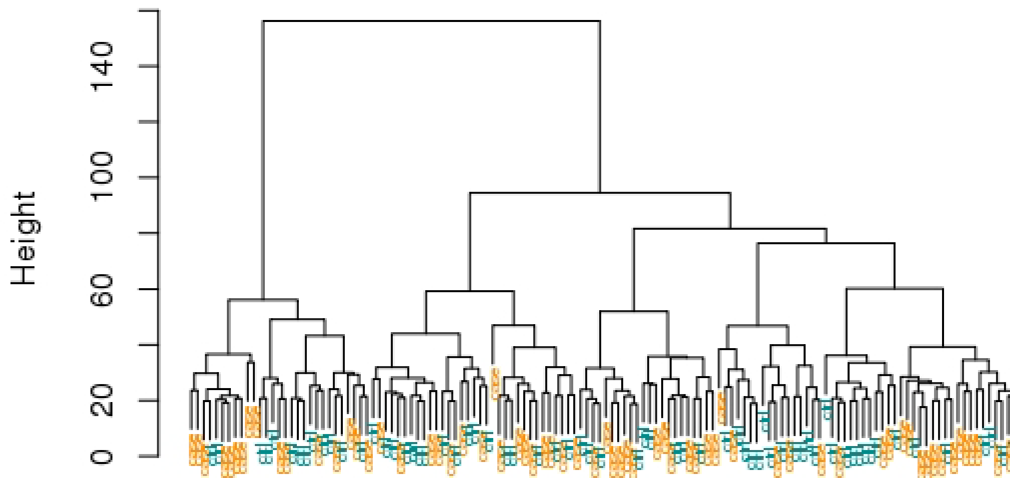


Fig S1. No separation of epithelial ovarian cancer cases and controls in hierarchical clustering of gene expression data. Cases shown in orange, controls in cyan. Dendrogram based on log_2FC values of the 500 probes with lowest p-values in single-gene linear models of each case-control pair in all EOC.

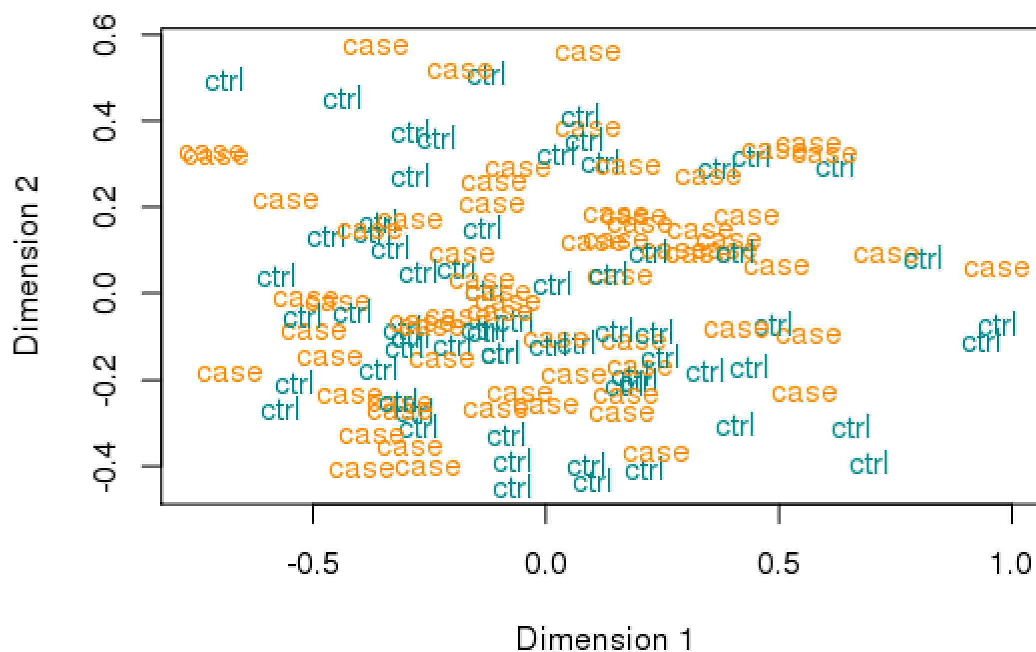


Fig S2. No separation of epithelial ovarian cancer cases and controls in multidimensional scaling of gene expression data. Cases shown in orange, controls in cyan. Plot based on log_2FC values of the 500 probes with lowest p-values in single-gene linear models of each case-control pair in all EOC.

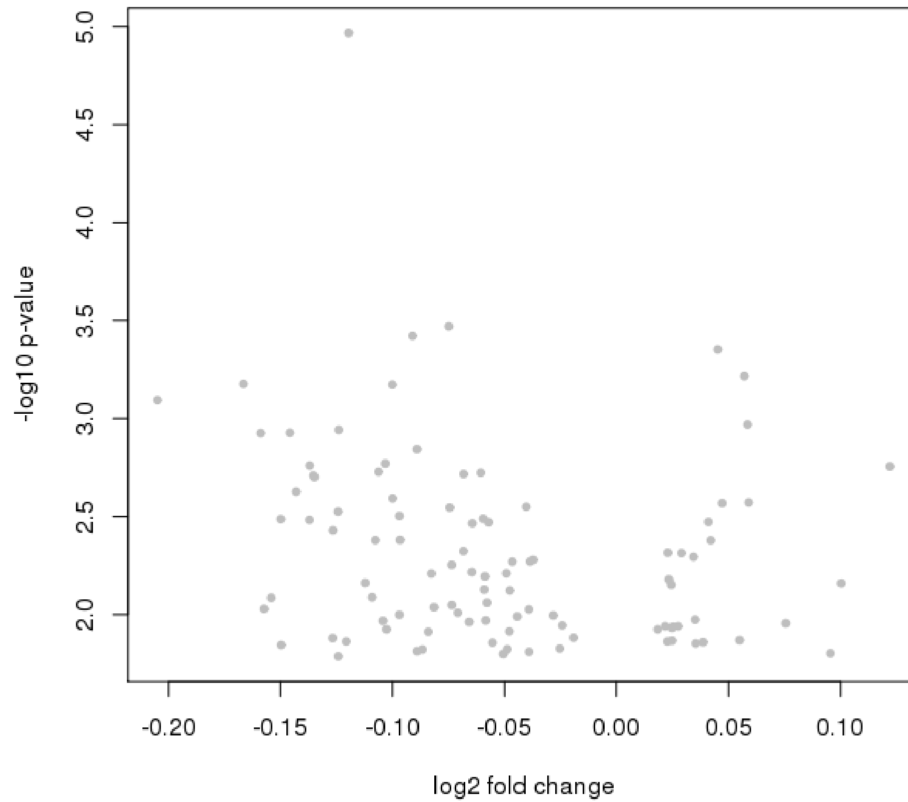


Fig S3. Small differences in gene expression among the probes with the lowest p-values. Few $\log_2$FC values exceeded ±0.2. Volcano plot of $\log_2$FC values and p-values of the 100 probes with lowest p-values in single-gene linear models of all EOC.

Supporting information

Jareid, Snapkov et al. (2021) The blood transcriptome prior to ovarian cancer diagnosis

S1 Table. Mean estimated fractions of leukocyte populations in blood samples from all cases of epithelial ovarian cancer and controls. Based on deconvolution of gene expression values. P-value from a two-sided t-test of the mean difference.

Leukocyte type (LM22 matrix)	Population in EOC cases	Population in controls	p-value
Neutrophils	0.21	0.24	**0.06**
CD8+ T cells	0.24	0.21	**0.08**
Monocytes	0.15	0.16	**0.08**
Regulatory T cells (Tregs)	0.1	0.09	0.28
Naive CD4+ T cells	0.09	0.09	0.68
Activated memory CD4+ T cells	0.06	0.06	0.85
Resting NK cells	0.05	0.04	0.41
Activated NK cells	0.03	0.02	0.7
Memory B cells	0.02	0.03	0.13
Gamma delta T cells	0.02	0.01	0.62
M0 macrophages	0.02	0.02	0.47
M2 macrophages	0.01	0.003	0.22
Resting mast cells	0.004	0.01	**0.02**
Activated mast cells	0.003	0.003	0.83
Macrophages M1	0.001	0.001	0.46
Resting dendritic cells	0.001	0.0002	0.23
Activated dendritic cells	0.001	0.001	0.78
Naive B cells	0.001	0.001	0.83
Eosinophils	0.001	0.001	0.9
Plasma cells	0.0004	0.00003	**0.07**
Resting CD4+ memory T cells	0	0	-
Follicular helper T cells	0	0	-

S2 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of gene expression in blood samples from cases of epithelial ovarian cancer (66 pairs). The presented p-values are not adjusted for multiple testing. All FDR q-values >0.96.

Gene name	log2 FC all EOC	average log2-expression	t-statistic	p-value	EntrezID	Accession number
ENSA	0.06	7.02	3.47	0.001	2029	NM_207043
B3GAT1	0.07	6.54	3.46	0.001	27087	NM_018644
LOC100131253	-0.10	6.97	-3.38	0.001	100131253	XR_038910
CYSLTR1	-0.13	7.55	-3.27	0.001	10800	NM_006639
LOC642161	0.14	7.06	3.14	0.002	642161	XM_936316
FBLN5	0.06	6.59	3.09	0.002	10516	NM_006329
SAMD3	0.07	6.61	2.97	0.004	154075	NM_001017373
CD93	-0.17	8.92	-2.94	0.004	22918	NM_012072
PSAT1	0.04	6.50	2.92	0.004	29968	NM_021154
KCTD12	-0.20	8.73	-2.90	0.004	115207	NM_138444
CYBRD1	-0.12	7.27	-2.82	0.006	79901	NM_024843
LOC728537	0.03	6.56	2.79	0.006	728537	XR_041703
CLN6	-0.04	6.55	-2.77	0.006	54982	NM_017882
LOC100128269	-0.08	6.86	-2.76	0.007	100128269	XR_038661
EZH2	0.04	6.58	2.76	0.007	2146	NM_152998
CENPE	0.02	6.46	2.75	0.007	1062	NM_001813
UBE2E2	-0.05	6.94	-2.73	0.007	7325	NM_152653
FXC1	-0.03	6.63	-2.72	0.007	26515	NM_012192
LOC728855	0.11	8.95	2.72	0.007	728855	NR_024510
TXNL4B	-0.04	6.74	-2.72	0.007	54957	NM_017853
CD8A	0.12	6.91	2.71	0.008	925	NM_001768
NR4A2	-0.06	6.72	-2.70	0.008	4929	NM_006186
CD300LB	-0.08	6.97	-2.66	0.009	124599	NM_174892
USF1	-0.14	7.95	-2.66	0.009	7391	NM_207005
HMBOX1	-0.13	7.35	-2.65	0.009	79618	NM_024567
ZNF235	0.02	6.48	2.60	0.010	9310	NM_004234
CRYZL1	0.03	6.56	2.60	0.010	9946	NM_145858
INPP4A	0.03	6.52	2.60	0.010	3631	NM_004027
PPT1	-0.13	10.48	-2.58	0.011	5538	NM_000310
FLJ20850	0.03	6.55	2.58	0.011	55049	NM_017967
WSB1	-0.04	6.54	-2.57	0.011	26118	NM_134264
MIAT	0.15	7.59	2.57	0.011	440823	NR_003491
CDK5R1	-0.09	7.06	-2.54	0.012	8851	NM_003885
C10orf32	0.06	6.59	2.54	0.012	119032	NM_144591
FLJ10916	0.06	6.52	2.53	0.013	55258	NM_018271
FAM156A	0.02	6.48	2.53	0.013	29057	NM_014138
PRICKLE1	-0.06	6.66	-2.53	0.013	144165	NM_153026
C10orf32-dupl	0.06	7.26	2.53	0.013	119032	NM_144591
SLC36A4	-0.06	6.75	-2.51	0.013	120103	NM_152313
HAGHL	0.03	6.59	2.51	0.013	84264	NM_032304
SLC7A7	-0.11	9.50	-2.50	0.014	9056	NM_003982

INADL	-0.02	6.46	-2.50	0.014	10207	NM_176877
TAOK1	-0.13	7.52	-2.49	0.014	57551	NM_020791
LOC440043	-0.05	9.97	-2.47	0.015	440043	XR_015812
RPL8	0.11	11.85	2.46	0.015	6132	NM_033301
LINS1	0.05	7.13	2.46	0.015	55180	NM_001040614
HSPBL2	0.04	6.67	2.45	0.015	653553	NR_024392
FCRL6	0.07	6.63	2.44	0.016	343413	NM_001004310
CDC14A	-0.08	7.13	-2.44	0.016	8556	NM_033313
AP4E1	0.03	6.69	2.42	0.017	23431	NM_007347
LOC387882	0.08	7.19	2.41	0.017	387882	NM_207376
HMOX2	0.03	6.51	2.41	0.017	3163	NM_002134
SNHG5	0.25	8.99	2.41	0.017	387066	NR_003038
X	-0.09	7.79	-2.40	0.018	-	AK094914
KIAA1671	0.07	6.77	2.40	0.018	85379	XM_371461
LPP	-0.09	9.34	-2.39	0.018	4026	NM_005578
AGPAT9	-0.12	7.47	-2.39	0.018	84803	NM_032717
ETFA	0.06	7.98	2.37	0.019	2108	NM_000126
PKP4	0.04	6.77	2.37	0.019	8502	NM_003628
X	-0.13	7.40	-2.36	0.019	-	AL080095
MPPE1	-0.13	8.21	-2.36	0.020	65258	NM_023075
PDE1B	0.03	6.48	2.36	0.020	5153	NM_000924
ATMIN	0.03	6.53	2.35	0.020	23300	NM_015251
NFAT5	-0.10	7.34	-2.35	0.020	10725	NM_173215
RAB11FIP5	0.08	6.75	2.35	0.020	26056	NM_015470
ABCC5	-0.06	6.94	-2.35	0.020	10057	NM_001023587
LAT2	-0.11	8.96	-2.34	0.021	7462	NM_022040
C1orf71	-0.11	8.15	-2.33	0.021	163882	NM_152609
LRRC56	0.03	6.54	2.32	0.022	115399	NM_198075
RPL39L	0.03	6.57	2.32	0.022	116832	NM_052969
LOC642083	0.05	6.55	2.32	0.022	642083	XM_942728
RABIF	-0.02	6.53	-2.32	0.022	5877	NM_002871
KIAA1875	0.04	6.60	2.32	0.022	340390	NM_032529
GZMH	0.31	9.79	2.31	0.023	2999	NM_033423
APOBEC3G	0.09	7.73	2.31	0.023	60489	NM_021822
GMCL1	-0.06	6.98	-2.30	0.023	64395	NM_178439
TECPR1	-0.08	7.60	-2.29	0.023	25851	NM_015395
MAGEH1	0.04	6.78	2.29	0.024	28986	NM_014061
LAIR2	-0.16	7.16	-2.28	0.024	3904	NM_021270
TMEM154	-0.13	9.43	-2.28	0.024	201799	NM_152680
DIAPH2	-0.05	7.04	-2.28	0.024	1730	NM_006729
X	0.02	6.54	2.28	0.024	-	BX337332
C12orf31	0.04	6.87	2.27	0.025	84298	NM_032338
STX6	-0.05	7.41	-2.27	0.025	10228	NM_005819
IER3	-0.11	7.54	-2.27	0.025	8870	NM_003897
PLA2G7	-0.06	6.74	-2.27	0.025	7941	NM_005084
CD163	-0.09	7.11	-2.26	0.026	9332	NM_203416
CHKA	-0.03	6.75	-2.26	0.026	1119	NM_212469

X	-0.09	7.12	-2.25	0.026	-	DA371742
MAK10	0.02	6.60	2.25	0.026	60560	NM_024635
LOC100132707	-0.06	6.96	-2.24	0.027	100132707	NR_024476
LOC654191	-0.05	6.73	-2.24	0.027	654191	XM_940642
STK24	-0.06	9.82	-2.23	0.027	8428	NM_003576
DIABLO	0.02	6.57	2.23	0.027	56616	NM_138930
RFFL	-0.06	7.42	-2.23	0.027	117584	NM_057178
RMND1	0.02	6.47	2.23	0.028	55005	NM_017909
HTRA2	-0.04	6.91	-2.23	0.028	27429	NM_145074
SERPINB8	-0.04	6.77	-2.23	0.028	5271	NM_198833
LAIR2-dupl	-0.17	7.18	-2.22	0.028	3904	NM_002288
CTSH	-0.10	9.69	-2.22	0.028	1512	NM_004390

Gene names marked '-dupl' denote that there were more probes annotated to the same gene

S3 Table: The 100 probes with the lowest p-values in single-gene linear models (case-control) of gene expression in blood samples from cases of metastatic epithelial ovarian cancer (56 pairs). The presented p-values are not adjusted for multiple testing. All FDR q-values >0.96.

Gene name	log2 FC metastatic	average log2-expression	t-statistic	p-value	EntrezID	Accession number
FBLN5	0.07	6.59	3.92	0.000	10516	NM_006329
CYSLTR1	-0.14	7.55	-3.63	0.000	10800	NM_006639
CD93	-0.20	8.92	-3.40	0.001	22918	NM_012072
LOC100128269	-0.10	6.87	-3.39	0.001	100128269	XR_038661
B3GAT1	0.07	6.53	3.29	0.001	27087	NM_018644
KCTD12	-0.24	8.72	-3.28	0.001	115207	NM_138444
SAMD3	0.08	6.61	3.21	0.002	154075	NM_001017373
TAOK1	-0.18	7.51	-3.20	0.002	57551	NM_020791
EZH2	0.05	6.58	3.18	0.002	2146	NM_152998
LOC100131253	-0.11	6.98	-3.17	0.002	100131253	XR_038910
LOC642161	0.16	7.06	3.06	0.003	642161	XM_936316
SCPEP1	-0.10	7.94	-3.05	0.003	59342	NM_021626
LOC440043	-0.07	9.97	-3.00	0.003	440043	XR_015812
HMOX2	0.03	6.50	2.98	0.004	3163	NM_002134
CYBRD1	-0.14	7.28	-2.93	0.004	79901	NM_024843
GABPB2	-0.03	6.53	-2.92	0.004	2553	NM_016655
NR4A2	-0.07	6.73	-2.91	0.004	4929	NM_006186
GMCL1	-0.08	7.09	-2.90	0.004	64395	NM_178439
PLA2G7	-0.08	6.74	-2.87	0.005	7941	NM_005084
ENSA	0.05	7.01	2.83	0.005	2029	NM_207043
MPPE1	-0.17	8.23	-2.81	0.006	65258	NM_023075
SLC7A7	-0.13	9.50	-2.77	0.007	9056	NM_003982
MEF2C	-0.10	7.00	-2.77	0.007	4208	NM_002397
TMEM154	-0.18	9.42	-2.76	0.007	201799	NM_152680
FXC1	-0.03	6.62	-2.74	0.007	26515	NM_012192
C10orf32	0.07	7.26	2.70	0.008	119032	NM_144591
RPL8	0.12	11.85	2.70	0.008	6132	NM_033301
HSPBL2	0.05	6.66	2.69	0.008	653553	NR_024392
RBMS1	-0.09	7.47	-2.69	0.008	5937	NM_002897
RABIF	-0.03	6.53	-2.67	0.009	5877	NM_002871
STX6	-0.06	7.42	-2.67	0.009	10228	NM_005819
AGPAT9	-0.15	7.48	-2.66	0.009	84803	NM_032717
HAGHL	0.04	6.59	2.64	0.009	84264	NM_032304
LOC728537	0.04	6.56	2.62	0.010	728537	XR_041703
LCK	0.08	7.39	2.62	0.010	3932	NM_005356
SFT2D2	0.03	6.48	2.62	0.010	375035	NM_199344
GMCL1-dupl	-0.07	6.98	-2.59	0.011	64395	NM_178439
WSB1	-0.04	6.55	-2.59	0.011	26118	NM_134264
PPT1	-0.15	10.49	-2.59	0.011	5538	NM_000310
RHOQ	-0.15	9.35	-2.59	0.011	23433	NM_012249
TCTN1	-0.05	6.87	-2.58	0.011	79600	NM_001082538

CENPE	0.03	6.46	2.56	0.012	1062	NM_001813
KCNAB2	0.03	6.48	2.56	0.012	8514	NM_003636
APOBEC3G	0.11	7.72	2.54	0.012	60489	NM_021822
C19orf29	0.03	6.57	2.53	0.013	58509	NM_001080543
CLN6	-0.04	6.55	-2.53	0.013	54982	NM_017882
EEF1G	0.13	11.16	2.53	0.013	1937	NM_001404
FBLN5-dupl	0.08	6.80	2.52	0.013	10516	NM_006329
PDE1B	0.03	6.48	2.52	0.013	5153	NM_000924
FLJ20850	0.04	6.55	2.52	0.013	55049	NM_017967
CYBRD1-dupl	-0.10	7.05	-2.52	0.013	79901	NM_024843
CDC14A	-0.08	7.13	-2.51	0.014	8556	NM_033313
GIMAP5	0.13	9.54	2.50	0.014	55340	NM_018384
PRICKLE1	-0.07	6.67	-2.49	0.014	144165	NM_153026
LRRC56	0.03	6.54	2.49	0.014	115399	NM_198075
TCF7	0.04	6.61	2.49	0.014	6932	NM_201632
FLJ22662	-0.15	9.90	-2.49	0.014	79887	NM_024829
CHKA	-0.04	6.76	-2.48	0.015	1119	NM_212469
PLCXD1	0.08	7.07	2.47	0.015	55344	NM_018390
LOC728640	-0.06	7.07	-2.47	0.015	728640	XR_015400
TMEM154-dupl	-0.18	10.23	-2.46	0.015	201799	NM_152680
IL13RA1	-0.14	9.00	-2.46	0.015	3597	NM_001560
KIAA1875	0.04	6.60	2.46	0.015	340390	NM_032529
CD82	-0.12	7.99	-2.46	0.015	3732	NM_001024844
CD8A	0.12	6.89	2.46	0.016	925	NM_001768
FLJ10916	0.06	6.52	2.46	0.016	55258	NM_018271
TERF1	-0.03	6.48	-2.46	0.016	7013	NM_017489
CRYZL1	0.03	6.56	2.45	0.016	9946	NM_145858
ZNF195	0.03	6.55	2.45	0.016	7748	NM_007152
C12orf43	0.03	6.68	2.45	0.016	64897	NM_022895
LOC730324	0.07	7.32	2.44	0.016	730324	XM_001722095
CDK5R1	-0.09	7.07	-2.44	0.016	8851	NM_003885
PUS1	0.04	6.65	2.44	0.016	80324	NM_001002019
CPT1B	0.10	7.10	2.43	0.017	1375	NM_152246
NA	-0.10	7.79	-2.43	0.017	-	AK094914
AP4E1	0.04	6.69	2.42	0.017	23431	NM_007347
ASGR2	-0.12	7.18	-2.42	0.017	433	NM_080914
UBE2E2	-0.05	6.93	-2.42	0.017	7325	NM_152653
LOC649553	0.03	6.56	2.41	0.017	649553	XR_038252
NA	0.03	6.54	2.41	0.018	-	BX337332
NA	-0.15	7.40	-2.41	0.018	-	AL080095
CD2	0.15	9.73	2.41	0.018	914	NM_001767
NELF (NSMF)	0.09	7.55	2.40	0.018	26012	NM_015537
LTBR	-0.11	8.03	-2.40	0.018	4055	NM_002342
NFAT5	-0.11	7.35	-2.39	0.019	10725	NM_173215
EXOSC8	0.07	7.09	2.39	0.019	11340	NM_181503
CD3E	0.13	8.29	2.39	0.019	916	NM_000733
CTSH	-0.12	9.70	-2.39	0.019	1512	NM_004390

KLHDC4	0.09	7.33	2.38	0.019	54758	NM_017566
TXNL4B	-0.04	6.74	-2.38	0.019	54957	NM_017853
FCRL6	0.08	6.63	2.38	0.019	343413	NM_001004310
CCNY	-0.09	7.91	-2.38	0.019	219771	NM_145012
LGR6	0.06	6.62	2.36	0.020	59352	NM_001017404
RPGRIP1	-0.07	6.72	-2.36	0.020	57096	NM_020366
DSTYK	-0.03	6.84	-2.36	0.020	25778	NM_199462
MOSPD2	-0.12	7.71	-2.35	0.020	158747	NM_152581
FCGR3B	-0.21	11.62	-2.35	0.020	2215	NM_000570
CD300LB	-0.08	6.96	-2.35	0.020	124599	NM_174892
NA	-0.06	7.03	-2.35	0.020	-	AL133627
POLE	0.04	6.64	2.35	0.021	5426	NM_006231

Gene names marked '-dupl' denote that there were more probes annotated to the same gene

S4 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of gene expression in blood samples from cases of serous epithelial ovarian cancer (45 pairs). Almost all serous cases were metastatic. The presented p-values are not adjusted for multiple testing. All FDR q-values >0.96.

Gene name	log2 FC serous	average log2-expression	t-statistic	p-value	EntrezID	Accession number
FBLN5	0.08	6.59	3.85	0.000	10516	NM_006329
CYBRD1	-0.17	7.29	-3.57	0.001	79901	NM_024843
CYBRD1-dupl	-0.13	7.04	-3.33	0.001	79901	NM_024843
LOC100128269	-0.10	6.87	-3.33	0.001	100128269	XR_038661
CD93	-0.23	8.92	-3.30	0.001	22918	NM_012072
KCTD12	-0.25	8.73	-3.25	0.002	115207	NM_138444
TAOK1	-0.19	7.51	-3.23	0.002	57551	NM_020791
SAMD3	0.09	6.60	3.16	0.002	154075	NM_001017373
LOC642161	0.19	7.08	3.10	0.003	642161	XM_936316
RBMS1	-0.12	7.47	-3.10	0.003	5937	NM_002897
TXNL4B	-0.05	6.74	-3.09	0.003	54957	NM_017853
SCPEP1	-0.12	7.94	-3.04	0.003	59342	NM_021626
ZNF195	0.04	6.54	3.01	0.003	7748	NM_007152
SLC7A7	-0.16	9.50	-3.01	0.003	9056	NM_003982
LOC440043	-0.08	9.98	-2.99	0.004	440043	XR_015812
PPT1	-0.19	10.47	-2.96	0.004	5538	NM_000310
CD300LB	-0.10	6.97	-2.90	0.005	124599	NM_174892
CRYZL1	0.04	6.57	2.89	0.005	9946	NM_145858
LOC100131253	-0.10	6.97	-2.88	0.005	100131253	XR_038910
ALB	-0.03	6.46	-2.88	0.005	213	NM_000477
CYSLTR1	-0.11	7.54	-2.85	0.005	10800	NM_006639
CCDC28B	0.05	6.74	2.83	0.006	79140	NM_024296
STX6	-0.07	7.41	-2.83	0.006	10228	NM_005819
IL13RA1	-0.17	8.98	-2.81	0.006	3597	NM_001560
LRRC56	0.05	6.54	2.81	0.006	115399	NM_198075
ANKRD16	0.03	6.55	2.77	0.007	54522	NM_019046
NA	0.04	6.54	2.77	0.007	-	BX337332
RPGRIP1	-0.08	6.72	-2.75	0.007	57096	NM_020366
FLJ22662	-0.20	9.91	-2.73	0.008	79887	NM_024829
CD163	-0.10	7.12	-2.72	0.008	9332	NM_203416
LOC728537	0.04	6.55	2.72	0.008	728537	XR_041703
TCERG1	0.04	6.50	2.72	0.008	10915	NM_006706
GABPB2	-0.03	6.52	-2.72	0.008	2553	NM_016655
CD82	-0.15	8.00	-2.70	0.008	3732	NM_001024844
SERPINE2	0.06	6.52	2.70	0.008	5270	NM_006216
FLJ20850	0.04	6.55	2.70	0.008	55049	NM_017967
KIAA1875	0.05	6.60	2.68	0.009	340390	NM_032529
CDK5R1	-0.11	7.07	-2.67	0.009	8851	NM_003885
CD7	0.16	9.33	2.67	0.009	924	NM_006137
HAGHL	0.04	6.59	2.64	0.010	84264	NM_032304

PAK1	-0.14	8.30	-2.64	0.010	5058	NM_002576
CALU	-0.05	6.68	-2.63	0.010	813	NM_001219
GIMAP5	0.16	9.54	2.62	0.010	55340	NM_018384
RFFL	-0.08	7.42	-2.62	0.010	117584	NM_057178
AGPAT9	-0.15	7.48	-2.62	0.010	84803	NM_032717
ENSA	0.05	7.01	2.60	0.011	2029	NM_207043
SNHG7	0.06	6.80	2.59	0.011	84973	NR_024542
CTSH	-0.15	9.70	-2.58	0.011	1512	NM_004390
JDP2	-0.07	6.86	-2.57	0.012	122953	NM_130469
LOC441253	0.03	6.49	2.54	0.013	441253	XM_379877
FBN2	-0.07	6.67	-2.53	0.013	2201	NM_001999
FLJ10916	0.07	6.53	2.53	0.013	55258	NM_018271
AGMAT	0.04	6.55	2.53	0.013	79814	NM_024758
TMEM154	-0.19	9.42	-2.53	0.013	201799	NM_152680
KLHDC4	0.10	7.33	2.51	0.014	54758	NM_017566
HMOX2	0.03	6.50	2.51	0.014	3163	NM_002134
X.1	-0.11	7.79	-2.50	0.014	0	AK094914
SFXN5	-0.06	6.73	-2.50	0.014	94097	NM_144579
MTFMT	-0.04	6.70	-2.48	0.015	123263	NM_139242
DIRC2	-0.09	7.03	-2.48	0.015	84925	NM_032839
RYBP	-0.21	8.41	-2.48	0.015	23429	NM_012234
SPEN	-0.07	8.15	-2.47	0.015	23013	NM_015001
EZH2	0.05	6.58	2.47	0.016	2146	NM_152998
LOC729774	0.03	6.49	2.47	0.016	729774	XM_001715945
MPPE1	-0.17	8.25	-2.47	0.016	65258	NM_023075
CEP78	0.07	6.80	2.46	0.016	84131	NM_032171
MEF2C	-0.10	6.99	-2.46	0.016	4208	NM_002397
PDE1B	0.03	6.48	2.46	0.016	5153	NM_000924
RPL8	0.13	11.84	2.46	0.016	6132	NM_033301
PLCXD1	0.08	7.07	2.45	0.016	55344	NM_018390
PI3	-0.40	10.16	-2.44	0.016	5266	NM_002638
HERC1	0.09	7.44	2.44	0.016	8925	NM_003922
LOC387882	0.10	7.18	2.43	0.017	387882	NM_207376
ASGR2	-0.14	7.19	-2.43	0.017	433	NM_080914
CD33	-0.13	7.87	-2.43	0.017	945	NM_001772
IKBIP	-0.07	6.83	-2.43	0.017	121457	NM_201612
CUL1	0.05	7.29	2.42	0.017	8454	NM_003592
BTN3A2	0.20	8.69	2.42	0.017	11118	NM_007047
TKT	-0.16	10.83	-2.42	0.018	7086	NM_001064
SAMD3-dupl	0.06	6.59	2.41	0.018	154075	NM_152552
LTBR	-0.13	8.04	-2.40	0.019	4055	NM_002342
LOC644936	-0.53	8.74	-2.40	0.019	644936	NR_004845
ATMIN	0.04	6.52	2.39	0.019	23300	NM_015251
FES	-0.15	8.50	-2.38	0.019	2242	NM_002005
FCGR3B	-0.25	11.64	-2.38	0.019	2215	NM_000570
AP4E1	0.04	6.69	2.38	0.019	23431	NM_007347
LINS1	0.06	7.13	2.38	0.019	55180	NM_001040614

DNAJA3	0.07	7.98	2.38	0.020	9093	NM_005147
ZNF509	0.03	6.52	2.38	0.020	166793	NM_145291
KDM3B	-0.07	7.60	-2.37	0.020	51780	NM_016604
TNFSF4	0.06	6.69	2.37	0.020	7292	NM_003326
PLXDC2	-0.12	7.60	-2.37	0.020	84898	NM_032812
ASAP1	-0.08	7.30	-2.36	0.020	50807	NM_018482
USF1	-0.14	7.96	-2.36	0.020	7391	NM_207005
ATP6V1B2	-0.13	11.13	-2.36	0.020	526	NM_001693
TSEN54	0.09	7.13	2.36	0.020	283989	NM_207346
LOC649553	0.03	6.56	2.36	0.020	649553	XR_038252
CD163-dupl	-0.08	7.08	-2.36	0.020	9332	NM_203416
NA	-0.08	6.68	-2.36	0.021	-	AK025332
IGSF6	-0.20	8.55	-2.36	0.021	10261	NM_005849

Gene names marked '-dupl' denote that there were more probes annotated to the same gene

S5 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of gene expression in blood samples collected ≤3 years before diagnosis (34 pairs). The presented p-values are not adjusted for multiple testing. All FDR q-values >0.96.

Gene name	log2 FC ≤3 years	average log2-expression	t-statistic	p-value	EntrezID	Accession number
FAM50B	-0.07	6.59	-3.02	0.004	26240	NM_012135
NA	0.04	6.54	2.97	0.004	-	BX337332
LOC644133	0.07	6.52	2.97	0.004	644133	XM_927346
B3GAT1	0.09	6.54	2.92	0.005	27087	NM_018644
LOC728537	0.05	6.56	2.89	0.005	728537	XR_041703
TSTD1	-0.10	7.36	-2.86	0.006	100131187	NM_001113206
PPT1	-0.21	10.44	-2.82	0.006	5538	NM_000310
PLCXD1	0.10	7.07	2.81	0.007	55344	NM_018390
LOC731049	0.09	7.55	2.79	0.007	731049	XM_001129232
PKP4	0.06	6.78	2.77	0.007	8502	NM_003628
C20orf111	-0.14	8.80	-2.77	0.007	51526	NM_016470
FBLN5	0.07	6.58	2.73	0.008	10516	NM_006329
PTTG3P	0.06	6.70	2.73	0.008	26255	NR_002734
BDKRB2	0.04	6.47	2.72	0.008	624	NM_000623
TSPAN17	0.12	8.15	2.68	0.009	26262	NM_012171
DAB2	0.13	6.98	2.68	0.009	1601	NM_001343
RBMS1	-0.10	7.47	-2.67	0.009	5937	NM_002897
APOBEC3H	0.09	6.65	2.65	0.010	164668	NM_181773
LOC642161	0.15	7.05	2.64	0.010	642161	XM_936316
KRTAP10-2	0.05	6.48	2.63	0.011	386679	NM_198693
TNFSF4	0.08	6.70	2.63	0.011	7292	NM_003326
KLHL12	-0.06	6.77	-2.63	0.011	59349	NM_021633
DEFA1B	0.64	9.06	2.62	0.011	728358	NM_001042500
OSBPL8	-0.15	7.43	-2.60	0.011	114882	NM_020841
KAT5	0.05	6.67	2.59	0.012	10524	NM_182710
C17orf101	0.04	6.55	2.58	0.012	79701	NM_175902
ACP2	0.07	6.90	2.57	0.012	53	NM_001610
DGKQ	0.15	7.79	2.57	0.012	1609	NM_001347
C16orf68	0.08	7.27	2.57	0.012	79091	NM_024109
CYBRD1	-0.16	7.26	-2.56	0.013	79901	NM_024843
DAB2-dupl	0.12	6.93	2.54	0.013	1601	NM_001343
LUC7L2	-0.03	6.53	-2.53	0.014	51631	NM_016019
RANBP1	0.05	7.13	2.53	0.014	5902	NM_002882
HAUS8	0.03	6.50	2.52	0.014	93323	NM_033417
PCDHGB6	0.06	6.67	2.52	0.014	56100	NM_018926
CENPE	0.03	6.46	2.52	0.014	1062	NM_001813
CHMP7	-0.06	6.64	-2.51	0.014	91782	NM_152272
SDHC	-0.06	6.77	-2.51	0.015	6391	NM_001035513
FAM13B	-0.12	8.06	-2.50	0.015	51306	NM_001101800
STX6	-0.08	7.41	-2.50	0.015	10228	NM_005819
DLK2	0.05	6.50	2.50	0.015	65989	NM_023932

ODF2	0.03	6.47	2.50	0.015	4957	NM_002540
CRELD2	0.10	8.04	2.49	0.015	79174	NM_024324
MCOLN2	0.19	7.54	2.48	0.016	255231	NM_153259
NELF	0.11	7.55	2.48	0.016	26012	NM_015537
PAPSS1	-0.13	7.69	-2.47	0.016	9061	NM_005443
TAP2	0.10	6.67	2.45	0.017	6891	NM_018833
BEND5	-0.07	6.69	-2.45	0.017	79656	NM_024603
LOC643856	0.06	6.86	2.42	0.018	643856	XR_037586
ABHD12	0.03	6.53	2.42	0.018	26090	NM_001042472
FLJ22662	-0.18	9.90	-2.42	0.018	79887	NM_024829
LOC220686	0.06	6.90	2.41	0.019	220686	NM_199283
TRO	0.04	6.50	2.41	0.019	7216	NM_001039705
RRP7A	-0.15	7.37	-2.40	0.019	27341	NM_015703
PMVK	0.07	7.28	2.40	0.019	10654	NM_006556
CCDC146	-0.05	6.50	-2.40	0.019	57639	NM_020879
LOC641298	0.04	6.62	2.39	0.019	641298	XR_041850
MT1E	0.13	7.07	2.39	0.019	4493	NM_175617
CASZ1	0.08	6.95	2.39	0.020	54897	NM_017766
SDHC-dupl	0.07	7.65	2.39	0.020	6391	NM_003001
PHF19	0.06	6.87	2.39	0.020	26147	NM_001009936
EZH2	0.06	6.58	2.38	0.020	2146	NM_152998
TSC22D1	0.08	6.60	2.38	0.020	8848	NM_006022
PLA2G7	-0.07	6.73	-2.38	0.020	7941	NM_005084
MTMR11	0.04	6.47	2.38	0.020	10903	NM_006697
C20orf27	0.13	7.65	2.37	0.020	54976	NM_001039140
ABCC5	-0.07	6.94	-2.36	0.021	10057	NM_001023587
SKAP2	-0.18	7.72	-2.36	0.021	8935	NM_003930
STK4	-0.08	10.90	-2.35	0.021	6789	NM_006282
KLHDC4	0.11	7.34	2.35	0.021	54758	NM_017566
RPL39L	0.04	6.57	2.35	0.022	116832	NM_052969
TNFRSF14	0.11	10.37	2.35	0.022	8764	NM_003820
SHISA4	0.06	6.51	2.35	0.022	149345	NM_198149
NA	0.07	6.53	2.34	0.022	-	AK096898
C21orf7	0.25	8.28	2.34	0.022	56911	NM_020152
ASCL2	0.16	7.90	2.33	0.023	430	NM_005170
RAB3IP	-0.07	6.90	-2.33	0.023	117177	NM_175624
KCNAB2	0.04	6.50	2.32	0.023	8514	NM_003636
C16orf72	-0.12	8.04	-2.32	0.023	29035	NM_014117
C20orf20	-0.07	7.22	-2.32	0.023	55257	NM_018270
DKFZp434N035	0.03	6.47	2.32	0.023	84222	NM_032262
LAT2	-0.14	8.99	-2.32	0.024	7462	NM_022040
CXCR5	-0.17	7.71	-2.31	0.024	643	NM_032966
SAMD3	0.07	6.59	2.31	0.024	154075	NM_001017373
GCC2	-0.06	6.62	-2.31	0.024	9648	NM_181453
MIAT	0.20	7.59	2.31	0.024	440823	NR_003491
MT1X	0.13	7.93	2.30	0.025	4501	NM_005952
PLGLB1	0.04	6.49	2.29	0.025	5343	NM_001032392

TRIB1	-0.15	7.78	-2.29	0.025	10221	NM_025195
GTF3C6	0.05	7.05	2.29	0.025	112495	NM_138408
INPP4A	0.03	6.52	2.29	0.025	3631	NM_004027
TSEN54	0.10	7.14	2.29	0.025	283989	NM_207346
MDH2	0.08	10.66	2.29	0.025	4191	NM_005918
FNIP2	0.05	6.54	2.29	0.025	57600	NM_020840
LOC387882	0.11	7.18	2.29	0.025	387882	NM_207376
PTTG1	0.08	7.16	2.28	0.026	9232	NM_004219
HOOK1	-0.07	6.62	-2.28	0.026	51361	NM_015888
DEFA4	0.23	6.83	2.28	0.026	1669	NM_001925
SFXN3	0.04	6.51	2.27	0.026	81855	NM_030971
NA	0.04	6.50	2.27	0.027	-	AW297854

Gene names marked '-dupl' denote that there were more probes annotated to the same gene

S6 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of gene expression in blood samples collected >3 years before diagnosis (31 pairs). The presented p-values are not adjusted for multiple testing. All FDR q-values >0.96.

Gene symbol	**log2 FC >3 years**	**average log2-expression**	**t-statistic**	**p-value**	**EntrezID**	**Accession number**
C10orf32	0.11	6.61	3.75	0.000	119032	NM_144591
FAM119A	0.08	6.53	3.59	0.001	151194	NM_001127395
EDG1	0.13	7.19	3.54	0.001	1901	NM_001400
NFAT5	-0.17	7.32	-3.40	0.001	10725	NM_173215
ENSA	0.08	7.03	3.39	0.001	2029	NM_207043
TRPC4AP	-0.18	8.24	-3.34	0.001	26133	NM_015638
USF1	-0.25	7.97	-3.34	0.001	7391	NM_207005
CTSG	-0.14	6.59	-3.27	0.002	1511	NM_001911
METAP1	0.10	7.75	3.26	0.002	23173	NM_015143
CCDC149	-0.09	6.58	-3.20	0.002	91050	NM_173463
FAM119A-dupl	0.10	6.70	3.15	0.003	151194	NM_145280
ANXA11	-0.19	8.43	-3.09	0.003	311	NM_001157
GLO1	0.16	7.73	3.07	0.003	2739	NM_006708
TAOK1	-0.22	7.49	-3.03	0.004	57551	NM_020791
PXK	-0.05	6.56	-3.00	0.004	54899	NM_017771
TSPAN9	-0.21	7.12	-2.97	0.004	10867	NM_006675
TSGA14	0.05	6.52	2.93	0.005	95681	NM_018718
PSAT1	0.06	6.51	2.90	0.005	29968	NM_021154
CCS	-0.09	7.11	-2.87	0.006	9973	NM_005125
ICAM1	-0.05	6.51	-2.87	0.006	3383	NM_000201
MAP4	-0.04	6.48	-2.86	0.006	4134	NM_002375
LOC727948	-0.11	6.66	-2.85	0.006	727948	XM_001126216
INADL	-0.04	6.46	-2.84	0.006	10207	NM_176877
ELANE	-0.19	6.71	-2.84	0.006	1991	NM_001972
VCL	-0.19	9.90	-2.82	0.006	7414	NM_014000
CAST	-0.11	6.80	-2.82	0.006	831	NM_001042443
CRYZL1	0.04	6.56	2.81	0.007	9946	NM_145858
USF1-dupl	-0.12	6.72	-2.76	0.008	7391	NM_007122
ETS1	0.20	9.69	2.75	0.008	2113	NM_005238
PIGH	0.05	6.80	2.74	0.008	5283	NM_004569
SCAP	-0.17	9.18	-2.74	0.008	22937	NM_012235
CD163	-0.13	7.08	-2.72	0.009	9332	NM_203416
PMS2CL	-0.04	6.50	-2.72	0.009	441194	NR_002217
GABARAP	-0.12	7.27	-2.71	0.009	11337	NM_007278
RERE	-0.14	8.01	-2.71	0.009	473	NM_001042682
KCNH3	-0.10	6.64	-2.71	0.009	23416	NM_012284
EEF1G	0.17	11.19	2.69	0.009	1937	NM_001404
SUOX	-0.07	6.91	-2.67	0.010	6821	NM_000456
DIP2C	-0.04	6.47	-2.67	0.010	22982	NM_014974
CCT2	0.11	7.62	2.66	0.010	10576	NM_006431
PF4V1	-0.12	6.56	-2.66	0.010	5197	NM_002620

HMBOX1	-0.17	7.33	-2.66	0.010	79618	NM_024567
ALKBH3	0.06	6.82	2.66	0.010	221120	NM_139178
C10orf32-dupl	0.08	7.27	2.65	0.010	119032	NM_144591
AZU1	-0.07	6.52	-2.65	0.010	566	NM_001700
C20orf107	-0.03	6.58	-2.65	0.010	388799	NM_001013646
FOSL2	-0.07	6.75	-2.65	0.010	2355	NM_005253
HABP4	0.07	6.67	2.64	0.011	22927	NM_014282
MPST	-0.04	6.50	-2.64	0.011	4357	NM_021126
ZIK1	0.04	6.56	2.63	0.011	284307	NM_001010879
IL18BP	-0.04	6.51	-2.63	0.011	10068	NM_173044
CD93	-0.22	8.92	-2.61	0.011	22918	NM_012072
ZNF7	0.06	6.70	2.60	0.012	7553	NM_003416
CLN6	-0.06	6.55	-2.60	0.012	54982	NM_017882
EXOSC8	0.11	7.08	2.59	0.012	11340	NM_181503
MAX	-0.13	7.39	-2.59	0.012	4149	NM_145113
NUP88	0.12	7.67	2.59	0.012	4927	NM_002532
AP1G1	-0.06	7.04	-2.58	0.012	164	NM_001128
SNHG7	0.07	6.81	2.57	0.012	84973	NR_024542
MOBKL3	0.06	6.52	2.57	0.013	25843	NM_001100819
MGA	0.06	6.72	2.57	0.013	23269	NM_001080541
ZNF613	0.08	6.74	2.56	0.013	79898	NM_024840
LEF1	0.21	8.61	2.56	0.013	51176	NM_016269
C3orf21	-0.06	6.87	-2.56	0.013	152002	NM_152531
CD163-dupl	-0.14	7.11	-2.56	0.013	9332	NM_203416
MRPS30	0.07	7.24	2.55	0.013	10884	NM_016640
GP9	-0.38	8.63	-2.55	0.013	2815	NM_000174
H2AFY	-0.15	7.90	-2.55	0.013	9555	NM_138609
DNAJC24	0.06	6.61	2.54	0.014	120526	NM_181706
SERPINB8	-0.06	6.77	-2.54	0.014	5271	NM_198833
CD33	-0.14	7.89	-2.53	0.014	945	NM_001772
KIFC3	-0.07	6.51	-2.53	0.014	3801	NM_005550
TMEM185A	-0.08	7.04	-2.53	0.014	84548	NM_032508
C20orf191	-0.10	7.10	-2.53	0.014	149934	NM_001039379
EIF2C2	-0.17	7.67	-2.52	0.014	27161	NM_012154
CHORDC1	0.05	6.54	2.52	0.014	26973	NM_012124
CSRP2BP	0.05	6.59	2.52	0.014	57325	NM_020536
CCT8	0.11	8.55	2.52	0.015	10694	NM_006585
SMYD4	0.05	6.79	2.51	0.015	114826	NM_052928
PSMD2	-0.09	7.64	-2.51	0.015	5708	NM_002808
FOXJ3	0.09	7.68	2.51	0.015	22887	NM_014947
BOLA3	0.09	7.45	2.51	0.015	388962	NM_212552
AMMECR1	0.07	6.87	2.50	0.015	9949	NM_015365
ITPK1	-0.16	8.18	-2.50	0.015	3705	NM_014216
TOM1L2	-0.04	6.50	-2.49	0.016	146691	NM_001082968
LOC653034	-0.04	6.50	-2.49	0.016	653034	XM_930587
WEE1	0.04	6.48	2.49	0.016	7465	NM_003390
LINS1	0.07	7.14	2.48	0.016	55180	NM_001040614

LOC124512	0.10	8.22	2.48	0.016	124512	XM_940962
MAPKAPK5	0.04	6.56	2.48	0.016	8550	NM_139078
FAM103A1	-0.04	6.85	-2.48	0.016	83640	NM_031452
PRPS1	0.09	7.75	2.47	0.016	5631	NM_002764
NA	-0.05	6.64	-2.47	0.016	-	AW962683
RIC8B	0.07	6.93	2.47	0.017	55188	NM_018157
LOC283953	-0.09	7.11	-2.46	0.017	283953	XM_208930
LOC100131253	-0.11	6.96	-2.46	0.017	100131253	XR_038910
LOC100128269	-0.10	6.84	-2.46	0.017	100128269	XR_038661
EEF1A1	0.14	12.99	2.45	0.017	1915	NM_001402
TSGA14-dupl	0.04	6.55	2.45	0.017	95681	NM_018718
LOC654053	-0.12	6.79	-2.45	0.017	654053	XM_943677

Gene names marked '-dupl' denote that there were more probes annotated to the same gene

S7 Table. The 100 probes with the lowest p-values in single-gene linear models (case-control) of blood samples from all cases of epithelial ovarian cancer (EOC) (66 pairs) in models adjusted for leukocyte populations. Adjusted for estimated fractions of resting mast cells, plasma cells, neutrophils, monocytes, and CD8+ T cells (Table S1). The presented p-values are not adjusted for multiple testing; all FDR q-values >0.96.

Gene symbol	log2 FC all EOC adj.	average log2-expression	t-statistic	p-value	EntrezID	Accession number
ENSA	-0.06	7.02	-3.58	0.000	2029	NM_207043
CIB1	0.10	10.38	3.34	0.001	10519	NM_006384
CENPE	-0.03	6.46	-3.28	0.001	1062	NM_001813
ANKRD36B	0.13	7.42	3.12	0.002	57730	NM_025190
CDC14A	0.10	7.13	3.02	0.003	8556	NM_033313
CBLB	0.10	7.85	3.00	0.003	868	NM_170662
TCEAL3	0.07	7.08	2.99	0.003	85012	NM_001006933
NKAP	-0.04	6.58	-2.98	0.003	79576	NM_024528
CIRH1A	0.07	7.66	2.97	0.004	84916	NM_032830
FLJ10916	-0.07	6.52	-2.94	0.004	55258	NM_018271
C10orf32	-0.07	7.26	-2.92	0.004	119032	NM_144591
CYSLTR1	0.12	7.54	2.91	0.004	10800	NM_006639
TYW1B	0.06	7.01	2.91	0.004	441250	XR_015176
HMBOX1	0.14	7.35	2.88	0.005	79618	NM_024567
FCRL3	0.17	8.20	2.86	0.005	115352	NM_001024667
LOC100131253	0.09	6.97	2.86	0.005	100131253	XR_038910
C1orf71	0.13	8.15	2.81	0.006	163882	NM_152609
RNF125	0.05	6.84	2.80	0.006	54941	NM_017831
SAE1	0.06	9.23	2.79	0.006	10055	NM_005500
LOC400986	0.12	7.91	2.76	0.007	400986	XM_001126815
C10orf32-dupl	-0.06	6.59	-2.74	0.007	119032	NM_144591
DDX39	0.06	8.72	2.74	0.007	10212	NM_005804
PTPLAD1	0.05	6.76	2.73	0.007	51495	NM_016395
TMEM99	0.05	6.93	2.73	0.007	147184	NM_145274
FXC1	0.04	6.63	2.72	0.007	26515	NM_012192
FAM156A	-0.03	6.48	-2.72	0.008	29057	NM_014138
NA	0.10	7.12	2.69	0.008		DA371742
LOC100132707	0.07	6.95	2.68	0.008	100132707	NR_024476
FAM50B	0.04	6.59	2.66	0.009	26240	NM_012135
TAOK1	0.15	7.51	2.65	0.009	57551	NM_020791
ZNF235	-0.02	6.48	-2.65	0.009	9310	NM_004234
LOC654191	0.06	6.74	2.65	0.009	654191	XM_940642
SMAD3	0.06	7.52	2.65	0.009	4088	NM_005902
PKP4	-0.05	6.77	-2.63	0.010	8502	NM_003628
X.1	0.07	7.03	2.61	0.010		AL133627
CCR6	0.09	7.31	2.61	0.010	1235	NM_031409
LOC728537	-0.03	6.56	-2.61	0.010	728537	XR_041703
NUP205	0.05	7.46	2.60	0.010	23165	NM_015135
PPP1R16B	0.06	7.51	2.59	0.011	26051	NM_015568
DNMT3A	0.03	6.51	2.58	0.011	1788	NM_153759

FBLN5	-0.05	6.59	-2.58	0.011	10516	NM_006329
CBX7	0.07	8.49	2.57	0.011	23492	NM_175709
CD247	0.11	10.82	2.56	0.012	919	NM_198053
LOC728014	0.08	7.50	2.55	0.012	728014	XM_001127981
B3GAT1	-0.05	6.54	-2.55	0.012	27087	NM_018644
X.2	0.07	7.29	2.54	0.012		AK091091
RORA	0.09	7.32	2.54	0.012	6095	NM_002943
UNG	0.05	7.20	2.50	0.014	7374	NM_003362
EZH2	-0.04	6.59	-2.50	0.014	2146	NM_152998
MED17	-0.02	6.47	-2.49	0.014	9440	NM_004268
LOC731275	-0.04	6.52	-2.49	0.014	731275	XM_001732853
SH3KBP1	0.07	7.08	2.48	0.014	30011	NM_001024666
CAPN12	0.08	6.77	2.48	0.014	147968	NM_144691
LOC440043	0.06	9.97	2.48	0.015	440043	XR_015812
PLEKHA1	0.09	7.56	2.47	0.015	59338	NM_021622
SRFBP1	0.05	6.72	2.47	0.015	153443	NM_152546
LOC642755	0.07	8.38	2.46	0.015	642755	XM_926382
OPN3	0.03	6.62	2.46	0.015	23596	NM_014322
SFT2D2	-0.03	6.48	-2.46	0.015	375035	NM_199344
PSAT1	-0.04	6.50	-2.45	0.016	29968	NM_021154
CREB3L2	0.05	7.57	2.43	0.017	64764	NM_194071
HEATR2	0.04	7.03	2.43	0.017	54919	NM_017802
CD82	0.10	7.99	2.42	0.017	3732	NM_001024844
NPAL3	0.06	7.33	2.42	0.017	57185	NM_020448
PMS2L3	0.06	6.93	2.42	0.017	5387	NM_005395
WNT10A	0.03	6.48	2.41	0.017	80326	NM_025216
CXXC5	0.09	8.13	2.41	0.017	51523	NM_016463
RMND1	-0.02	6.47	-2.41	0.017	55005	NM_017909
BEND5	0.05	6.70	2.41	0.018	79656	NM_024603
C20orf94	0.07	7.19	2.40	0.018	128710	NM_001009608
ZDHHC8	0.08	8.77	2.40	0.018	29801	NM_013373
C16orf68	-0.07	7.24	-2.39	0.018	79091	NM_024109
CXXC5-dupl	0.15	8.75	2.39	0.018	51523	NM_016463
FAR2	-0.03	6.54	-2.38	0.019	55711	NM_018099
CLN6	0.04	6.55	2.38	0.019	54982	NM_017882
AKAP11	0.04	6.59	2.36	0.020	11215	NM_144490
PSMC1	0.08	9.25	2.36	0.020	5700	NM_002802
TCF20	0.05	7.29	2.36	0.020	6942	NM_181492
STX12	-0.03	6.98	-2.36	0.020	23673	NM_177424
RNF216	0.09	8.02	2.35	0.020	54476	NM_207111
ANKRD36	0.06	6.78	2.35	0.020	375248	NM_198555
RBMX	0.05	7.62	2.35	0.020	27316	NM_002139
FKBP1A	-0.05	6.65	-2.35	0.021	2280	NM_054014
TAPBP	-0.04	6.51	-2.34	0.021	6892	NM_172209
TROVE2	0.04	6.71	2.34	0.021	6738	NM_001042370
IAH1	-0.06	7.28	-2.33	0.021	285148	NM_001039613
CHCHD8	-0.03	6.60	-2.33	0.021	51287	NM_016565

MRPL2	0.05	6.93	2.33	0.021	51069	NM_015950
FANCE	0.03	6.93	2.33	0.022	2178	NM_021922
AAK1	0.12	7.90	2.33	0.022	22848	NM_014911
NUCKS1	0.12	9.43	2.32	0.022	64710	NM_022731
TATDN1	-0.04	6.66	-2.31	0.022	83940	NM_032026
ZDHHC14	0.04	6.57	2.31	0.022	79683	NM_024630
LOC642197	-0.04	6.74	-2.31	0.023	642197	XM_936354
SPIN1	0.04	6.61	2.30	0.023	10927	NM_006717
AADACL1	-0.03	6.52	-2.30	0.023	57552	NM_020792
NSUN5B	0.05	6.64	2.29	0.024	155400	NM_001039575
CNOT1	0.06	8.89	2.29	0.024	23019	NM_016284
ZMYND8	0.04	6.73	2.29	0.024	23613	NM_183048
HSPBL2	-0.04	6.67	-2.28	0.024	653553	NR_024392

Gene names marked '-dupl' denote that there were more probes annotated to the same gene

S8 Table. Background data for Figure 2. Enriched Gene Ontology (GO) categories for biological processes among the 100 probes with the lowest p-values in single-gene linear models (case-control) of blood samples from all cases of epithelial ovarian cancer (EOC), metastatic EOC (56 pairs), serous EOC (45 pairs, almost all were metastatic), and from blood samples collected ≤3 years or >3 years before diagnosis (34 and 31 pairs, respectively).

Model	Gene Ontology ID	Biological process	Gene ratio	Background ratio	p-value	q-value	Gene names
All EOC	GO:0097194	execution phase of apoptosis	4/72	42/7903	0.0005	0.40	*TAOK1/STK24/RFFL/HTRA2*
All EOC	GO:0007626	locomotory behavior	5/72	75/7903	0.0006	0.40	*CLN6/NR4A2/PPT1/PDE1B/HTRA2*
All EOC	GO:0008344	adult locomotory behavior	3/72	30/7903	0.0025	0.71	*NR4A2/PPT1/HTRA2*
All EOC	GO:0035751	regulation of lysosomal lumen pH	2/72	10/7903	0.0035	0.71	*CLN6/PPT1*
All EOC	GO:0008631	intrinsic apoptotic signaling pathway in response to oxidative stress	3/72	34/7903	0.0035	0.71	*STK24/DIABLO/HTRA2*
All EOC	GO:0042417	dopamine metabolic process	2/72	11/7903	0.0043	0.71	*NR4A2/PDE1B*
All EOC	GO:0034599	cellular response to oxidative stress	6/72	181/7903	0.0059	0.71	*FBLN5/EZH2/NR4A2/STK24/DIABLO/HTRA2*
All EOC	GO:0044106	cellular amine metabolic process	4/72	80/7903	0.0059	0.71	*NR4A2/SLC7A7/PDE1B/CHKA*
All EOC	GO:0030534	adult behavior	3/72	42/7903	0.0065	0.71	*NR4A2/PPT1/HTRA2*
All EOC	GO:0009308	amine metabolic process	4/72	82/7903	0.0065	0.71	*NR4A2/SLC7A7/PDE1B/CHKA*
All EOC	GO:0001975	response to amphetamine	2/72	14/7903	0.0069	0.71	*NR4A2/PDE1B*
All EOC	GO:0007035	vacuolar acidification	2/72	15/7903	0.0080	0.71	*CLN6/PPT1*
All EOC	GO:0032467	positive regulation of cytokinesis	2/72	16/7903	0.0090	0.71	*CDC14A/PKP4*
All EOC	GO:0009636	response to toxic substance	7/72	264/7903	0.0099	0.71	*FBLN5/EZH2/NR4A2/PDE1B/CHKA/STK24/HTRA2*
All EOC	GO:0006979	response to oxidative stress	7/72	266/7903	0.0103	0.71	*FBLN5/EZH2/NR4A2/HMOX2/STK24/DIABLO/HTRA2*
All EOC	GO:0090068	positive regulation of cell cycle process	5/72	146/7903	0.0104	0.71	*EZH2/UBE2E2/CDK5R1/CDC14A/PKP4*
All EOC	GO:0006584	catecholamine metabolic process	2/72	19/7903	0.0127	0.71	*NR4A2/PDE1B*
All EOC	GO:0009712	catechol-containing compound metabolic process	2/72	19/7903	0.0127	0.71	*NR4A2/PDE1B*
All EOC	GO:0097164	ammonium ion metabolic process	4/72	100/7903	0.0129	0.71	*NR4A2/PDE1B/PLA2G7/CHKA*
All EOC	GO:0014075	response to amine	2/72	20/7903	0.0140	0.71	*NR4A2/PDE1B*
All EOC	GO:0033238	regulation of cellular amine metabolic process	3/72	58/7903	0.0156	0.71	*NR4A2/SLC7A7/PDE1B*
All EOC	GO:0036473	cell death in response to oxidative stress	3/72	58/7903	0.0156	0.71	*STK24/DIABLO/HTRA2*
All EOC	GO:0007585	respiratory gaseous exchange	2/72	23/7903	0.0183	0.71	*CYSLTR1/NR4A2*
All EOC	GO:0097366	response to bronchodilator	2/72	24/7903	0.0199	0.71	*NR4A2/PDE1B*
All EOC	GO:1902475	L-alpha-amino acid transmembrane transport	2/72	24/7903	0.0199	0.71	*SLC36A4/SLC7A7*
All EOC	GO:0009069	serine family amino acid metabolic process	2/72	25/7903	0.0215	0.71	*PSAT1/THNSL2*
All EOC	GO:1901615	organic hydroxy compound metabolic process	6/72	240/7903	0.0217	0.71	*PSAT1/CLN6/NR4A2/INPP4A/PDE1B/CHKA*
All EOC	GO:0006643	membrane lipid metabolic process	4/72	118/7903	0.0223	0.71	*CLN6/PPT1/MPPE1/HTRA2*
All EOC	GO:0051402	neuron apoptotic process	4/72	118/7903	0.0223	0.71	*NR4A2/PPT1/CDK5R1/DIABLO*
All EOC	GO:1902808	positive regulation of cell cycle G1/S phase transition	2/72	26/7903	0.0231	0.71	*EZH2/UBE2E2*
All EOC	GO:0070997	neuron death	5/72	183/7903	0.0253	0.71	*NR4A2/PPT1/CDK5R1/DIABLO/HTRA2*
All EOC	GO:0030203	glycosaminoglycan metabolic process	3/72	70/7903	0.0257	0.71	*B3GAT1/CLN6/ABCC5*
All EOC	GO:0015807	L-amino acid transport	2/72	28/7903	0.0266	0.71	*SLC36A4/SLC7A7*
All EOC	GO:0050773	regulation of dendrite development	3/72	71/7903	0.0267	0.71	*EZH2/CDK5R1/LLPH*
All EOC	GO:0006022	aminoglycan metabolic process	3/72	73/7903	0.0286	0.71	*B3GAT1/CLN6/ABCC5*
All EOC	GO:0045787	positive regulation of cell cycle	5/72	190/7903	0.0291	0.71	*EZH2/UBE2E2/CDK5R1/CDC14A/PKP4*
All EOC	GO:0046474	glycerophospholipid biosynthetic process	4/72	132/7903	0.0320	0.71	*INPP4A/GPAT3/MPPE1/CHKA*
All EOC	GO:0006650	glycerophospholipid metabolic process	5/72	195/7903	0.0321	0.71	*INPP4A/GPAT3/MPPE1/PLA2G7/CHKA*
All EOC	GO:0008306	associative learning	2/72	32/7903	0.0340	0.71	*PPT1/PDE1B*
All EOC	GO:0018958	phenol-containing compound metabolic process	2/72	32/7903	0.0340	0.71	*NR4A2/PDE1B*
All EOC	GO:0051452	intracellular pH reduction	2/72	32/7903	0.0340	0.71	*CLN6/PPT1*
All EOC	GO:0007610	behavior	5/72	199/7903	0.0346	0.71	*CLN6/NR4A2/PPT1/PDE1B/HTRA2*
All EOC	GO:0043281	regulation of cysteine-type endopeptidase activity involved in apoptotic process	4/72	136/7903	0.0351	0.71	*DIABLO/RFFL/HTRA2/CTSH*
All EOC	GO:0030900	forebrain development	4/72	137/7903	0.0359	0.71	*EZH2/NR4A2/CDK5R1/HTRA2*
All EOC	GO:0003333	amino acid transmembrane transport	2/72	33/7903	0.0360	0.71	*SLC36A4/SLC7A7*
All EOC	GO:0045851	pH reduction	2/72	33/7903	0.0360	0.71	*CLN6/PPT1*
All EOC	GO:0051781	positive regulation of cell division	2/72	33/7903	0.0360	0.71	*CDC14A/PKP4*
All EOC	GO:0034394	protein localization to cell surface	2/72	34/7903	0.0381	0.71	*FBLN5/RAB11FIP5*
All EOC	GO:0052548	regulation of endopeptidase activity	5/72	206/7903	0.0393	0.71	*DIABLO/RFFL/HTRA2/SERPINB8/CTSH*
All EOC	GO:0006665	sphingolipid metabolic process	3/72	84/7903	0.0409	0.71	*CLN6/PPT1/HTRA2*
All EOC	GO:0032526	response to retinoic acid	2/72	36/7903	0.0423	0.71	*HTRA2/CTSH*
All EOC	GO:0043280	positive regulation of cysteine-type endopeptidase activity involved in apoptotic process	3/72	86/7903	0.0434	0.71	*DIABLO/HTRA2/CTSH*
All EOC	GO:0045017	glycerolipid biosynthetic process	4/72	146/7903	0.0438	0.71	*INPP4A/GPAT3/MPPE1/CHKA*
All EOC	GO:0021766	hippocampus development	2/72	37/7903	0.0444	0.71	*EZH2/CDK5R1*
All EOC	GO:1900006	positive regulation of dendrite development	2/72	37/7903	0.0444	0.71	*EZH2/LLPH*
All EOC	GO:1901607	alpha-amino acid biosynthetic process	2/72	37/7903	0.0444	0.71	*PSAT1/THNSL2*
All EOC	GO:0006879	cellular iron ion homeostasis	2/72	38/7903	0.0466	0.71	*CYBRD1/HMOX2*
All EOC	GO:0060998	regulation of dendritic spine development	2/72	38/7903	0.0466	0.71	*CDK5R1/LLPH*
All EOC	GO:2000116	regulation of cysteine-type endopeptidase activity	4/72	150/7903	0.0475	0.71	*DIABLO/RFFL/HTRA2/CTSH*
All EOC	GO:0021549	cerebellum development	2/72	39/7903	0.0488	0.71	*EZH2/CDK5R1*
All EOC	GO:0052547	regulation of peptidase activity	5/72	220/7903	0.0497	0.71	*DIABLO/RFFL/HTRA2/SERPINB8/CTSH*
Metastatic	GO:0097164	ammonium ion metabolic process	6/72	100/7903	0.0003	0.50	*NR4A2/PLA2G7/PDE1B/PLBD1/CHKA/CPT1B*
Metastatic	GO:0001764	neuron migration	4/72	48/7903	0.0009	0.56	*NR4A2/MEF2C/CDK5R1/NSMF*
Metastatic	GO:0043523	regulation of neuron apoptotic process	5/72	101/7903	0.0022	0.56	*NR4A2/MEF2C/PPT1/CDK5R1/NSMF*
Metastatic	GO:0016358	dendrite development	5/72	112/7903	0.0034	0.56	*EZH2/MEF2C/CDK5R1/NSMF/CD3E*
Metastatic	GO:0003299	muscle hypertrophy in response to stress	2/72	10/7903	0.0035	0.56	*EZH2/MEF2C*
Metastatic	GO:0014887	cardiac muscle adaptation	2/72	10/7903	0.0035	0.56	*EZH2/MEF2C*
Metastatic	GO:0014898	cardiac muscle hypertrophy in response to stress	2/72	10/7903	0.0035	0.56	*EZH2/MEF2C*
Metastatic	GO:0035751	regulation of lysosomal lumen pH	2/72	10/7903	0.0035	0.56	*PPT1/CLN6*
Metastatic	GO:0090026	positive regulation of monocyte chemotaxis	2/72	10/7903	0.0035	0.56	*PLA2G7/MOSPD2*
Metastatic	GO:0050773	regulation of dendrite development	4/72	71/7903	0.0039	0.56	*EZH2/MEF2C/CDK5R1/NSMF*
Metastatic	GO:0042417	dopamine metabolic process	2/72	11/7903	0.0043	0.56	*NR4A2/PDE1B*
Metastatic	GO:0051402	neuron apoptotic process	5/72	118/7903	0.0043	0.56	*NR4A2/MEF2C/PPT1/CDK5R1/NSMF*
Metastatic	GO:0007626	locomotory behavior	4/72	75/7903	0.0047	0.56	*NR4A2/PPT1/CLN6/PDE1B*
Metastatic	GO:0021549	cerebellum development	3/72	39/7903	0.0052	0.56	*EZH2/CDK5R1/CD3E*
Metastatic	GO:0044106	cellular amine metabolic process	4/72	80/7903	0.0059	0.56	*NR4A2/SLC7A7/PDE1B/CHKA*
Metastatic	GO:0090025	regulation of monocyte chemotaxis	2/72	13/7903	0.0060	0.56	*PLA2G7/MOSPD2*
Metastatic	GO:0002521	leukocyte differentiation	8/72	305/7903	0.0063	0.56	*MEF2C/LCK/PDE1B/TCF7/CD8A/CD2/LTBR/CD3E*
Metastatic	GO:0009308	amine metabolic process	4/72	82/7903	0.0065	0.56	*NR4A2/SLC7A7/PDE1B/CHKA*
Metastatic	GO:0022037	metencephalon development	3/72	43/7903	0.0069	0.56	*EZH2/CDK5R1/CD3E*
Metastatic	GO:0001975	response to amphetamine	2/72	14/7903	0.0069	0.56	*NR4A2/PDE1B*
Metastatic	GO:0071677	positive regulation of mononuclear cell migration	2/72	14/7903	0.0069	0.56	*PLA2G7/MOSPD2*
Metastatic	GO:0031110	regulation of microtubule polymerization or depolymerization	3/72	45/7903	0.0078	0.56	*TAOK1/TERF1/CDK5R1*
Metastatic	GO:0007035	vacuolar acidification	2/72	15/7903	0.0080	0.56	*PPT1/CLN6*
Metastatic	GO:0014888	striated muscle adaptation	2/72	15/7903	0.0080	0.56	*EZH2/MEF2C*
Metastatic	GO:0031579	membrane raft organization	2/72	15/7903	0.0080	0.56	*PPT1/CD2*
Metastatic	GO:0007601	visual perception	3/72	47/7903	0.0088	0.57	*PPT1/CLN6/RPGRIP1*
Metastatic	GO:0048666	neuron development	10/72	465/7903	0.0090	0.57	*EZH2/NR4A2/MEF2C/PPT1/TCTN1/CDK5R1/NSMF/CD3E/LGR6/RPGRIP1*
Metastatic	GO:0001773	myeloid dendritic cell activation	2/72	16/7903	0.0090	0.57	*CD2/LTBR*

Metastatic	GO:0050953	sensory perception of light stimulus	3/72	48/7903	0.0094	0.57	*PPT1/CLN6/RPGRIP1*
Metastatic	GO:1905207	regulation of cardiocyte differentiation	2/72	17/7903	0.0102	0.57	*MEF2C/PRICKLE1*
Metastatic	GO:0045737	positive regulation of cyclin-dependent protein serine/threonine kinase activity	2/72	18/7903	0.0114	0.57	*CDK5R1/CCNY*
Metastatic	GO:0006584	catecholamine metabolic process	2/72	19/7903	0.0127	0.57	*NR4A2/PDE1B*
Metastatic	GO:0009712	catechol-containing compound metabolic process	2/72	19/7903	0.0127	0.57	*NR4A2/PDE1B*
Metastatic	GO:0021955	central nervous system neuron axonogenesis	2/72	19/7903	0.0127	0.57	*NR4A2/TCTN1*
Metastatic	GO:1904031	positive regulation of cyclin-dependent protein kinase activity	2/72	19/7903	0.0127	0.57	*CDK5R1/CCNY*
Metastatic	GO:0030902	hindbrain development	3/72	54/7903	0.0129	0.57	*EZH2/CDK5R1/CD3E*
Metastatic	GO:0030217	T cell differentiation	5/72	154/7903	0.0129	0.57	*LCK/TCF7/CD8A/CD2/CD3E*
Metastatic	GO:0014075	response to amine	2/72	20/7903	0.0140	0.57	*NR4A2/PDE1B*
Metastatic	GO:0060070	canonical Wnt signaling pathway	5/72	160/7903	0.0150	0.57	*NR4A2/PRICKLE1/TCF7/CCNY/LGR6*
Metastatic	GO:0044772	mitotic cell cycle phase transition	8/72	356/7903	0.0152	0.57	*TAOK1/EZH2/ENSA/CENPE/CDC14A/UBE2E2/CCNY/POLE*
Metastatic	GO:0031116	positive regulation of microtubule polymerization	2/72	21/7903	0.0154	0.57	*TERF1/CDK5R1*
Metastatic	GO:0033238	regulation of cellular amine metabolic process	3/72	58/7903	0.0156	0.57	*NR4A2/SLC7A7/PDE1B*
Metastatic	GO:1901214	regulation of neuron death	5/72	162/7903	0.0158	0.57	*NR4A2/MEF2C/PPT1/CDK5R1/NSMF*
Metastatic	GO:0030224	monocyte differentiation	2/72	22/7903	0.0168	0.57	*MEF2C/PDE1B*
Metastatic	GO:0071675	regulation of mononuclear cell migration	2/72	22/7903	0.0168	0.57	*PLA2G7/MOSPD2*
Metastatic	GO:1903131	mononuclear cell differentiation	2/72	22/7903	0.0168	0.57	*MEF2C/PDE1B*
Metastatic	GO:0050877	nervous system process	7/72	294/7903	0.0171	0.57	*MEF2C/PPT1/KCNAB2/CLN6/PDE1B/CDC14A/RPGRIP1*
Metastatic	GO:1905114	cell surface receptor signaling pathway involved in cell-cell signaling	7/72	294/7903	0.0171	0.57	*NR4A2/MEF2C/PRICKLE1/TCF7/C12orf43/CCNY/LGR6*
Metastatic	GO:0031109	microtubule polymerization or depolymerization	3/72	61/7903	0.0179	0.57	*TAOK1/TERF1/CDK5R1*
Metastatic	GO:0007585	respiratory gaseous exchange	2/72	23/7903	0.0183	0.57	*CYSLTR1/NR4A2*
Metastatic	GO:0008589	regulation of smoothened signaling pathway	2/72	23/7903	0.0183	0.57	*TCTN1/CD3E*
Metastatic	GO:0031112	positive regulation of microtubule polymerization or depolymerization	2/72	23/7903	0.0183	0.57	*TERF1/CDK5R1*
Metastatic	GO:0033555	multicellular organismal response to stress	2/72	23/7903	0.0183	0.57	*NR4A2/MEF2C*
Metastatic	GO:0002548	monocyte chemotaxis	2/72	24/7903	0.0199	0.57	*PLA2G7/MOSPD2*
Metastatic	GO:0097366	response to bronchodilator	2/72	24/7903	0.0199	0.57	*NR4A2/PDE1B*
Metastatic	GO:0046916	cellular transition metal ion homeostasis	3/72	64/7903	0.0203	0.57	*HMOX2/CYBRD1/LCK*
Metastatic	GO:0044770	cell cycle phase transition	8/72	377/7903	0.0207	0.57	*TAOK1/EZH2/ENSA/CENPE/CDC14A/UBE2E2/CCNY/POLE*
Metastatic	GO:0032201	telomere maintenance via semi-conservative replication	2/72	25/7903	0.0215	0.57	*TERF1/POLE*
Metastatic	GO:0045661	regulation of myoblast differentiation	2/72	25/7903	0.0215	0.57	*MEF2C/PRICKLE1*
Metastatic	GO:0043524	negative regulation of neuron apoptotic process	3/72	66/7903	0.0220	0.57	*NR4A2/MEF2C/PPT1*
Metastatic	GO:0048667	cell morphogenesis involved in neuron differentiation	6/72	241/7903	0.0221	0.57	*NR4A2/MEF2C/TCTN1/CDK5R1/NSMF/LGR6*
Metastatic	GO:0060562	epithelial tube morphogenesis	4/72	118/7903	0.0223	0.57	*MEF2C/TCTN1/PRICKLE1/CTSH*
Metastatic	GO:1902808	positive regulation of cell cycle G1/S phase transition	2/72	26/7903	0.0231	0.57	*EZH2/UBE2E2*
Metastatic	GO:0021915	neural tube development	3/72	69/7903	0.0247	0.57	*TCTN1/PRICKLE1/TCF7*
Metastatic	GO:0007215	glutamate receptor signaling pathway	2/72	27/7903	0.0248	0.57	*MEF2C/CDK5R1*
Metastatic	GO:0070997	neuron death	5/72	183/7903	0.0253	0.57	*NR4A2/MEF2C/PPT1/CDK5R1/NSMF*
Metastatic	GO:0019725	cellular homeostasis	9/72	471/7903	0.0264	0.57	*CYSLTR1/TAOK1/HMOX2/CYBRD1/LCK/PPT1/CLN6/TERF1/POLE*
Metastatic	GO:0034113	heterotypic cell-cell adhesion	2/72	29/7903	0.0284	0.57	*LCK/CD2*
Metastatic	GO:0045787	positive regulation of cell cycle	5/72	190/7903	0.0291	0.57	*EZH2/CDC14A/CDK5R1/UBE2E2/CCNY*
Metastatic	GO:0008344	adult locomotory behavior	2/72	30/7903	0.0302	0.57	*NR4A2/PPT1*
Metastatic	GO:0021954	central nervous system neuron development	2/72	30/7903	0.0302	0.57	*NR4A2/TCTN1*
Metastatic	GO:0055076	transition metal ion homeostasis	3/72	76/7903	0.0318	0.57	*HMOX2/CYBRD1/LCK*
Metastatic	GO:0006650	glycerophospholipid metabolic process	5/72	195/7903	0.0321	0.57	*PLA2G7/MPPE1/GPAT3/PLBD1/CHKA*
Metastatic	GO:0016055	Wnt signaling pathway	6/72	265/7903	0.0332	0.57	*NR4A2/PRICKLE1/TCF7/C12orf43/CCNY/LGR6*
Metastatic	GO:0198738	cell-cell signaling by wnt	6/72	266/7903	0.0337	0.57	*NR4A2/PRICKLE1/TCF7/C12orf43/CCNY/LGR6*
Metastatic	GO:0008306	associative learning	2/72	32/7903	0.0340	0.57	*PPT1/PDE1B*
Metastatic	GO:0018958	phenol-containing compound metabolic process	2/72	32/7903	0.0340	0.57	*NR4A2/PDE1B*
Metastatic	GO:0051452	intracellular pH reduction	2/72	32/7903	0.0340	0.57	*PPT1/CLN6*
Metastatic	GO:0007610	behavior	5/72	199/7903	0.0346	0.57	*NR4A2/MEF2C/PPT1/CLN6/PDE1B*
Metastatic	GO:0050803	regulation of synapse structure or activity	3/72	79/7903	0.0350	0.57	*MEF2C/PPT1/CDK5R1*
Metastatic	GO:0030900	forebrain development	4/72	137/7903	0.0359	0.57	*EZH2/NR4A2/TCTN1/CDK5R1*
Metastatic	GO:0031113	regulation of microtubule polymerization	2/72	33/7903	0.0360	0.57	*TERF1/CDK5R1*
Metastatic	GO:0045851	pH reduction	2/72	33/7903	0.0360	0.57	*PPT1/CLN6*
Metastatic	GO:0001505	regulation of neurotransmitter levels	4/72	138/7903	0.0368	0.57	*MEF2C/PPT1/PDE1B/CHKA*
Metastatic	GO:0007600	sensory perception	4/72	139/7903	0.0376	0.57	*PPT1/CLN6/CDC14A/RPGRIP1*
Metastatic	GO:0032729	positive regulation of interferon-gamma production	2/72	34/7903	0.0381	0.57	*CD2/CD3E*
Metastatic	GO:0034394	protein localization to cell surface	2/72	34/7903	0.0381	0.57	*FBLN5/KCNAB2*
Metastatic	GO:0042246	tissue regeneration	2/72	34/7903	0.0381	0.57	*EZH2/LGR6*
Metastatic	GO:0045445	myoblast differentiation	2/72	34/7903	0.0381	0.57	*MEF2C/PRICKLE1*
Metastatic	GO:0031175	neuron projection development	8/72	426/7903	0.0390	0.57	*EZH2/NR4A2/MEF2C/TCTN1/CDK5R1/NSMF/CD3E/LGR6*
Metastatic	GO:0016311	dephosphorylation	6/72	278/7903	0.0405	0.57	*ENSA/MEF2C/LCK/CDC14A/THNSL2/NSMF*
Metastatic	GO:0051153	regulation of striated muscle cell differentiation	2/72	36/7903	0.0423	0.57	*EZH2/MEF2C*
Metastatic	GO:0071674	mononuclear cell migration	2/72	36/7903	0.0423	0.57	*PLA2G7/MOSPD2*
Metastatic	GO:0090068	positive regulation of cell cycle process	4/72	146/7903	0.0438	0.57	*EZH2/CDC14A/CDK5R1/UBE2E2*
Metastatic	GO:0030098	lymphocyte differentiation	5/72	213/7903	0.0443	0.57	*LCK/TCF7/CD8A/CD2/CD3E*
Metastatic	GO:0021766	hippocampus development	2/72	37/7903	0.0444	0.57	*EZH2/CDK5R1*
Metastatic	GO:0006879	cellular iron ion homeostasis	2/72	38/7903	0.0466	0.57	*HMOX2/CYBRD1*
Metastatic	GO:0035306	positive regulation of dephosphorylation	2/72	38/7903	0.0466	0.57	*MEF2C/NSMF*
Metastatic	GO:0060998	regulation of dendritic spine development	2/72	38/7903	0.0466	0.57	*MEF2C/CDK5R1*
Metastatic	GO:0050900	leukocyte migration	5/72	218/7903	0.0481	0.57	*PLA2G7/SLC7A7/LCK/CD2/MOSPD2*
Metastatic	GO:0007160	cell-matrix adhesion	3/72	90/7903	0.0485	0.57	*FBLN5/TIMM10B/CD3E*
Metastatic	GO:0007611	learning or memory	3/72	90/7903	0.0485	0.57	*MEF2C/PPT1/PDE1B*
Metastatic	GO:0007224	smoothened signaling pathway	2/72	39/7903	0.0488	0.57	*TCTN1/CD3E*
Serous	GO:0021549	cerebellum development	5/73	39/7903	0.0000	0.04	*SERPINE2/CDK5R1/PAK1/EZH2/HERC1*
Serous	GO:0022037	metencephalon development	5/73	43/7903	0.0000	0.04	*SERPINE2/CDK5R1/PAK1/EZH2/HERC1*
Serous	GO:0030902	hindbrain development	5/73	54/7903	0.0001	0.07	*SERPINE2/CDK5R1/PAK1/EZH2/HERC1*
Serous	GO:0021695	cerebellar cortex development	3/73	18/7903	0.0006	0.22	*SERPINE2/EZH2/HERC1*
Serous	GO:0031110	regulation of microtubule polymerization or depolymerization	4/73	45/7903	0.0007	0.22	*TAOK1/CDK5R1/PAK1/FES*
Serous	GO:0031116	positive regulation of microtubule polymerization	3/73	21/7903	0.0009	0.22	*CDK5R1/PAK1/FES*
Serous	GO:0030224	monocyte differentiation	3/73	22/7903	0.0010	0.22	*MEF2C/PDE1B/FES*
Serous	GO:1903131	mononuclear cell differentiation	3/73	22/7903	0.0010	0.22	*MEF2C/PDE1B/FES*
Serous	GO:0031112	positive regulation of microtubule polymerization or depolymerization	3/73	23/7903	0.0012	0.22	*CDK5R1/PAK1/FES*
Serous	GO:0031109	microtubule polymerization or depolymerization	4/73	61/7903	0.0024	0.39	*TAOK1/CDK5R1/PAK1/FES*
Serous	GO:0031113	regulation of microtubule polymerization	3/73	33/7903	0.0034	0.40	*CDK5R1/PAK1/FES*
Serous	GO:0003299	muscle hypertrophy in response to stress	2/73	10/7903	0.0036	0.40	*EZH2/MEF2C*
Serous	GO:0014887	cardiac muscle adaptation	2/73	10/7903	0.0036	0.40	*EZH2/MEF2C*

Serous	GO:0014898	cardiac muscle hypertrophy in response to stress	2/73	10/7903	0.0036	0.40	*EZH2/MEF2C*
Serous	GO:0021696	cerebellar cortex morphogenesis	2/73	10/7903	0.0036	0.40	*SERPINE2/HERC1*
Serous	GO:0050773	regulation of dendrite development	4/73	71/7903	0.0041	0.40	*CDK5R1/EZH2/MEF2C/ASAP1*
Serous	GO:0051153	regulation of striated muscle cell differentiation	3/73	36/7903	0.0043	0.40	*PAK1/EZH2/MEF2C*
Serous	GO:0006525	arginine metabolic process	2/73	11/7903	0.0044	0.40	*SLC7A7/AGMAT*
Serous	GO:0006879	cellular iron ion homeostasis	3/73	38/7903	0.0051	0.40	*CYBRD1/HMOX2/CUL1*
Serous	GO:0060998	regulation of dendritic spine development	3/73	38/7903	0.0051	0.40	*CDK5R1/MEF2C/ASAP1*
Serous	GO:1904738	vascular associated smooth muscle cell migration	2/73	12/7903	0.0052	0.40	*PAK1/MEF2C*
Serous	GO:1904752	regulation of vascular associated smooth muscle cell migration	2/73	12/7903	0.0052	0.40	*PAK1/MEF2C*
Serous	GO:0051154	negative regulation of striated muscle cell differentiation	2/73	13/7903	0.0061	0.40	*PAK1/EZH2*
Serous	GO:0003300	cardiac muscle hypertrophy	3/73	41/7903	0.0063	0.40	*PAK1/EZH2/MEF2C*
Serous	GO:0014897	striated muscle hypertrophy	3/73	41/7903	0.0063	0.40	*PAK1/EZH2/MEF2C*
Serous	GO:0030308	negative regulation of cell growth	4/73	81/7903	0.0065	0.40	*PPT1/SERPINE2/CDK5R1/PAK1*
Serous	GO:0014896	muscle hypertrophy	3/73	42/7903	0.0067	0.40	*PAK1/EZH2/MEF2C*
Serous	GO:0007618	mating	2/73	14/7903	0.0071	0.40	*SERPINE2/PI3*
Serous	GO:0021575	hindbrain morphogenesis	2/73	14/7903	0.0071	0.40	*SERPINE2/HERC1*
Serous	GO:0021587	cerebellum morphogenesis	2/73	14/7903	0.0071	0.40	*SERPINE2/HERC1*
Serous	GO:0030282	bone mineralization	3/73	45/7903	0.0081	0.40	*FBN2/MEF2C/ASGR2*
Serous	GO:0014888	striated muscle adaptation	2/73	15/7903	0.0082	0.40	*EZH2/MEF2C*
Serous	GO:0030501	positive regulation of bone mineralization	2/73	15/7903	0.0082	0.40	*FBN2/MEF2C*
Serous	GO:2000725	regulation of cardiac muscle cell differentiation	2/73	15/7903	0.0082	0.40	*PAK1/MEF2C*
Serous	GO:0043500	muscle adaptation	3/73	46/7903	0.0086	0.41	*PAK1/EZH2/MEF2C*
Serous	GO:0046785	microtubule polymerization	3/73	47/7903	0.0092	0.42	*CDK5R1/PAK1/FES*
Serous	GO:0055072	iron ion homeostasis	3/73	47/7903	0.0092	0.42	*CYBRD1/HMOX2/CUL1*
Serous	GO:0010762	regulation of fibroblast migration	2/73	17/7903	0.0105	0.42	*PAK1/RFFL*
Serous	GO:0030195	negative regulation of blood coagulation	2/73	17/7903	0.0105	0.42	*SERPINE2/USF1*
Serous	GO:0050819	negative regulation of coagulation	2/73	17/7903	0.0105	0.42	*SERPINE2/USF1*
Serous	GO:1900047	negative regulation of hemostasis	2/73	17/7903	0.0105	0.42	*SERPINE2/USF1*
Serous	GO:1905207	regulation of cardiocyte differentiation	2/73	17/7903	0.0105	0.42	*PAK1/MEF2C*
Serous	GO:0050769	positive regulation of neurogenesis	6/73	205/7903	0.0114	0.44	*SERPINE2/PAK1/SPEN/EZH2/MEF2C/FES*
Serous	GO:0070169	positive regulation of biomineral tissue development	2/73	18/7903	0.0117	0.44	*FBN2/MEF2C*
Serous	GO:0090314	positive regulation of protein targeting to membrane	2/73	18/7903	0.0117	0.44	*CDK5R1/PAK1*
Serous	GO:0060996	dendritic spine development	3/73	53/7903	0.0127	0.46	*CDK5R1/MEF2C/ASAP1*
Serous	GO:0050767	regulation of neurogenesis	8/73	344/7903	0.0136	0.48	*SERPINE2/CDK5R1/PAK1/SPEN/EZH2/MEF2C/FES/ASAP1*
Serous	GO:0070507	regulation of microtubule cytoskeleton organization	4/73	101/7903	0.0140	0.48	*TAOK1/CDK5R1/PAK1/FES*
Serous	GO:0010975	regulation of neuron projection development	6/73	215/7903	0.0142	0.48	*CDK5R1/PAK1/EZH2/MEF2C/FES/ASAP1*
Serous	GO:0051966	regulation of synaptic transmission, glutamatergic	2/73	20/7903	0.0144	0.48	*SERPINE2/MEF2C*
Serous	GO:0051962	positive regulation of nervous system development	6/73	220/7903	0.0157	0.49	*SERPINE2/PAK1/SPEN/EZH2/MEF2C/FES*
Serous	GO:0010922	positive regulation of phosphatase activity	2/73	21/7903	0.0158	0.49	*MEF2C/CD33*
Serous	GO:0031638	zymogen activation	2/73	21/7903	0.0158	0.49	*SERPINE2/CTSH*
Serous	GO:0050890	cognition	4/73	106/7903	0.0164	0.49	*PPT1/MEF2C/PDE1B/LINS1*
Serous	GO:0045926	negative regulation of growth	4/73	107/7903	0.0169	0.49	*PPT1/SERPINE2/CDK5R1/PAK1*
Serous	GO:0010761	fibroblast migration	2/73	22/7903	0.0173	0.49	*PAK1/RFFL*
Serous	GO:0055021	regulation of cardiac muscle tissue growth	2/73	22/7903	0.0173	0.49	*PAK1/MEF2C*
Serous	GO:0090313	regulation of protein targeting to membrane	2/73	22/7903	0.0173	0.49	*CDK5R1/PAK1*
Serous	GO:0002456	T cell mediated immunity	3/73	60/7903	0.0178	0.49	*CTSH/BTN3A2/TNFSF4*
Serous	GO:0031214	biomineral tissue development	3/73	60/7903	0.0178	0.49	*FBN2/MEF2C/ASGR2*
Serous	GO:0050877	nervous system process	7/73	294/7903	0.0184	0.49	*PPT1/RPGRIP1/SERPINE2/MEF2C/PDE1B/HERC1/LINS1*
Serous	GO:0016358	dendrite development	4/73	112/7903	0.0197	0.49	*CDK5R1/EZH2/MEF2C/ASAP1*
Serous	GO:0032956	regulation of actin cytoskeleton organization	5/73	170/7903	0.0201	0.49	*TAOK1/CDK5R1/PAK1/MEF2C/FES*
Serous	GO:0120035	regulation of plasma membrane bounded cell projection organization	7/73	300/7903	0.0203	0.49	*CDK5R1/PAK1/EZH2/MEF2C/ATMIN/FES/ASAP1*
Serous	GO:0061045	negative regulation of wound healing	2/73	24/7903	0.0204	0.49	*SERPINE2/USF1*
Serous	GO:0051960	regulation of nervous system development	8/73	371/7903	0.0205	0.49	*SERPINE2/CDK5R1/PAK1/SPEN/EZH2/MEF2C/FES/ASAP1*
Serous	GO:0046916	cellular transition metal ion homeostasis	3/73	64/7903	0.0211	0.49	*CYBRD1/HMOX2/CUL1*
Serous	GO:0098869	cellular oxidant detoxification	3/73	64/7903	0.0211	0.49	*FBLN5/ALB/KDM3B*
Serous	GO:0031344	regulation of cell projection organization	7/73	304/7903	0.0216	0.49	*CDK5R1/PAK1/EZH2/MEF2C/ATMIN/FES/ASAP1*
Serous	GO:0035249	synaptic transmission, glutamatergic	2/73	25/7903	0.0220	0.49	*SERPINE2/MEF2C*
Serous	GO:0060420	regulation of heart growth	2/73	25/7903	0.0220	0.49	*PAK1/MEF2C*
Serous	GO:0070317	negative regulation of G0 to G1 transition	2/73	25/7903	0.0220	0.49	*RYBP/EZH2*
Serous	GO:0010720	positive regulation of cell development	6/73	238/7903	0.0222	0.49	*SERPINE2/PAK1/SPEN/EZH2/MEF2C/FES*
Serous	GO:0045669	positive regulation of osteoblast differentiation	2/73	26/7903	0.0237	0.49	*FBN2/MEF2C*
Serous	GO:0051148	negative regulation of muscle cell differentiation	2/73	26/7903	0.0237	0.49	*PAK1/EZH2*
Serous	GO:0040008	regulation of growth	7/73	312/7903	0.0245	0.49	*FBLN5/PPT1/SERPINE2/CDK5R1/PAK1/MEF2C/TKT*
Serous	GO:0002573	myeloid leukocyte differentiation	4/73	120/7903	0.0246	0.49	*MEF2C/PDE1B/LTBR/FES*
Serous	GO:0032886	regulation of microtubule-based process	4/73	120/7903	0.0246	0.49	*TAOK1/CDK5R1/PAK1/FES*
Serous	GO:0032946	positive regulation of mononuclear cell proliferation	3/73	68/7903	0.0247	0.49	*MEF2C/DNAJA3/TNFSF4*
Serous	GO:0050671	positive regulation of lymphocyte proliferation	3/73	68/7903	0.0247	0.49	*MEF2C/DNAJA3/TNFSF4*
Serous	GO:0051147	regulation of muscle cell differentiation	3/73	68/7903	0.0247	0.49	*PAK1/EZH2/MEF2C*
Serous	GO:0007215	glutamate receptor signaling pathway	2/73	27/7903	0.0255	0.49	*CDK5R1/MEF2C*
Serous	GO:0045023	G0 to G1 transition	2/73	27/7903	0.0255	0.49	*RYBP/EZH2*
Serous	GO:0055024	regulation of cardiac muscle tissue development	2/73	27/7903	0.0255	0.49	*PAK1/MEF2C*
Serous	GO:0070316	regulation of G0 to G1 transition	2/73	27/7903	0.0255	0.49	*RYBP/EZH2*
Serous	GO:1902903	regulation of supramolecular fiber organization	5/73	181/7903	0.0256	0.49	*TAOK1/CDK5R1/PAK1/MEF2C/FES*
Serous	GO:1990748	cellular detoxification	3/73	69/7903	0.0257	0.49	*FBLN5/ALB/KDM3B*
Serous	GO:0048666	neuron development	9/73	465/7903	0.0266	0.50	*PPT1/RPGRIP1/CDK5R1/PAK1/EZH2/MEF2C/HERC1/FES/ASAP1*
Serous	GO:0032970	regulation of actin filament-based process	5/73	183/7903	0.0267	0.50	*TAOK1/CDK5R1/PAK1/MEF2C/FES*
Serous	GO:0070665	positive regulation of leukocyte proliferation	3/73	71/7903	0.0276	0.51	*MEF2C/DNAJA3/TNFSF4*
Serous	GO:0014910	regulation of smooth muscle cell migration	2/73	29/7903	0.0291	0.51	*PAK1/MEF2C*
Serous	GO:1904705	regulation of vascular smooth muscle cell proliferation	2/73	29/7903	0.0291	0.51	*PAK1/MEF2C*
Serous	GO:1990874	vascular smooth muscle cell proliferation	2/73	29/7903	0.0291	0.51	*PAK1/MEF2C*
Serous	GO:0060284	regulation of cell development	8/73	398/7903	0.0296	0.51	*SERPINE2/CDK5R1/PAK1/SPEN/EZH2/MEF2C/FES/ASAP1*
Serous	GO:0030500	regulation of bone mineralization	2/73	30/7903	0.0310	0.51	*FBN2/MEF2C*
Serous	GO:0032273	positive regulation of protein polymerization	3/73	76/7903	0.0329	0.51	*CDK5R1/PAK1/FES*
Serous	GO:0055076	transition metal ion homeostasis	3/73	76/7903	0.0329	0.51	*CYBRD1/HMOX2/CUL1*
Serous	GO:0030193	regulation of blood coagulation	2/73	31/7903	0.0329	0.51	*SERPINE2/USF1*
Serous	GO:1900046	regulation of hemostasis	2/73	31/7903	0.0329	0.51	*SERPINE2/USF1*
Serous	GO:1903035	negative regulation of response to wounding	2/73	31/7903	0.0329	0.51	*SERPINE2/USF1*
Serous	GO:0098754	detoxification	3/73	77/7903	0.0340	0.51	*FBLN5/ALB/KDM3B*
Serous	GO:0008306	associative learning	2/73	32/7903	0.0349	0.51	*PPT1/PDE1B*
Serous	GO:0014909	smooth muscle cell migration	2/73	32/7903	0.0349	0.51	*PAK1/MEF2C*
Serous	GO:0050818	regulation of coagulation	2/73	32/7903	0.0349	0.51	*SERPINE2/USF1*
Serous	GO:0051452	intracellular pH reduction	2/73	32/7903	0.0349	0.51	*PPT1/ATP6V1B2*

Serous	GO:0055017	cardiac muscle tissue growth	2/73	32/7903	0.0349	0.51	*PAK1/MEF2C*
Serous	GO:0001558	regulation of cell growth	5/73	198/7903	0.0357	0.51	*FBLN5/PPT1/SERPINE2/CDK5R1/PAK1*
Serous	GO:0050803	regulation of synapse structure or activity	3/73	79/7903	0.0363	0.51	*PPT1/CDK5R1/MEF2C*
Serous	GO:0045851	pH reduction	2/73	33/7903	0.0370	0.51	*PPT1/ATP6V1B2*
Serous	GO:0044106	cellular amine metabolic process	3/73	80/7903	0.0375	0.51	*SLC7A7/AGMAT/PDE1B*
Serous	GO:0045664	regulation of neuron differentiation	6/73	270/7903	0.0380	0.51	*CDK5R1/PAK1/EZH2/MEF2C/FES/ASAP1*
Serous	GO:0046330	positive regulation of JNK cascade	3/73	81/7903	0.0386	0.51	*TAOK1/PAK1/LTBR*
Serous	GO:0009308	amine metabolic process	3/73	82/7903	0.0399	0.51	*SLC7A7/AGMAT/PDE1B*
Serous	GO:0031670	cellular response to nutrient	2/73	35/7903	0.0412	0.51	*FES/USF1*
Serous	GO:0045778	positive regulation of ossification	2/73	35/7903	0.0412	0.51	*FBN2/MEF2C*
Serous	GO:0060419	heart growth	2/73	35/7903	0.0412	0.51	*PAK1/MEF2C*
Serous	GO:0045597	positive regulation of cell differentiation	8/73	426/7903	0.0418	0.51	*SERPINE2/PAK1/FBN2/SPEN/EZH2/MEF2C/FES/TNFSF4*
Serous	GO:0009064	glutamine family amino acid metabolic process	2/73	36/7903	0.0433	0.51	*SLC7A7/AGMAT*
Serous	GO:0002460	adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	4/73	144/7903	0.0438	0.51	*CTSH/MEF2C/BTN3A2/TNFSF4*
Serous	GO:0098609	cell-cell adhesion	7/73	355/7903	0.0448	0.51	*CD93/SERPINE2/CDK5R1/PAK1/CD33/DNAJA3/TNFSF4*
Serous	GO:0021766	hippocampus development	2/73	37/7903	0.0455	0.51	*CDK5R1/EZH2*
Serous	GO:0070167	regulation of biomineral tissue development	2/73	37/7903	0.0455	0.51	*FBN2/MEF2C*
Serous	GO:0032870	cellular response to hormone stimulus	7/73	358/7903	0.0465	0.51	*CDK5R1/PAK1/CTSH/MEF2C/TNFSF4/USF1/ATP6V1B2*
Serous	GO:0097237	cellular response to toxic substance	4/73	147/7903	0.0466	0.51	*FBLN5/ALB/EZH2/KDM3B*
Serous	GO:0035306	positive regulation of dephosphorylation	2/73	38/7903	0.0478	0.51	*MEF2C/CD33*
Serous	GO:0055007	cardiac muscle cell differentiation	2/73	38/7903	0.0478	0.51	*PAK1/MEF2C*
Serous	GO:0040007	growth	8/73	440/7903	0.0490	0.51	*FBLN5/PPT1/SERPINE2/CDK5R1/PAK1/EZH2/MEF2C/TKT*
≤3 years	GO:0010965	regulation of mitotic sister chromatid separation	3/76	28/7903	0.0024	0.53	*PTTG3P/CENPE/PTTG1*
≤3 years	GO:0050829	defense response to Gram-negative bacterium	3/76	28/7903	0.0024	0.53	*DEFA1B/TNFRSF14/DEFA4*
≤3 years	GO:0051306	mitotic sister chromatid separation	3/76	31/7903	0.0032	0.53	*PTTG3P/CENPE/PTTG1*
≤3 years	GO:1905818	regulation of chromosome separation	3/76	32/7903	0.0035	0.53	*PTTG3P/CENPE/PTTG1*
≤3 years	GO:0002227	innate immune response in mucosa	2/76	10/7903	0.0039	0.53	*DEFA1B/DEFA4*
≤3 years	GO:0033047	regulation of mitotic sister chromatid segregation	3/76	36/7903	0.0049	0.53	*PTTG3P/CENPE/PTTG1*
≤3 years	GO:0050830	defense response to Gram-positive bacterium	3/76	36/7903	0.0049	0.53	*DEFA1B/TNFRSF14/DEFA4*
≤3 years	GO:0031023	microtubule organizing center organization	4/76	73/7903	0.0052	0.53	*RANBP1/HAUS8/ODF2/GCC2*
≤3 years	GO:0002385	mucosal immune response	2/76	12/7903	0.0057	0.53	*DEFA1B/DEFA4*
≤3 years	GO:0060706	cell differentiation involved in embryonic placenta development	2/76	12/7903	0.0057	0.53	*STK4/ASCL2*
≤3 years	GO:0009913	epidermal cell differentiation	4/76	75/7903	0.0057	0.53	*PKP4/KRTAP10-2/EZH2/STK4*
≤3 years	GO:0002251	organ or tissue specific immune response	2/76	13/7903	0.0066	0.53	*DEFA1B/DEFA4*
≤3 years	GO:0070734	histone H3-K27 methylation	2/76	13/7903	0.0066	0.53	*PHF19/EZH2*
≤3 years	GO:0071280	cellular response to copper ion	2/76	13/7903	0.0066	0.53	*MT1E/MT1X*
≤3 years	GO:0006658	phosphatidylserine metabolic process	2/76	14/7903	0.0077	0.53	*OSBPL8/ABHD12*
≤3 years	GO:0045143	homologous chromosome segregation	2/76	14/7903	0.0077	0.53	*PTTG3P/PTTG1*
≤3 years	GO:0050832	defense response to fungus	2/76	14/7903	0.0077	0.53	*DEFA1B/DEFA4*
≤3 years	GO:0007040	lysosome organization	3/76	43/7903	0.0080	0.53	*PPT1/ACP2/HOOK1*
≤3 years	GO:0080171	lytic vacuole organization	3/76	43/7903	0.0080	0.53	*PPT1/ACP2/HOOK1*
≤3 years	GO:0000070	mitotic sister chromatid segregation	4/76	83/7903	0.0082	0.53	*PTTG3P/CENPE/CHMP7/PTTG1*
≤3 years	GO:0051304	chromosome separation	3/76	44/7903	0.0085	0.53	*PTTG3P/CENPE/PTTG1*
≤3 years	GO:0007088	regulation of mitotic nuclear division	4/76	86/7903	0.0093	0.53	*PTTG3P/RANBP1/CENPE/PTTG1*
≤3 years	GO:0006644	phospholipid metabolic process	7/76	247/7903	0.0093	0.53	*OSBPL8/DGKQ/ABHD12/PLBD1/PMVK/PLA2G7/INPP4A*
≤3 years	GO:0033045	regulation of sister chromatid segregation	3/76	46/7903	0.0096	0.53	*PTTG3P/CENPE/PTTG1*
≤3 years	GO:0032467	positive regulation of cytokinesis	2/76	16/7903	0.0100	0.53	*PKP4/CXCR5*
≤3 years	GO:0006650	glycerophospholipid metabolic process	6/76	195/7903	0.0109	0.53	*OSBPL8/DGKQ/ABHD12/PLBD1/PLA2G7/INPP4A*
≤3 years	GO:0019731	antibacterial humoral response	2/76	17/7903	0.0113	0.53	*DEFA1B/DEFA4*
≤3 years	GO:0140014	mitotic nuclear division	5/76	142/7903	0.0116	0.53	*PTTG3P/RANBP1/CENPE/CHMP7/PTTG1*
≤3 years	GO:0051783	regulation of nuclear division	4/76	92/7903	0.0117	0.53	*PTTG3P/RANBP1/CENPE/PTTG1*
≤3 years	GO:0006882	cellular zinc ion homeostasis	2/76	18/7903	0.0126	0.53	*MT1E/MT1X*
≤3 years	GO:0046640	regulation of alpha-beta T cell proliferation	2/76	18/7903	0.0126	0.53	*TNFSF4/TNFRSF14*
≤3 years	GO:2000816	negative regulation of mitotic sister chromatid separation	2/76	18/7903	0.0126	0.53	*PTTG3P/PTTG1*
≤3 years	GO:0046688	response to copper ion	2/76	19/7903	0.0140	0.53	*MT1E/MT1X*
≤3 years	GO:0055069	zinc ion homeostasis	2/76	19/7903	0.0140	0.53	*MT1E/MT1X*
≤3 years	GO:0071168	protein localization to chromatin	2/76	19/7903	0.0140	0.53	*CHMP7/EZH2*
≤3 years	GO:1905819	negative regulation of chromosome separation	2/76	19/7903	0.0140	0.53	*PTTG3P/PTTG1*
≤3 years	GO:0140056	organelle localization by membrane tethering	4/76	100/7903	0.0155	0.53	*HAUS8/STX6/ODF2/RAB3IP*
≤3 years	GO:0010043	response to zinc ion	2/76	20/7903	0.0155	0.53	*MT1E/MT1X*
≤3 years	GO:0033048	negative regulation of mitotic sister chromatid segregation	2/76	20/7903	0.0155	0.53	*PTTG3P/PTTG1*
≤3 years	GO:0071276	cellular response to cadmium ion	2/76	20/7903	0.0155	0.53	*MT1E/MT1X*
≤3 years	GO:0010035	response to inorganic substance	7/76	273/7903	0.0156	0.53	*OSER1/FBLN5/TNFSF4/CYBRD1/MT1E/EZH2/MT1X*
≤3 years	GO:0097711	ciliary basal body-plasma membrane docking	3/76	55/7903	0.0157	0.53	*HAUS8/ODF2/RAB3IP*
≤3 years	GO:0051983	regulation of chromosome segregation	3/76	56/7903	0.0165	0.53	*PTTG3P/CENPE/PTTG1*
≤3 years	GO:0033046	negative regulation of sister chromatid segregation	2/76	21/7903	0.0170	0.53	*PTTG3P/PTTG1*
≤3 years	GO:0046633	alpha-beta T cell proliferation	2/76	21/7903	0.0170	0.53	*TNFSF4/TNFRSF14*
≤3 years	GO:0030216	keratinocyte differentiation	3/76	57/7903	0.0172	0.53	*PKP4/KRTAP10-2/STK4*
≤3 years	GO:0001837	epithelial to mesenchymal transition	3/76	58/7903	0.0181	0.53	*DAB2/KLHL12/EZH2*
≤3 years	GO:0000819	sister chromatid segregation	4/76	105/7903	0.0182	0.53	*PTTG3P/CENPE/CHMP7/PTTG1*
≤3 years	GO:0031424	keratinization	2/76	22/7903	0.0186	0.53	*PKP4/KRTAP10-2*
≤3 years	GO:0051985	negative regulation of chromosome segregation	2/76	22/7903	0.0186	0.53	*PTTG3P/PTTG1*
≤3 years	GO:0016482	cytosolic transport	4/76	107/7903	0.0193	0.53	*DAB2/STX6/GCC2/HOOK1*
≤3 years	GO:0022406	membrane docking	4/76	107/7903	0.0193	0.53	*HAUS8/STX6/ODF2/RAB3IP*
≤3 years	GO:0045926	negative regulation of growth	4/76	107/7903	0.0193	0.53	*PPT1/MT1E/STK4/MT1X*
≤3 years	GO:0007032	endosome organization	3/76	60/7903	0.0198	0.53	*CHMP7/STX6/HOOK1*
≤3 years	GO:0008544	epidermis development	4/76	108/7903	0.0199	0.53	*PKP4/KRTAP10-2/EZH2/STK4*
≤3 years	GO:0009620	response to fungus	2/76	23/7903	0.0203	0.53	*DEFA1B/DEFA4*
≤3 years	GO:0010718	positive regulation of epithelial to mesenchymal transition	2/76	23/7903	0.0203	0.53	*DAB2/EZH2*
≤3 years	GO:0046636	negative regulation of alpha-beta T cell activation	2/76	23/7903	0.0203	0.53	*TNFSF4/TNFRSF14*
≤3 years	GO:0046486	glycerolipid metabolic process	6/76	225/7903	0.0208	0.53	*OSBPL8/DGKQ/ABHD12/PLBD1/PLA2G7/INPP4A*
≤3 years	GO:0045839	negative regulation of mitotic nuclear division	2/76	24/7903	0.0220	0.55	*PTTG3P/PTTG1*
≤3 years	GO:0030003	cellular cation homeostasis	7/76	295/7903	0.0228	0.55	*PPT1/BDKRB2/CYBRD1/MCOLN2/MT1E/CXCR5/MT1X*
≤3 years	GO:0046916	cellular transition metal ion homeostasis	3/76	64/7903	0.0234	0.55	*CYBRD1/MT1E/MT1X*
≤3 years	GO:0007156	homophilic cell adhesion via plasma membrane adhesion molecules	2/76	25/7903	0.0237	0.55	*PCDHGB6/TRO*
≤3 years	GO:0048260	positive regulation of receptor-mediated endocytosis	2/76	25/7903	0.0237	0.55	*PPT1/DAB2*

≤3 years	GO:1903432	regulation of TORC1 signaling	2/76	25/7903	0.0237	0.55	*DGKQ/FNIP2*
≤3 years	GO:0006873	cellular ion homeostasis	7/76	299/7903	0.0244	0.55	*PPT1/BDKRB2/CYBRD1/MCOLN2/MT1E/CXCR5/MT1X*
≤3 years	GO:0071222	cellular response to lipopolysaccharide	4/76	116/7903	0.0252	0.55	*TNFSF4/DEFA1B/TRIB1/DEFA4*
≤3 years	GO:0002820	negative regulation of adaptive immune response	2/76	26/7903	0.0256	0.55	*TNFSF4/TNFRSF14*
≤3 years	GO:0031640	killing of cells of other organism	2/76	26/7903	0.0256	0.55	*DEFA1B/DEFA4*
≤3 years	GO:0044364	disruption of cells of other organism	2/76	26/7903	0.0256	0.55	*DEFA1B/DEFA4*
≤3 years	GO:0010564	regulation of cell cycle process	9/76	445/7903	0.0262	0.56	*PKP4/PTTG3P/RANBP1/HAUS8/CENPE/ODF2/EZH2/CXCR5/PTTG1*
≤3 years	GO:0045132	meiotic chromosome segregation	2/76	27/7903	0.0274	0.57	*PTTG3P/PTTG1*
≤3 years	GO:0051784	negative regulation of nuclear division	2/76	27/7903	0.0274	0.57	*PTTG3P/PTTG1*
≤3 years	GO:0071219	cellular response to molecule of bacterial origin	4/76	120/7903	0.0281	0.57	*TNFSF4/DEFA1B/TRIB1/DEFA4*
≤3 years	GO:0007098	centrosome cycle	3/76	69/7903	0.0285	0.57	*RANBP1/HAUS8/ODF2*
≤3 years	GO:0006099	tricarboxylic acid cycle	2/76	28/7903	0.0294	0.57	*SDHC/MDH2*
≤3 years	GO:0007080	mitotic metaphase plate congression	2/76	28/7903	0.0294	0.57	*CENPE/CHMP7*
≤3 years	GO:0061844	antimicrobial humoral immune response mediated by antimicrobial peptide	2/76	28/7903	0.0294	0.57	*DEFA1B/DEFA4*
≤3 years	GO:0040008	regulation of growth	7/76	312/7903	0.0298	0.57	*PPT1/FBLN5/KAT5/MT1E/STK4/MRGBP/MT1X*
≤3 years	GO:0033135	regulation of peptidyl-serine phosphorylation	3/76	71/7903	0.0306	0.58	*BDKRB2/STK4/FNIP2*
≤3 years	GO:0006101	citrate metabolic process	2/76	29/7903	0.0313	0.58	*SDHC/MDH2*
≤3 years	GO:0008344	adult locomotory behavior	2/76	30/7903	0.0334	0.60	*PPT1/ABHD12*
≤3 years	GO:0045022	early endosome to late endosome transport	2/76	30/7903	0.0334	0.60	*DAB2/HOOK1*
≤3 years	GO:0000280	nuclear division	5/76	188/7903	0.0343	0.60	*PTTG3P/RANBP1/CENPE/CHMP7/PTTG1*
≤3 years	GO:0055080	cation homeostasis	7/76	322/7903	0.0346	0.60	*PPT1/BDKRB2/CYBRD1/MCOLN2/MT1E/CXCR5/MT1X*
≤3 years	GO:0098813	nuclear chromosome segregation	4/76	129/7903	0.0353	0.60	*PTTG3P/CENPE/CHMP7/PTTG1*
≤3 years	GO:0038202	TORC1 signaling	2/76	31/7903	0.0355	0.60	*DGKQ/FNIP2*
≤3 years	GO:0072350	tricarboxylic acid metabolic process	2/76	31/7903	0.0355	0.60	*SDHC/MDH2*
≤3 years	GO:0098771	inorganic ion homeostasis	7/76	325/7903	0.0361	0.60	*PPT1/BDKRB2/CYBRD1/MCOLN2/MT1E/CXCR5/MT1X*
≤3 years	GO:0006875	cellular metal ion homeostasis	6/76	256/7903	0.0361	0.60	*BDKRB2/CYBRD1/MCOLN2/MT1E/CXCR5/MT1X*
≤3 years	GO:0055076	transition metal ion homeostasis	3/76	76/7903	0.0364	0.60	*CYBRD1/MT1E/MT1X*
≤3 years	GO:0051640	organelle localization	8/76	399/7903	0.0370	0.60	*KLHL12/HAUS8/CENPE/CHMP7/STX6/ODF2/RAB3IP/LAT2*
≤3 years	GO:0098927	vesicle-mediated transport between endosomal compartments	2/76	32/7903	0.0376	0.60	*DAB2/HOOK1*
≤3 years	GO:0098754	detoxification	3/76	77/7903	0.0377	0.60	*FBLN5/MT1E/MT1X*
≤3 years	GO:0000226	microtubule cytoskeleton organization	6/76	260/7903	0.0385	0.60	*RANBP1/HAUS8/CENPE/ODF2/GCC2/HOOK1*
≤3 years	GO:0051310	metaphase plate congression	2/76	33/7903	0.0398	0.60	*CENPE/CHMP7*
≤3 years	GO:0051781	positive regulation of cell division	2/76	33/7903	0.0398	0.60	*PKP4/CXCR5*
≤3 years	GO:0048762	mesenchymal cell differentiation	3/76	80/7903	0.0414	0.60	*DAB2/KLHL12/EZH2*
≤3 years	GO:0002720	positive regulation of cytokine production involved in immune response	2/76	34/7903	0.0420	0.60	*TNFSF4/TNFRSF14*
≤3 years	GO:0034394	protein localization to cell surface	2/76	34/7903	0.0420	0.60	*FBLN5/KCNAB2*
≤3 years	GO:0071216	cellular response to biotic stimulus	4/76	138/7903	0.0435	0.60	*TNFSF4/DEFA1B/TRIB1/DEFA4*
≤3 years	GO:0018022	peptidyl-lysine methylation	3/76	82/7903	0.0441	0.60	*METTL22/PHF19/EZH2*
≤3 years	GO:0046686	response to cadmium ion	2/76	35/7903	0.0443	0.60	*MT1E/MT1X*
≤3 years	GO:0072678	T cell migration	2/76	35/7903	0.0443	0.60	*DEFA1B/TNFRSF14*
≤3 years	GO:0030518	intracellular steroid hormone receptor signaling pathway	3/76	83/7903	0.0454	0.60	*DAB2/DEFA1B/KAT5*
≤3 years	GO:0033865	nucleoside bisphosphate metabolic process	3/76	83/7903	0.0454	0.60	*PPT1/PAPSS1/PMVK*
≤3 years	GO:0033875	ribonucleoside bisphosphate metabolic process	3/76	83/7903	0.0454	0.60	*PPT1/PAPSS1/PMVK*
≤3 years	GO:0034032	purine nucleoside bisphosphate metabolic process	3/76	83/7903	0.0454	0.60	*PPT1/PAPSS1/PMVK*
≤3 years	GO:0043433	negative regulation of DNA-binding transcription factor activity	3/76	83/7903	0.0454	0.60	*TNFSF4/EZH2/TRIB1*
≤3 years	GO:0072503	cellular divalent inorganic cation homeostasis	5/76	206/7903	0.0477	0.60	*BDKRB2/MCOLN2/MT1E/CXCR5/MT1X*
≤3 years	GO:0007127	meiosis I	2/76	37/7903	0.0490	0.60	*PTTG3P/PTTG1*
≤3 years	GO:0090181	regulation of cholesterol metabolic process	2/76	37/7903	0.0490	0.60	*DGKQ/PMVK*
>3 years	GO:1904996	positive regulation of leukocyte adhesion to vascular endothelial cell	4/81	13/7903	0.0000	0.01	*NFAT5/ICAM1/ELANE/ETS1*
>3 years	GO:1904994	regulation of leukocyte adhesion to vascular endothelial cell	4/81	14/7903	0.0000	0.01	*NFAT5/ICAM1/ELANE/ETS1*
>3 years	GO:0032212	positive regulation of telomere maintenance via telomerase	4/81	20/7903	0.0000	0.02	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:0061756	leukocyte adhesion to vascular endothelial cell	4/81	21/7903	0.0001	0.02	*NFAT5/ICAM1/ELANE/ETS1*
>3 years	GO:1904358	positive regulation of telomere maintenance via telomere lengthening	4/81	23/7903	0.0001	0.03	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:0051818	disruption of cells of other organism involved in symbiotic interaction	3/81	10/7903	0.0001	0.03	*CTSG/ELANE/AZU1*
>3 years	GO:1904814	regulation of protein localization to chromosome, telomeric region	3/81	12/7903	0.0002	0.05	*CCT2/MACROH2A1/CCT8*
>3 years	GO:0032206	positive regulation of telomere maintenance	4/81	34/7903	0.0004	0.07	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:0001909	leukocyte mediated cytotoxicity	5/81	61/7903	0.0004	0.07	*CTSG/ICAM1/ELANE/AZU1/AP1G1*
>3 years	GO:0032210	regulation of telomere maintenance via telomerase	4/81	36/7903	0.0005	0.08	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:2000573	positive regulation of DNA biosynthetic process	4/81	40/7903	0.0007	0.11	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:1904356	regulation of telomere maintenance via telomere lengthening	4/81	42/7903	0.0009	0.11	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:0098609	cell-cell adhesion	11/81	355/7903	0.0009	0.11	*NFAT5/METAP1/ICAM1/ELANE/VCL/ETS1/CD93/LEF1/SERPINB8/CD33/KIFC3*
>3 years	GO:0019730	antimicrobial humoral response	4/81	43/7903	0.0009	0.11	*CTSG/ELANE/PF4V1/AZU1*
>3 years	GO:0070198	protein localization to chromosome, telomeric region	3/81	20/7903	0.0010	0.11	*CCT2/MACROH2A1/CCT8*
>3 years	GO:0070199	establishment of protein localization to chromosome	3/81	20/7903	0.0010	0.11	*CCT2/MACROH2A1/CCT8*
>3 years	GO:0007004	telomere maintenance via telomerase	4/81	48/7903	0.0014	0.13	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:0006278	RNA-dependent DNA biosynthetic process	4/81	49/7903	0.0015	0.13	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:0051851	modification by host of symbiont morphology or physiology	4/81	49/7903	0.0015	0.13	*CTSG/ELANE/AZU1/LEF1*
>3 years	GO:0001906	cell killing	5/81	83/7903	0.0016	0.13	*CTSG/ICAM1/ELANE/AZU1/AP1G1*
>3 years	GO:0051973	positive regulation of telomerase activity	3/81	24/7903	0.0018	0.14	*CCT2/HMBOX1/MAPKAPK5*
>3 years	GO:0051702	interaction with symbiont	4/81	52/7903	0.0019	0.14	*CTSG/ELANE/AZU1/LEF1*
>3 years	GO:0044839	cell cycle G2/M phase transition	7/81	174/7903	0.0020	0.14	*ENSA/TAOK1/CEP41/MACROH2A1/KAT14/PSMD2/WEE1*
>3 years	GO:0022617	extracellular matrix disassembly	3/81	26/7903	0.0023	0.15	*CTSG/ELANE/ETS1*
>3 years	GO:0031640	killing of cells of other organism	3/81	26/7903	0.0023	0.15	*CTSG/ELANE/AZU1*
>3 years	GO:0044364	disruption of cells of other organism	3/81	26/7903	0.0023	0.15	*CTSG/ELANE/AZU1*
>3 years	GO:0010833	telomere maintenance via telomere lengthening	4/81	56/7903	0.0025	0.15	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:0032204	regulation of telomere maintenance	4/81	56/7903	0.0025	0.15	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:0050829	defense response to Gram-negative bacterium	3/81	28/7903	0.0028	0.16	*CTSG/ELANE/AZU1*
>3 years	GO:0043299	leukocyte degranulation	11/81	412/7903	0.0030	0.17	*CTSG/ELANE/VCL/CCT2/AZU1/CD93/AP1G1/CD33/CCT8/PSMD2/EEF1A1*

>3 years	GO:0051972	regulation of telomerase activity	3/81	31/7903	0.0038	0.18	CCT2/HMBOX1/MAPKAPK5
>3 years	GO:2000278	regulation of DNA biosynthetic process	4/81	64/7903	0.0041	0.18	CCT2/HMBOX1/CCT8/MAPKAPK5
>3 years	GO:0002693	positive regulation of cellular extravasation	2/81	10/7903	0.0044	0.18	ICAM1/ELANE
>3 years	GO:0042033	chemokine biosynthetic process	2/81	10/7903	0.0044	0.18	ELANE/AZU1
>3 years	GO:0045073	regulation of chemokine biosynthetic process	2/81	10/7903	0.0044	0.18	ELANE/AZU1
>3 years	GO:0050755	chemokine metabolic process	2/81	10/7903	0.0044	0.18	ELANE/AZU1
>3 years	GO:0050926	regulation of positive chemotaxis	2/81	10/7903	0.0044	0.18	S1PR1/AZU1
>3 years	GO:0050927	positive regulation of positive chemotaxis	2/81	10/7903	0.0044	0.18	S1PR1/AZU1
>3 years	GO:0070203	regulation of establishment of protein localization to telomere	2/81	10/7903	0.0044	0.18	CCT2/CCT8
>3 years	GO:1904816	positive regulation of protein localization to chromosome, telomeric region	2/81	10/7903	0.0044	0.18	CCT2/CCT8
>3 years	GO:1990173	protein localization to nucleoplasm	2/81	10/7903	0.0044	0.18	CCT2/CCT8
>3 years	GO:0045123	cellular extravasation	3/81	33/7903	0.0045	0.18	ICAM1/ELANE/AZU1
>3 years	GO:0030575	nuclear body organization	2/81	11/7903	0.0054	0.21	ETS1/HABP4
>3 years	GO:0070202	regulation of establishment of protein localization to chromosome	2/81	11/7903	0.0054	0.21	CCT2/CCT8
>3 years	GO:0043312	neutrophil degranulation	10/81	385/7903	0.0057	0.21	CTSG/ELANE/VCL/CCT2/AZU1/CD93/CD33/CCT8/PSMD2/EEF1A1
>3 years	GO:0002283	neutrophil activation involved in immune response	10/81	386/7903	0.0058	0.21	CTSG/ELANE/VCL/CCT2/AZU1/CD93/CD33/CCT8/PSMD2/EEF1A1
>3 years	GO:0002446	neutrophil mediated immunity	10/81	389/7903	0.0061	0.21	CTSG/ELANE/VCL/CCT2/AZU1/CD93/CD33/CCT8/PSMD2/EEF1A1
>3 years	GO:0051817	modification of morphology or physiology of other organism involved in symbiotic interaction	4/81	72/7903	0.0062	0.21	CTSG/ELANE/AZU1/LEF1
>3 years	GO:0010948	negative regulation of cell cycle process	7/81	215/7903	0.0063	0.21	TAOK1/MAX/MGA/MACROH2A1/PSMD2/TOM1L2/WEE1
>3 years	GO:0070200	establishment of protein localization to telomere	2/81	12/7903	0.0064	0.21	CCT2/CCT8
>3 years	GO:1904874	positive regulation of telomerase RNA localization to Cajal body	2/81	12/7903	0.0064	0.21	CCT2/CCT8
>3 years	GO:0042119	neutrophil activation	10/81	394/7903	0.0067	0.21	CTSG/ELANE/VCL/CCT2/AZU1/CD93/CD33/CCT8/PSMD2/EEF1A1
>3 years	GO:0000086	G2/M transition of mitotic cell cycle	6/81	165/7903	0.0068	0.21	ENSA/TAOK1/CEP41/KAT14/PSMD2/WEE1
>3 years	GO:0036230	granulocyte activation	10/81	398/7903	0.0072	0.21	CTSG/ELANE/VCL/CCT2/AZU1/CD93/CD33/CCT8/PSMD2/EEF1A1
>3 years	GO:0007596	blood coagulation	6/81	168/7903	0.0074	0.21	USF1/METAP1/VCL/PF4V1/GP9/ITPK1
>3 years	GO:0002438	acute inflammatory response to antigenic stimulus	2/81	13/7903	0.0075	0.21	ICAM1/ELANE
>3 years	GO:0007339	binding of sperm to zona pellucida	2/81	13/7903	0.0075	0.21	CCT2/CCT8
>3 years	GO:0032717	negative regulation of interleukin-8 production	2/81	13/7903	0.0075	0.21	ELANE/CD33
>3 years	GO:0050817	coagulation	6/81	169/7903	0.0076	0.21	USF1/METAP1/VCL/PF4V1/GP9/ITPK1
>3 years	GO:2001252	positive regulation of chromosome organization	5/81	120/7903	0.0076	0.21	CCT2/HMBOX1/MACROH2A1/CCT8/MAPKAPK5
>3 years	GO:0007599	hemostasis	6/81	172/7903	0.0082	0.23	USF1/METAP1/VCL/PF4V1/GP9/ITPK1
>3 years	GO:0061077	chaperone-mediated protein folding	3/81	41/7903	0.0084	0.23	CCT2/DNAJC24/CHORDC1
>3 years	GO:0050832	defense response to fungus	2/81	14/7903	0.0087	0.23	CTSG/ELANE
>3 years	GO:0002275	myeloid cell activation involved in immune response	10/81	415/7903	0.0095	0.25	CTSG/ELANE/VCL/CCT2/AZU1/CD93/CD33/CCT8/PSMD2/EEF1A1
>3 years	GO:0002444	myeloid leukocyte mediated immunity	10/81	418/7903	0.0100	0.26	CTSG/ELANE/VCL/CCT2/AZU1/CD93/CD33/CCT8/PSMD2/EEF1A1
>3 years	GO:1904872	regulation of telomerase RNA localization to Cajal body	2/81	15/7903	0.0100	0.26	CCT2/CCT8
>3 years	GO:0002691	regulation of cellular extravasation	2/81	16/7903	0.0113	0.27	ICAM1/ELANE
>3 years	GO:0090670	RNA localization to Cajal body	2/81	16/7903	0.0113	0.27	CCT2/CCT8
>3 years	GO:0090671	telomerase RNA localization to Cajal body	2/81	16/7903	0.0113	0.27	CCT2/CCT8
>3 years	GO:0090672	telomerase RNA localization	2/81	16/7903	0.0113	0.27	CCT2/CCT8
>3 years	GO:0090685	RNA localization to nucleus	2/81	16/7903	0.0113	0.27	CCT2/CCT8
>3 years	GO:0019731	antibacterial humoral response	2/81	17/7903	0.0128	0.30	CTSG/ELANE
>3 years	GO:0030168	platelet activation	4/81	89/7903	0.0129	0.30	METAP1/VCL/PF4V1/GP9
>3 years	GO:0009611	response to wounding	8/81	307/7903	0.0130	0.30	USF1/METAP1/VCL/ETS1/PF4V1/MAX/GP9/ITPK1
>3 years	GO:0035821	modification of morphology or physiology of other organism	4/81	91/7903	0.0140	0.31	CTSG/ELANE/AZU1/LEF1
>3 years	GO:0035036	sperm-egg recognition	2/81	18/7903	0.0143	0.32	CCT2/CCT8
>3 years	GO:0034502	protein localization to chromosome	3/81	51/7903	0.0152	0.33	CCT2/MACROH2A1/CCT8
>3 years	GO:0042060	wound healing	7/81	256/7903	0.0156	0.34	USF1/METAP1/VCL/ETS1/PF4V1/GP9/ITPK1
>3 years	GO:1904036	negative regulation of epithelial cell apoptotic process	2/81	19/7903	0.0158	0.34	ICAM1/CAST
>3 years	GO:0045216	cell-cell junction organization	3/81	52/7903	0.0160	0.34	PATJ/VCL/KIFC3
>3 years	GO:0006959	humoral immune response	4/81	97/7903	0.0173	0.36	CTSG/ELANE/PF4V1/AZU1
>3 years	GO:0007223	Wnt signaling pathway, calcium modulating pathway	2/81	20/7903	0.0175	0.36	LEF1/AGO2
>3 years	GO:0042088	T-helper 1 type immune response	2/81	20/7903	0.0175	0.36	IL18BP/LEF1
>3 years	GO:0040017	positive regulation of locomotion	7/81	265/7903	0.0185	0.37	S1PR1/ICAM1/ELANE/ETS1/AZU1/LEF1/AGO2
>3 years	GO:0048066	developmental pigmentation	2/81	21/7903	0.0192	0.38	AP1G1/LEF1
>3 years	GO:0002274	myeloid leukocyte activation	10/81	469/7903	0.0209	0.41	CTSG/ELANE/VCL/CCT2/AZU1/CD93/CD33/CCT8/PSMD2/EEF1A1
>3 years	GO:1901998	toxin transport	2/81	22/7903	0.0210	0.41	CCT2/CCT8
>3 years	GO:0006414	translational elongation	4/81	104/7903	0.0217	0.42	EEF1G/MRPS30/DNAJC24/EEF1A1
>3 years	GO:0002526	acute inflammatory response	3/81	59/7903	0.0224	0.42	ICAM1/ELANE/CD163
>3 years	GO:0000723	telomere maintenance	4/81	105/7903	0.0224	0.42	CCT2/HMBOX1/CCT8/MAPKAPK5
>3 years	GO:0009620	response to fungus	2/81	23/7903	0.0228	0.42	CTSG/ELANE
>3 years	GO:0043271	negative regulation of ion transport	3/81	60/7903	0.0234	0.43	PXK/ICAM1/CD33
>3 years	GO:0030198	extracellular matrix organization	4/81	109/7903	0.0253	0.46	CTSG/ICAM1/ELANE/ETS1
>3 years	GO:0060249	anatomical structure homeostasis	6/81	221/7903	0.0255	0.46	S1PR1/TAOK1/CCT2/HMBOX1/CCT8/MAPKAPK5
>3 years	GO:0009069	serine family amino acid metabolic process	2/81	25/7903	0.0267	0.47	PSAT1/MPST
>3 years	GO:0070317	negative regulation of G0 to G1 transition	2/81	25/7903	0.0267	0.47	MAX/MGA
>3 years	GO:0032200	telomere organization	4/81	111/7903	0.0268	0.47	CCT2/HMBOX1/CCT8/MAPKAPK5
>3 years	GO:0050918	positive chemotaxis	2/81	26/7903	0.0288	0.50	S1PR1/AZU1
>3 years	GO:0021700	developmental maturation	4/81	114/7903	0.0292	0.50	S1PR1/TRPC4AP/RERE/AP1G1
>3 years	GO:0050877	nervous system process	7/81	294/7903	0.0306	0.51	S1PR1/ICAM1/CLN6/LEF1/KIFC3/LINS1/METTL23
>3 years	GO:0045023	G0 to G1 transition	2/81	27/7903	0.0309	0.51	MAX/MGA
>3 years	GO:0070316	regulation of G0 to G1 transition	2/81	27/7903	0.0309	0.51	MAX/MGA
>3 years	GO:0071897	DNA biosynthetic process	4/81	116/7903	0.0309	0.51	CCT2/HMBOX1/CCT8/MAPKAPK5
>3 years	GO:0050878	regulation of body fluid levels	6/81	232/7903	0.0313	0.51	USF1/METAP1/VCL/PF4V1/GP9/ITPK1
>3 years	GO:0061844	antimicrobial humoral immune response mediated by antimicrobial peptide	2/81	28/7903	0.0330	0.53	ELANE/PF4V1
>3 years	GO:0009988	cell-cell recognition	2/81	29/7903	0.0352	0.56	CCT2/CCT8
>3 years	GO:0051926	negative regulation of calcium ion transport	2/81	29/7903	0.0352	0.56	ICAM1/CD33
>3 years	GO:0043297	apical junction assembly	2/81	30/7903	0.0375	0.58	PATJ/VCL
>3 years	GO:0010564	regulation of cell cycle process	9/81	445/7903	0.0376	0.58	TAOK1/CEP41/MAX/MGA/MACROH2A1/CHORDC1/PSMD2/TOM1L2/WEE1
>3 years	GO:0044770	cell cycle phase transition	8/81	377/7903	0.0386	0.59	ENSA/TAOK1/CEP41/MAX/MACROH2A1/KAT14/PSMD2/WEE1
>3 years	GO:0030335	positive regulation of cell migration	6/81	244/7903	0.0386	0.59	S1PR1/ICAM1/ELANE/ETS1/LEF1/AGO2
>3 years	GO:0071695	anatomical structure maturation	3/81	74/7903	0.0400	0.60	S1PR1/TRPC4AP/RERE
>3 years	GO:1902750	negative regulation of cell cycle G2/M phase transition	3/81	75/7903	0.0414	0.61	TAOK1/MACROH2A1/PSMD2
>3 years	GO:2000147	positive regulation of cell motility	6/81	249/7903	0.0420	0.61	S1PR1/ICAM1/ELANE/ETS1/LEF1/AGO2
>3 years	GO:0043062	extracellular structure organization	4/81	128/7903	0.0420	0.61	CTSG/ICAM1/ELANE/ETS1

>3 years	GO:0002437	inflammatory response to antigenic stimulus	2/81	32/7903	0.0422	0.61	*ICAM1/ELANE*
>3 years	GO:0060429	epithelium development	9/81	455/7903	0.0424	0.61	*S1PR1/TRPC4AP/ICAM1/VCL/FOSL2/LEF1/MACROH2A1/PSMD2/ITPK1*
>3 years	GO:0043392	negative regulation of DNA binding	2/81	33/7903	0.0446	0.64	*HABP4/LEF1*
>3 years	GO:0051272	positive regulation of cellular component movement	6/81	255/7903	0.0462	0.66	*S1PR1/ICAM1/ELANE/ETS1/LEF1/AGO2*
>3 years	GO:0051054	positive regulation of DNA metabolic process	4/81	133/7903	0.0473	0.66	*CCT2/HMBOX1/CCT8/MAPKAPK5*
>3 years	GO:1903039	positive regulation of leukocyte cell-cell adhesion	4/81	135/7903	0.0494	0.68	*NFAT5/ICAM1/ELANE/ETS1*
>3 years	GO:0072678	T cell migration	2/81	35/7903	0.0496	0.68	*S1PR1/ICAM1*

S9 Table. Summary of tests of genes identified in published functional genomics studies. Results from targeted tests of single genes identified in published studies investigating gene expression in peripheral whole blood or DNA methylation in circulating leukocytes from women with epithelial ovarian cancer.

Citation and genes	**Data type in publication**	**Direction of effects (+ = up, - = down)**	**CpG location**	**Methylation - direction of effects (+ = hyper, - = hypo)**	**log2 FC present study (metastatic EOC vs. controls; prospective)**	**p-value present study**
Isaksson et al. 2012	Comparison: patients with macroscopic residual tumor post cytoreductive surgery, relative to patients without residual tumor					
CTNNA1	Gene expression	-			-0.05	0.09
IL1B	Gene expression	-			-0.04	0.54
KISS1	Gene expression	-				
MMP10	Gene expression	-				
MTA2	Gene expression	-				
TNF	Gene expression	-				
Isaksson et al. 2014	Comparison: patients with more aggressive and more advanced disease relative to patients with less aggressive and less advanced disease					
PDIA3	Gene expression	-				
LYAR	Gene expression	-			0.06	0.33
NOP14	Gene expression	-			0.04	0.11
NCALD	Gene expression	-			0.08	0.08
MTSS1	Gene expression	-			0.02	0.33
CYP1B1	Gene expression	+			-0.05	0.49
Teschendorff et al. 2009	Comparison: cases relative to controls, cancer predisposition CpGs, not adjusted for leukocyte composition					
LIME1	DNA methylation		island	+	0.11	**0.05**
FUT7	DNA methylation			-	-0.05	0.07
MPHOSPH9	DNA methylation			+	0.02	0.11
PC	DNA methylation			+		
ZNF364	DNA methylation			-	-0.05	0.22
CHMP7	DNA methylation			-	-0.02	0.25
SFRS6	DNA methylation		island	+	0.02	0.72
CFI	DNA methylation			-	0.00	0.90
HK2	DNA methylation			-	-0.03	0.43
RFPL3	DNA methylation			+		
DST	DNA methylation		island	-		
GPR162	DNA methylation		island	+	-0.17	**0.04**
MEPE	DNA methylation			-		
MAS1L	DNA methylation			+		
MAS1L	DNA methylation			+		
WBSCR16	DNA methylation		island	+	-0.01	0.48
SLC16A6	DNA methylation		island	+	-0.01	0.62
TSG101	DNA methylation		island		-0.01	0.56
Fridley et al. 2014	Comparison: Cases relative to controls, disease status; adjusted for leukocyte composition					
DUSP13	DNA methylation		Shelf	+		
APC2	DNA methylation		Shore	+		
RELL2	DNA methylation			+	0.00	0.89
HDAC3	DNA methylation		Shore	+	0.03	0.13
HHIP	DNA methylation		Island	+		
ENTHD1	DNA methylation			+		
C19orf18	DNA methylation			+		
GCNT3	DNA methylation			+		
SPACA5	DNA methylation		Island	+		
ZNF182	DNA methylation			+		
ENTPD8	DNA methylation		Island	+		
PROM2	DNA methylation			+		
PAG1	DNA methylation		Shore	-	-0.02	0.61
SCARA5	DNA methylation			+		
PI4KA	DNA methylation			+	0.01	0.49

CUL7	DNA methylation			+		
MRPL2	DNA methylation		Shore	+	-0.02	0.42
KCTD4	DNA methylation			+		
GTF2F2	DNA methylation			+	0.01	0.80
IL4	DNA methylation			+		
MARCH1	DNA methylation			+		
SREBF1	DNA methylation		Shore	+	-0.02	0.45
ACTL7A	DNA methylation			+		
ESX1	DNA methylation		Island	+		
TMSB4X	DNA methylation		Shore	+		
SLC16A7	DNA methylation			+		
ESX1	DNA methylation		Island	+		
ARMCX1	DNA methylation		Shelf	+		
ABCB7	DNA methylation			+	-0.01	0.50
CSRNP1	DNA methylation		Shore	+		
SCML2	DNA methylation		Island	+		
GBP6	DNA methylation			+		
SRC	DNA methylation		Shore	+	-0.01	0.75
NTF4	DNA methylation			+		
Li et al. 2017	Comparison: cases relative to controls; cancer diagnostic CpGs					
C5orf63	DNA methylation		N_Shore	-		
WDR90	DNA methylation		Island	-		
IL17RA	DNA methylation		N_Shelf	-	-0.05	0.35
POMP	DNA methylation			-	0.03	0.20
NAV1	DNA methylation		S_Shelf	-		
GNAS	DNA methylation		Island	-	0.03	0.57
Koestler et al. 2014	Comparison: cases relative to controls; methylation-mediated genetic risk					
TMOD4	DNA methylation			-	0.00	0.83
APOA1BP	DNA methylation			+	0.04	0.22
AIM2	DNA methylation			-	-0.07	0.16
LRPPRC	DNA methylation			+		
STAB1	DNA methylation			-	-0.05	**0.01**
HMGB2	DNA methylation			+	-0.03	0.37
ATG10	DNA methylation			+		
KCNMB1	DNA methylation			+	0.01	0.66
FLJ22318	DNA methylation			+		
ARID1B	DNA methylation			+		
TCP1	DNA methylation			+	0.01	0.52
EVI2B	DNA methylation			+	-0.02	0.56
TAF15	DNA methylation			+	-0.04	0.49
Yang et al. 2018	Comparison: cases relative to controls; methylation-mediated genetic risk					
MAPT	DNA methylation		5'UTR	-		
HOXB3	DNA methylation		Intronic	-		
ABHD8	DNA methylation		Intronic	+		
ARHGAP27	DNA methylation		3'UTR	+	-0.03	0.35
SKAP1	DNA methylation		Intronic	+	0.07	**0.04**

Isaksson, et al 2012. 'Whole blood RNA expression profiles in ovarian cancer patients with or without residual tumors after primary cytoreductive surgery', *Oncology Reports* , 27: 1331-5.

Isaksson, et al. 2014. 'Whole genome expression profiling of blood cells in ovarian cancer patients -prognostic impact of the CYP1B1, MTSS1, NCALD, and NOP14', *Oncotarget* , 5: 4040-9.

Teschendorff, et al. 2009. 'An epigenetic signature in peripheral blood predicts active ovarian cancer', *PloS One* , 4: e8274.

Fridley, et al. 2014. 'Methylation of leukocyte DNA and ovarian cancer: relationships with disease status and outcome', *BMC Medical Genomics* , 7: 21.

Li, et al. 2017. 'DNA methylation signatures and coagulation factors in the peripheral blood leucocytes of epithelial ovarian cancer', *Carcinogenesis* , 38: 797-805.

Koestler, et al. 2014. 'Integrative genomic analysis identifies epigenetic marks that mediate genetic risk for epithelial ovarian cancer', BMC Medical Genomics, 7: 8.

Appendix I

Basis for Paper I and Paper II

Letter requesting participation in the NOWAC Study

Questionnaire

Photo pamphlet

Reminder card

INSTITUTT FOR SAMFUNNSMEDISIN
UNIVERSITETET I TROMSØ
9037 TROMSØ
Telefon 77 64 48 16/77 64 66 38

KVINNER OG KREFT

Institutt for samfunnsmedisin ved Universitetet i Tromsø gjennomfører en spørreundersøkelse om levesett og kreft blant kvinner i Norge. En slik undersøkelse gir et verdifullt grunnlag for å studere mulige sammenhenger mellom f.eks. kosthold, barnefødsler, p-piller, solvaner og utviklingen av kreft. Resultatet vil bli publisert i dagspressen og i internasjonale fagtidsskrifter. Ansvarlig for undersøkelsen er professor Eiliv Lund.

Du forespørres hermed om å delta i undersøkelsen. Alle som blir forespurt er trukket ut tilfeldig. Statistisk Sentralbyrå har trukket utvalget og står for utsending av spørreskjemaene.

Med noen års mellomrom fram til 2035 vil vi sammenholde opplysningene som er gitt i undersøkelsen mot opplysninger fra Kreftregisteret, Mammografiregisteret og Dødsårsaksregisteret. Samtykket fra deg for dette vil være ensbetydende med returnering av utfylt spørreskjema. Alle opplysninger fra undersøkelsen og fra registrene vil bli behandlet konfidensielt og etter regler Datatilsynet har gitt i sin tillatelse, samt tillatelse fra Sosial- og helsedirektoratet. På spørreskjemaet er navn og fødselsnummer erstattet med et løpenummer slik at ingen av de som mottar og tar hånd om skjemaene vil kjenne din identitet. Undersøkelsen er tilrådd av Regional komité for medisinsk forskningsetikk i Nord-Norge.

Hvis du vil delta i undersøkelsen, ber vi deg besvare det vedlagte spørreskjemaet så riktig som mulig. Dersom ingen av de oppgitte svaralternativ dekker din situasjon, sett kryss for det alternativet som ligger nærmest. Gi eventuelle tilleggsopplysninger i skjemaet.

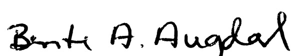
Det vil senere bli aktuelt å samle inn blodprøver fra noen av deltakerne. Dette vil skje hos lege, og vil være gratis. Det vil også bli aktuelt å spørre noen av deltakerne om å være med på et kostholdsintervju over telefon. Bare de av deltakerne som på forhånd har krysset av for at de er villig til å bli kontaktet på nytt og/eller til å bli spurt om å avgi blodprøve, vil få henvendelse om dette. Det vil da bli gitt nærmere informasjon og innhentet samtykke til dette.

Det er frivillig å være med i undersøkelsen. Det er adgang til å trekke seg senere, hvis du skulle ønske det. Du får slettet dine opplysninger hvis du krever det. Undersøkelsen avsluttes 31.12.2035

For spørsmål om hormoner og p-pillebruk finner du bilder i denne brosjyren som skal være et hjelpemiddel til å svare riktig (brosjyren skal ikke returneres). Spørreskjemaet returneres i vedlagte konvolutt med betalt svarporto.

Med hilsen

Eiliv Lund
professor dr.med.



Bente A. Augdal
prosjektmedarbeider

Du kan finne mer informasjon om "Kvinner og kreft" på våre nettsider: www.ism.uit.no/kk/

KVINNER OG KREFT

KONFIDENSIELT

Vinter 2005

Vi ber deg fylle ut spørreskjemaet så nøye som mulig.
Dersom du ikke ønsker å delta kan du unngå purring ved å sette kryss for NEI og returnere skjemaet i vedlagte svarkonvolutt.

Skjemaet skal leses optisk. Vennligst bruk blå eller sort penn.
Du kan ikke bruke komma, bruk blokkbokstaver.

Med vennlig hilsen
Eiliv Lund
Professor dr. med

Jeg samtykker i å delta i spørreskjemaundersøkelsen JA ☐ NEI ☐

Sosiale forhold

Er du: (Sett ett kryss)
☐ gift ☐ samboer ☐ ugift ☐ skilt ☐ enke

Hvor mange års skolegang/yrkesutdannelse har du i alt, ta med folkeskole og ungdomsskole? ☐

Hvor mange personer er det i ditt hushold? ☐

Hvor høy er bruttoinntekten i husholdet pr. år?

under 150.000 kr.	☐	151.000-300.000 kr.	☐
301.000-450.000 kr.	☐	451.000-600.000 kr.	☐
601.000-750.000 kr.	☐	over 750.000 kr.	☐

Hva er din arbeidssituasjon? (sett kryss)
☐ Arbeider heltid ☐ Arbeider deltid ☐ Pensjonist
☐ Hjemmearbeidende ☐ Under utdanning ☐ Uføretrygdet
☐ Under attføring ☐ Arbeidssøkende

Yrke: ☐

Høyde og vekt

Hvor høy er du?(i hele cm.) ☐

Hvor mye veide du da du var 18 år?(i hele kg.) ☐

Hvor mye veier du i dag?(i hele kg.) ☐

Kroppstype i 1. klasse. (Sett ett kryss)
☐ veldig tynn ☐ tynn ☐ normal ☐ tykk ☐ veldig tykk

Menstruasjonsforhold

Hvor gammel var du da du fikk menstruasjon første gang? ☐

Hvor mange år tok det før menstruasjonen ble regelmessig?
☐ Ett år eller mindre ☐ Mer enn ett år
☐ Aldri ☐ Husker ikke

Overgangsalder

Har du regelmessig menstruasjon fremdeles?
☐ Ja ☐ Har uregelmessig menstruasjon
☐ Vet ikke (menstruasjon uteblitt pga. sykdom o.l.)
☐ Bruk av hormonpreparat med østrogen
☐ Nei

Hvis Nei;
har den stoppet av seg selv? ☐
operert vekk eggstokkene? ☐
operert vekk livmoren? ☐
annet? ☐

Alder da menstruasjonen opphørte? ☐

Graviditeter, fødsler og amming

Har du noen gang vært gravid? Ja ☐ Nei ☐

Hvis Ja; fyll ut for hvert barn du har født opplysninger om fødselsår og antall måneder du ammet (fylles også ut for dødfødte eller for barn som er døde senere i livet). Dersom du ikke har født barn fortsetter du ved neste spørsmål.

Barn	Fødselsår	Antall måneder med amming	Barn	Fødselsår	Antall måneder med amming
1	☐	☐	5	☐	☐
2	☐	☐	6	☐	☐
3	☐	☐	7	☐	☐
4	☐	☐	8	☐	☐

Bruk av hormonpreparater med østrogen i overgangsalderen

Har du noen gang brukt østrogen-tabletter/plaster? Ja ☐ Nei ☐

Hvis Ja; hvor mange år har du brukt østrogentabletter/plaster i alt? ☐

Hvor gammel var du første gang du brukte østrogentabletter/plaster? ☐

Bruker du tabletter/plaster nå? Ja ☐ Nei ☐

UTFYLLENDE SPØRSMÅL TIL ALLE SOM HAR BRUKT ELLER BRUKER PREPARATER MED ØSTROGEN I FORM AV TABLETTER ELLER PLASTER.

Hvis du har svart «nei» på spørsmålene om hormonbruk i overgangsalderen, kan du gå videre til spørsmålene under «**P-pillebruk**». Har du svart «ja», ber vi deg utdype dette nærmere ved å svare på spørsmålene nedenfor. For hver periode med sammenhengende bruk av samme hormonpreparat håper vi du kan si oss hvor gammel du var da du startet, hvor lenge du brukte det samme hormonpreparatet og navnet på dette. Dersom du har hatt opphold eller skiftet merke skal du besvare spørsmålene for en ny periode. Dersom du ikke husker navnet på hormonpreparatet, sett «usikker». For å hjelpe deg til å huske navnet på hormonpreparatene ber vi deg bruke den vedlagte brosjyre som viser bilder av hormonpreparater som har vært solgt i Norge. Vennligst oppgi også nummer på hormontabletten/plasteret som står i brosjyren.

Periode	Alder ved start	Brukt samme hormontablett/plaster/ sammenhengende år	måned	Nr.	Navn på hormontablett/ plaster/ (se brosjyre)
1.					
Angi nr. her dersom du bruker to preparater					
2					
Angi nr. her dersom du bruker to preparater					
3.					
Angi nr. her dersom du bruker to preparater					

P-pillebruk

Har du brukt p-piller eller minipiller? .... Ja ☐ Nei ☐

Hvis ja, hvor mange år har du brukt p-piller i alt .... ☐

Bruker du p-piller nå? .... Ja ☐ Nei ☐

For p-pillebruk ønsker vi å få vite navnet på p-pillen, årstallet du startet å bruke den og hvor lenge du brukte dette merket sammenhengende. Dersom du har hatt opphold eller skiftet merke start på ny linje. For å hjelpe deg å huske navnet ber vi deg bruke den vedlagte brosjyren. Vennligst oppgi nummeret på p-pillen.

Periode	Alder ved start	Brukt samme p-piller sammenhengende år	måned	Nr.	P-piller (se brosjyre) Navn
1.					
2.					
3.					
4.					

Hormonspiral

Har du noen gang brukt <u>hormonspiral</u> (Levonova)? .... Ja ☐ Nei ☐

Hvis Ja; hvor mange hele år har du brukt hormonspiral i alt? .... ☐

Hvor gammel var du første gang du fikk innsatt <u>hormonspiral</u>? ☐

Bruker du <u>hormonspiral</u> nå? .... Ja ☐ Nei ☐

Østrogenpreparat til lokal bruk i skjeden

Har du noen gang brukt østrogenkrem/stikkpille? .... Ja ☐ Nei ☐

Hvis Ja; bruker du krem/stikkpille nå? .... Ja ☐ Nei ☐

Andre legemidler

Bruker du noen av disse legemidlene daglig nå?

	Ja	Nei
Fontex, Fluoxetin	☐	☐
Cipramil, Citalopram, Desital	☐	☐
Seroxat, Paroxetin	☐	☐
Zoloft	☐	☐
Fevarin	☐	☐
Cipralex	☐	☐

Hvis Ja; hvor lenge har du brukt dette legemidlet sammenhengede? Måneder ☐ År ☐

Har du benyttet noen av disse legemidlene tidligere? Ja ☐ Nei ☐

Hvis Ja; hvor lenge har du benyttet disse legemidlene i alt? År ☐

Sykdom

Har du eller har du hatt noen av følgende sykdommer?

	Ja	Nei	Hvis ja: Alder ved start
Kreft	☐	☐	
Høyt blodtrykk	☐	☐	
Hjertesvikt/hjertekrampe	☐	☐	
Hjerteinfarkt	☐	☐	
Slag	☐	☐	
Sukkersyke (diabetes)	☐	☐	
Depresjon (oppsøkt lege)	☐	☐	

Røykevaner

Har du i løpet av livet røykt mer enn 100 sigaretter til sammen? Ja ☐ Nei ☐

Hvor gammel var du da du tok din første sigarett? ☐

Hvis Ja, ber vi deg om å fylle ut for hver aldersgruppe i livet hvor mange sigaretter du i gjennomsnitt røykte pr. dag i den perioden.

Antall sigaretter hver dag

Alder	0	1-4	5-9	10-14	15-19	20-24	25+
10-14	☐	☐	☐	☐	☐	☐	☐
15-19	☐	☐	☐	☐	☐	☐	☐
20-29	☐	☐	☐	☐	☐	☐	☐
30-39	☐	☐	☐	☐	☐	☐	☐
40-49	☐	☐	☐	☐	☐	☐	☐
50+	☐	☐	☐	☐	☐	☐	☐

	Ja	Nei
Røyker du daglig nå?	☐	☐
Røykte noen av dine foreldre da du var barn?	☐	☐

Hvis Ja, hvor mange sigaretter røykte de til sammen pr. dag? ☐

Brystkreft i nærmeste familie

Har noen nære slektninger hatt brystkreft?

	Ja	Nei	Vet ikke	Alder ved start
Din datter	☐	☐	☐	☐
Din mor	☐	☐	☐	☐
Din søster	☐	☐	☐	☐

Hvor mange søstre har du (ta med evt. døde) ☐

Hvor mange døtre har du (ta med evt. døde) ☐

Selvopplevd helse

Oppfatter du din egen helse som; (Sett ett kryss)

Meget god ☐ God ☐ Dårlig ☐ Meget dårlig

Mammografiundersøkelse

Har du vært til undersøkelse av brystene med mammografi Nei ☐ Ja ☐

Hvis Ja;
hvor mange år er det siden du sist var til mammografi? (hele år) ☐

Har du hatt noen form for operasjon av bryst(ene)?

	Alder (år)
Godartet kul (angi alder for første gang)	☐
Brystreduksjon (angi alder)	☐
Brystinnlegg (silikon)	☐
Annet (angi)	☐

Fysisk aktivitet

Vi ber deg angi din fysiske aktivitet etter en skala fra svært lite til svært mye. Skalaen nedenfor går fra 1-10. Med fysisk aktivitet mener vi både arbeid i hjemmet og i yrkeslivet, samt trening og annen fysisk aktivitet som turgåing o.l. Sett kryss over det tallet som best angir ditt nivå av fysisk aktivitet.

Alder	Svært lite									Svært mye
14 år	1	2	3	4	5	6	7	8	9	10
30 år	1	2	3	4	5	6	7	8	9	10
I dag	1	2	3	4	5	6	7	8	9	10

Hvor mange timer pr. dag i gjennomsnitt går eller spaserer du utendørs?

	sjelden/ aldri	mindre enn 1/2 time	1/2-1 time	1-2 timer	mer enn 2 timer
Vinter	☐	☐	☐	☐	☐
Vår	☐	☐	☐	☐	☐
Sommer	☐	☐	☐	☐	☐
Høst	☐	☐	☐	☐	☐

Alkohol

Er du totalavholdskvinne? Ja ☐ Nei ☐

Hvis Nei; hvor ofte og hvor mye drakk du i gjennomsnitt siste året? (Sett ett kryss for hver linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2-4 pr. uke	5-6 pr. uke	1 pr. dag	2+ pr. dag
Øl (1/2 l.)	☐	☐	☐	☐	☐	☐	☐	☐
Vin (glass)	☐	☐	☐	☐	☐	☐	☐	☐
Brennevin (drink)	☐	☐	☐	☐	☐	☐	☐	☐
Likør/Hetvin (glass)	☐	☐	☐	☐	☐	☐	☐	☐

Solvaner

Får du fregner når du soler deg? ....Ja ☐ Nei ☐

Hvilken øyefarge har du? (sett ett kryss)

☐ brun ☐ grå, grønn eller blanding ☐ blå

Hva er din opprinnelige hårfarge? (sett ett kryss)

☐ mørkbrun, svart ☐ brun ☐ blond, gul ☐ rød

For å kunne studere effekten av soling på risiko for hudkreft ber vi deg gi opplysninger om hudfarge
Sett ett kryss på det tallet under fargen som best passer din naturlige hudfarge (uten soling)

1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Hvor mange ganger pr. år er du blitt forbrent av solen slik at du har fått svie og blemmer med avflassing etterpå? (ett kryss for hver aldersgruppe)

Alder	Aldri	Høyst 1 gang pr. år	2-3 g. pr. år	4-5 g. pr. år	6 eller flere ganger
Før 10 år	☐	☐	☐	☐	☐
10-19 år	☐	☐	☐	☐	☐
20-29 år	☐	☐	☐	☐	☐
30-39 år	☐	☐	☐	☐	☐
40+ år	☐	☐	☐	☐	☐

Hvor mange uker soler du deg pr. år i syden?

Alder	Aldri	1 uke	2-3 uker	4-5 uker	6 uker eller mer
Før 10 år	☐	☐	☐	☐	☐
10-19 år	☐	☐	☐	☐	☐
20-29 år	☐	☐	☐	☐	☐
30-39 år	☐	☐	☐	☐	☐
40+ år	☐	☐	☐	☐	☐
Siste 12 mnd.	☐	☐	☐	☐	☐

Hvor mange uker pr. år soler du deg i Norge eller utenfor syden?

Alder	Aldri	1 uke	2-3 uker	4-5 uker	6 uker eller mer
Før 10 år	☐	☐	☐	☐	☐
10-19 år	☐	☐	☐	☐	☐
20-29 år	☐	☐	☐	☐	☐
30-39 år	☐	☐	☐	☐	☐
40+ år	☐	☐	☐	☐	☐
Siste 12 mnd.	☐	☐	☐	☐	☐

Hvor ofte dusjer eller bader du?

	mer enn 1 g. dagl.	1 g. dagl.	4-6 g. pr. uke	2-3 g. pr. uke	1 g. pr. uke	2-3 g. pr. mnd	sjel-den/ aldri
Med såpe/shampo	☐	☐	☐	☐	☐	☐	☐
Uten såpe/shampo	☐	☐	☐	☐	☐	☐	☐

Når bruker du krem med solfaktor? (sett evt. flere kryss):

☐ i påsken ☐ i Norge eller utenfor syden ☐ solferie i syden
☐ aldri

Hvilken solfaktor bruker du i disse periodene?

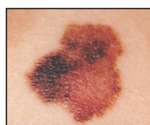
	påsken	i Norge eller utenfor syden	solferie i syden
I dag	☐	☐	☐
For 10 år siden	☐	☐	☐

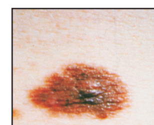
Hvor ofte har du solt deg i solarium?

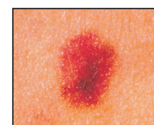
Alder	Aldri	Sjelden	1 gang pr. mnd.	2 ganger pr. mnd.	3-4 ganger pr. mnd	oftere enn1 gang pr. uke
Før 10 år	☐	☐	☐	☐	☐	☐
10-19 år	☐	☐	☐	☐	☐	☐
20-29 år	☐	☐	☐	☐	☐	☐
30-39 år	☐	☐	☐	☐	☐	☐
40+ år	☐	☐	☐	☐	☐	☐
Siste 12 mnd.	☐	☐	☐	☐	☐	☐

Hvor mange uregelmessige føflekker større enn 5 mm har du sammenlagt på begge beina (fra tærne til lysken)? Tre eksempler på føflekker større enn 5 mm med uregelmessig form er vist i nedenfor.

☐ 0 ☐ 1 ☐ 2-3 ☐ 4-6 ☐ 7-12 ☐ 13-24 ☐ 25+







5 mm

Hvor ofte bruker du følgende hudpleiemidler?

(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr.mnd.	1 pr.uke	2-4 pr.uke	5-6 pr.uke	1 pr.dag	2+ pr. dag
Ansiktskrem	☐	☐	☐	☐	☐	☐	☐
Håndkrem	☐	☐	☐	☐	☐	☐	☐
Body lotion	☐	☐	☐	☐	☐	☐	☐
Parfyme	☐	☐	☐	☐	☐	☐	☐

Til slutt vil vi spørre deg om ditt samtykke til å kontakte deg på nytt pr. post. Vi vil hente adressen fra det sentrale personregister.

Ja ☐ Nei ☐

Er du villig til å avgi en blodprøve?

Ja ☐ Nei ☐

Takk for at du ville delta i undersøkelsen

LUNDBLAD MEDIA AS, TROMSØ

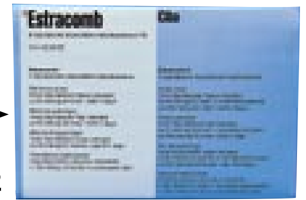
INSTITUTT FOR SAMFUNNSMEDISIN
UNIVERSITETET I TROMSØ
9037 TROMSØ
Telefon 77 64 48 16

UNIVERSITETET I TROMSØ

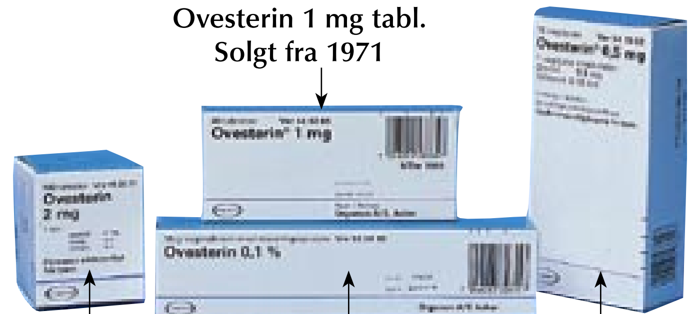
Bilder av hormoner til bruk i og etter overgangsalderen (østrogen)

Denne brosjyren er et hjelpemiddel for å huske riktig navn på de hormontabletter/plaster du har brukt. Alle som er nevnt eller avbildet nedenfor har vært i salg mellom 1998 og 2004. Under bildene er det oppgitt hvilke år disse var i salg. For noen hormontabletter/plaster finnes det esker med samme utseende, men med ulik styrke av hormonene. Vi ber deg tenke nøye gjennom navnet på de hormon-tabletter/plaster du har brukt. Enkelte preparater er ikke gjengitt med bilder, det gjelder:

Nr. 104 Etifollin 50 mcg tabletter, solgt fra 1953-2000
Nr. 121 Menorest 37,5 mcg/24t plaster, solgt fra 1996-2002
Nr. 122 Menorest 50 mcg/24t plaster, solgt fra 1996-2002
Nr. 123 Menorest 75 mcg/24t plaster, solgt fra 1996-2002
Nr. 124 Menorest 100 mcg/24t plaster, solgt fra 1996-2002
Nr. 196 Primolut tabletter, solgt fra 1958-
Nr. 197 Perlutex tabletter, solgt fra 1960-
Nr. 199 Provera 5 og 10 mg tabletter, solgt fra 1964-



Nr. 110
Estracomb
Solgt fra 1994-2002

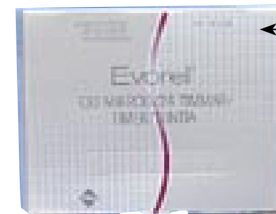


Nr. 111
Ovesterin 1 mg tabl.
Solgt fra 1971

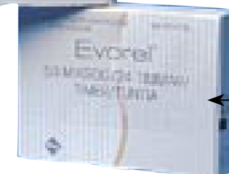
Nr. 112
Ovesterin 2 mg tabl.
Solgt fra 1989

Nr. 113
Ovesterin krem
Solgt fra 1983

Nr. 114
Ovesterin vag.
Solgt fra 1984



Nr. 115
Evorel 100 mcg
Solgt fra 1995



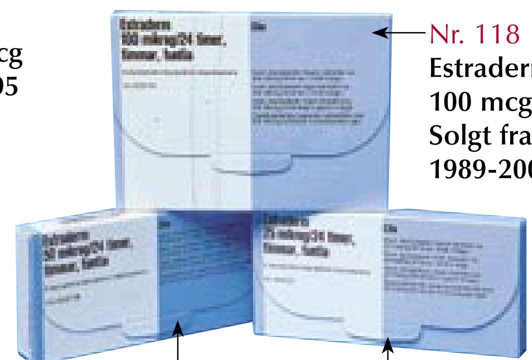
Nr. 116
Evorel 25 mcg
Solgt fra 1995

Nr. 117
Evorel 50 mcg
Solgt fra 1994

Nr. 101 **Cyclabil**
Solgt fra 1978

Nr. 102
Trisekvens
Solgt fra 1978

Nr. 103
Trisekvens Forte
Solgt fra 1978



Nr. 118
Estraderm 100 mcg
Solgt fra 1989-2002

Nr. 119
Estraderm 50 mcg
Solgt fra 1989-2002

Nr. 120
Estraderm 25 mcg
Solgt fra 1989

Nr. 105
Kliogest
Solgt fra 1988

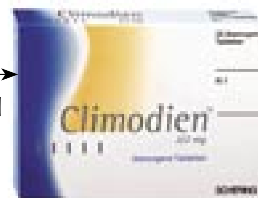
Nr. 107 **Progynova 2mg**
Solgt fra 1967

Nr. 106 **Progynova 1mg Solgt fra 1970**

Estraderm produseres av "Novartis". Fantes og som Estraderm Matrix.

Nr. 125
Estring
Solgt fra 1996

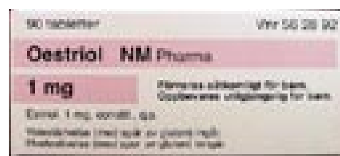
Nr. 136 Vagifem
Solgt fra 2000



Nr. 138
Climodien
Solgt fra 2001

Nr. 126
Climara
50 mcg
Solgt fra 1997

Nr. 127
Climara 100 mcg
Solgt fra 1997



Nr. 139 Oestriol 1 mg
Solgt fra 1999

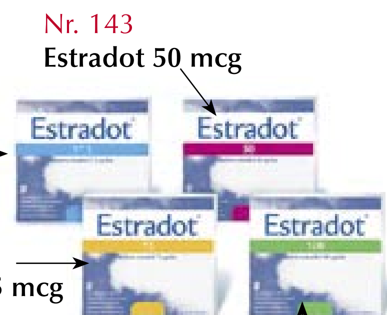
Nr. 140 Oestriol 2 mg
Solgt fra 1999

Nr. 128
Livial
Solgt fra
1999

Nr. 143
Estradot 50 mcg

Nr. 142
Estradot
37,5 mcg

Nr. 144
Estradot 75 mcg



Solgt fra 2002

Nr. 145
Estradot 100 mcg

Nr. 130
Indivina 1mg/2,5 mg
Solgt fra
2001

Nr. 141
Novofem
Solgt fra 2002

Estradot

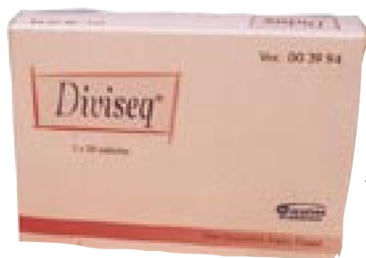
Nr. 149
Solgt fra 2004
Estradot 25mcg

Nr. 132
Indivina 2 mg/5 mg
Solgt fra 2001

Nr. 131
Indivina 1mg/5 mg
Solgt fra 2001

Nr. 146
Estalis
Solgt fra 2002

Nr. 147
Estalis Sekvens
Solgt fra 2003



Nr. 133 Diviseq
Solgt fra 2001-2003

Nr. 134
Climen
Solgt fra 1999

Nr. 135 Activelle
Solgt fra 1999

Nr. 148
Totelle Sekvens
Solgt fra 2003

Bilder av P-pille merker i salg 1965-2003

Denne brosjyren er et hjelpemiddel for å huske riktig navn på de p-piller du har brukt. Under bildene er det oppgitt hvilke år p-pillene var i salg. For noen p-piller finnes det esker med samme utseende, men med ulik størrelse, anhengig av om de inneholder p-piller for en eller flere måneder. Vi ber deg tenke nøye gjennom navnet på de p-pillene du har brukt. Av noen p-piller/ merker har vi ikke bilder, det gjelder:

Nr. 1. Follistrel, solgt fra 1973–76
Nr. 2. Menokvens, solgt fra 1971–72
Nr. 3. Novokvens, solgt fra 1969–70
Nr. 5. Anovlar Mite, solgt fra 1967–69
Nr. 8. Consan, solgt fra 1968–70
Nr. 9. Delpregnin, solgt fra 1968–71
Nr. 14. Kombikvens, solgt fra 1971–75
Nr. 20. Micronor, solgt fra 1971–79
Nr. 22. Norlestrin, solgt fra 1965–80
Nr. 23. Nyo-Kon, solgt fra 1968–70
Nr. 26. Ortho-Novin Mite, solgt fra 1968–72
Nr. 39. Implanon, solgt fra 2002
Nr. 43. Jadelle, solgt fra 2004

Nr. 4 **Solgt fra 1965-68**

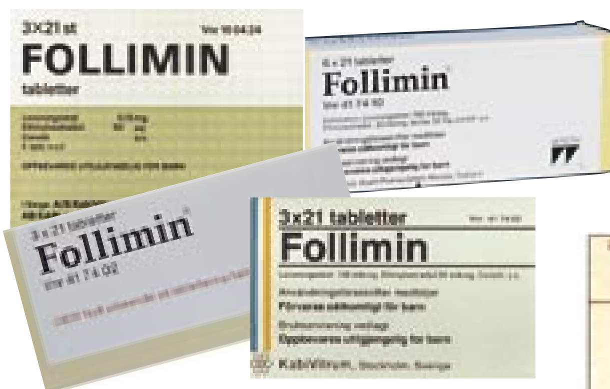
Nr. 6. **Solgt fra 1980**

Nr. 7 **Solgt fra 1971**

Nr. 10 **Solgt fra 1980**

Nr. 11 **Solgt fra 1969**

Nr. 12 **Solgt fra 1973**



Nr. 13 **Solgt fra 1978**

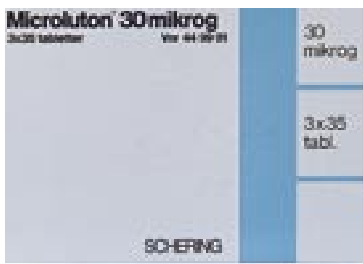
LYNDIOL MITE

Nr. 15 **Solgt fra 1966-72**

Nr. 16 **Solgt fra 1965**

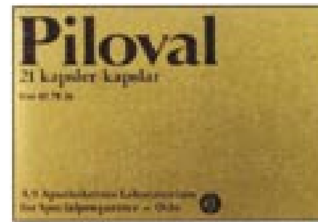
Nr. 17 **Solgt fra 1985**

Nr. 18 **Solgt fra 1975**

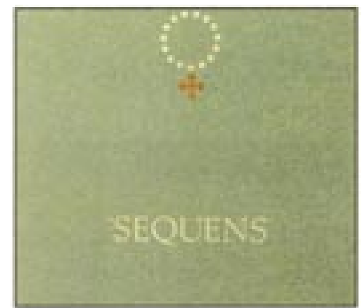


Nr. 19 Solgt fra 1973

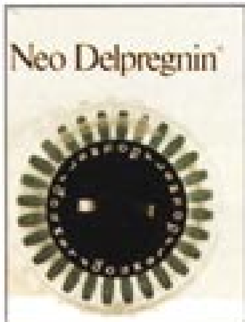
Nr. 29 Solgt fra 1973-82



Nr. 30 Solgt fra 1968-84



Nr. 33 Solgt fra 1967-69

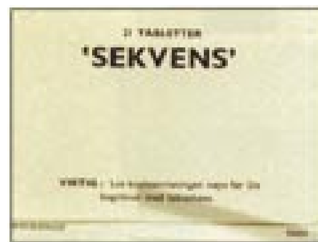


Nr. 21 Solgt fra 1971-79

Nr. 31 Solgt fra 1977

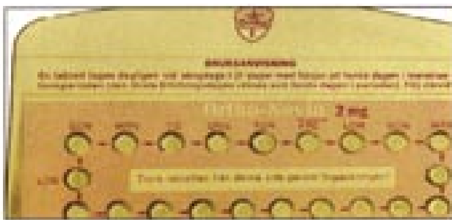
Nr. 34
Solgt fra 1990

Nr. 24 Solgt fra 1971-81

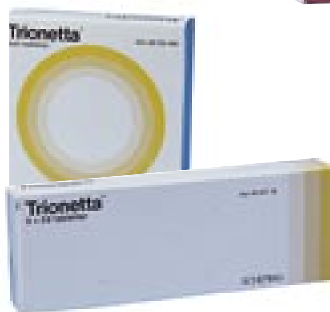


Nr. 32 Solgt fra 1969-70

Nr. 35
Solgt fra
1981

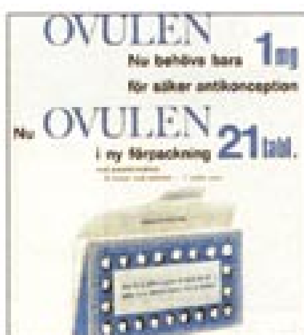


Nr. 25 Solgt fra 1966-69



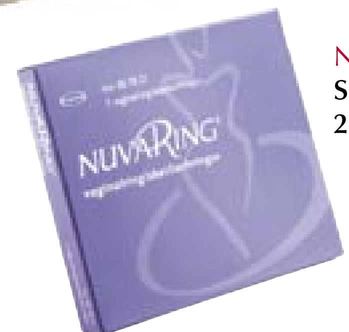
Nr. 36 Solgt fra 1981

Nr. 38 Solgt fra 2002



Nr. 27 Solgt fra 1965-71

Nr. 37
Solgt fra
2001



Nr. 40
Solgt fra
2003

Nr. 28 Solgt fra 1970

TAKK FOR INNSATSEN!

Nr. 41 Solgt fra 2003

Nr. 42 Solgt fra 2004

LUNDBLAD MEDIA AS, TROMSØ - O.nr. 50253 - 40.000 Uts. nr. 40

Undersøkelsen
"KVINNER OG KREFT"

Vi minner om at vi nylig har sendt deg et spørreskjema som vi håper du tar deg tid til å svare på. Ditt svar er et viktig bidrag for oss, fordi slutningene vi kan trekke ut fra undersøkelsen vil være mer pålitelige dersom mange har svart.

Vi ønsker at resultatene fra undersøkelsen skal komme deg og andre kvinner til gode. Du velger likevel selv om du vil delta i undersøkelsen.

Hvis du nylig har returnert skjemaet, ber vi deg se bort fra denne hendvendelsen. Vi takker for verdifull bistand.

Alle opplysninger fra undersøkelsen behandles konfidensielt og etter Datatilsynets regler.

Har du spørsmål om undersøkelsen, eller trenger du et nytt spørreskjema, kan du kontakte Institutt for samfunnsmedisin, Universitetet i Tromsø, 9037 Tromsø,
Bente A. Augdal tlf. 77 64 66 38

Med vennlig hilsen

Eiliv Lund
professor dr.med.

LUNDBLAD MEDIA AS, TROMSØ – 032163

UNIVERSITETET I TROMSØ
INSTITUTT FOR SAMFUNNSMEDISIN

B

Appendix II

Basis for Paper III

Letter requesting a blood sample

Instruction regarding sample collection

Questionnaire completed on the day of blood sample collection

Reminder car

KVINNER OG KREFT

Du sendte i 2003 eller 2004 et utfylt spørreskjema til Institutt for samfunnsmedisin som del av den landsdekkende undersøkelsen "Kvinner og kreft". Spørsmålene var særlig rettet mot kosthold.
Vi ønsker å studere hvilken betydning våre matvaner har for kreftutvikling hos kvinner. I følgeskrivet til spørreskjemaet informerte vi om at en del kvinner senere ville bli forespurt om de var villig til å avgi blodprøve. Blodprøvene vil bli avidentifisert ved ankomst Institutt for samfunnsmedisin.

Formålet med blodprøven vil være:

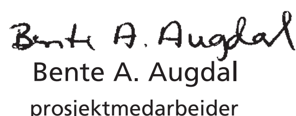
- Måle nivå av vitaminer, mineraler og andre stoffer i blodet som kan settes i forbindelse med kostholdet.
- I fremtiden kunne studere de såkalte genetiske markører dvs. egenskaper i arvestoffet som kan disponere for kreft.
- Teste nye ideer eller hypoteser som oppstår i fremtiden.

Det er frivillig om du vil delta. Du kan trekke deg uten begrunnelse, og du kan be om at opplysninger du har gitt blir slettet, uten at dette vil få konsekvenser for deg.
Blodprøven vil kun bli benyttet til forskning og ingen resultater vil bli utlevert til deg eller noen andre. Blodprøven vil bli lagret i 30 år.

Ansvarlig for undersøkelsen er professor Eiliv Lund. Undersøkelsen er tilrådd av Regional komité for medisinsk forskningsetikk, Nord-Norge (REK NORD), og Datatilsynet har gitt konsesjon for oppbevaring av opplysninger.
Fremtidige forskningsprosjekter som vil benytte de lagrete blodprøvene vil forelegges Regional komité for medisinsk forskningsetikk, Nord-Norge (REK NORD).

Du kan finne mer informasjon om "Kvinner og kreft"og om forskningsresultatene på våre nettsider: www.ism.uit.no/kk/

Med vennlig hilsen



Eiliv Lund
professor dr.med.

Bente A. Augdal
prosjektmedarbeider

Ønsker du ikke å delta og vil slippe påminning pr. brev ber vi deg fylle ut svar-slippen og returnere denne sammen med utstyret tilbake til oss (forseglet utstyr **må** ikke åpnes).

Jeg ønsker **ikke** å delta i blodprøvetakingen. ....

Underskrift

INFORMASJON
TIL DEG SOM ØNSKER Å DELTA

Hvis du ønsker å delta, må du ta kontakt med ditt legekontor, bedriftshelsetjeneste eller annen kyndig person og avtale tid for blodprøvetaking. **Det er viktig for prøvens holdbarhet at den tas mandag, tirsdag eller onsdag, slik at den kan nå oss via post innen fredag.**
Vedlagt utstyr og informasjon om prøvetakingen leveres til den som tar prøven.

Spørreskjema fylles ut prøvetakingsdagen og returneres til oss sammen med blodglassene.
Du vil ikke bli belastet med noen utgifter i forbindelse med prøvetakingen.

Utstyr:

- To prøveglass (1 stk rød kork, 1 stk blå kork)
- Nål til prøvetaking (kun et stikk i armen)
- Ett spørreskjema (til utfylling prøvedagen)
- Returkonvolutt for prøvene og spørreskjema

TIL PRØVETAKEREN

Vi ber om hjelp med prøvetaking av 2 blodglass, som skal benyttes til forskning i den nasjonale studien av brystkreft "Kvinner og kreft".

Deltakeren har mottatt det utstyr og de glass du behøver for å kunne hjelpe oss til å utføre denne delen av studien.

- Glassene merkes med ID-nr. til deltakeren.
- Fyll først det **røde** og deretter det **blå** prøveglasset med vanlig venepunksjon. Vær tålmodig, det røde glasset fylles sakte. Vend rørene forsiktig 8 – 10 ganger.
- Blodprøvene skal ikke sentrifugeres.
- Glassene legges i transporthylstrene og pakkes i returkonvolutten sammen med spørreskjemaet som deltakeren har fylt ut, konvolutten sendes oss snarest mulig.

Deltakeren skal ikke belastes med noen utgifter i forbindelse med blodprøvetakingen.
Betaling tilsvarende takst 701a (se baksiden) refunderes ved at det fylles ut en giro med kontonummer, og at denne sendes sammen med glassene tilbake til oss.

Takk for hjelpen!

Ønsker du mer informasjon kan du kontakte Bente A. Augdal telefon 77 64 66 38 eller Merethe Kumle telefon 77 64 48 84.

Prosjektet støttes av Norges forskningsråd.

Den norske lægeforening

Professor Eiliv Lund
Det medisinske fakultet
Institutt for samfunnsmedisin
Universitetet i Tromsø
9037 Tromsø

AM/3594/98/560.0 Oslo, 1.6.2003

Taking og sending av blodprøver i forb.m. undersøkelsen "Kvinner og kreft"

Vi viser til henvendelsen fra Det medisinske fakultet.

Legeforeningen anser at alle leger bør være positive til å delta i undersøkelsen "Kvinner og kreft" som jo kan få stor helsemessig og faglig betydning.

Vi vil også oppfordre til at leger aksepterer en betaling svarende til takst 701a for taking og innsending av blodprøvene, og håper selvsagt dermed at undersøkelsen får den nødvendige oppslutning. Legeforeningen forutsetter at kvinnen er klar over at hun skal informere om hva ærendet gjelder ved første henvendelse til legen.

Med vennlig hilsen

Terje Vigen
fung. generalsekretær

Øyvind Sæbø
forhandlingssjef

Vennligst oppgi vår ref. ved henvendelse

Postadresse
Postboks 1152 Sentrum, 0107 Oslo

Besøksadresse
Legenes hus, Akersgata 2, Oslo

Telefon
23 10 90 00

Telefaks
23 10 90 10

Postgiro
0805 5114707

Bankgiro
5005 05 48802

Organisasjonsnr.
NO 960 474 341 MVA

E-post
legeforeningen@legeforeningen.no

KVINNER OG KREFT

2004/2005
KONFIDENSIELT

Følgende opplysninger fylles ut i forbindelse med blodprøvetaking.

DETTE SKJEMA **MÅ** FØLGE BLODPRØVEN!

Skjemaet skal leses optisk. Vennligst bruk blå eller sort penn. Du kan ikke bruke komma, bruk blokkbokstaver.

ID-nr:

LAB-kobling.

Jeg har lest informasjonen om blodprøveundersøkelsen og samtykker i å delta i denne: Ja: ☐

PRØVETAKINGSDAGEN

Fyll inn tidspunkt når blodprøven er tatt: Dato: dag ☐☐ mnd ☐☐

Klokkeslett: ☐☐ ☐☐

STILLING NÅR BLODPRØVEN BLE TATT

Sittende ☐
Liggende ☐

MENSTRUASJONSFORHOLD

Har du menstruasjon?

Ja ☐
Nei ☐
Uregelmessig ☐
Er gravid ☐

Hvis ja:
Angi dato for første dag i siste menstruasjon: dag ☐☐ mnd ☐☐

Når spiste du siste måltid før blodprøven ble tatt: Dato: dag ☐☐ mnd ☐☐

Klokkeslett: ☐☐ ☐☐

RØYKEVANER SISTE UKEN

Har du røkt i løpet av siste uke?

Ja ☐
Nei ☐

Hvis ja: Hvor mange sigaretter røkte du?

Antall i går: ☐☐

Antall i dag: ☐☐

VEKT OG HØYDE

Hvor mye veier du i dag? kg ☐☐☐

Hvor høy er du? cm ☐☐☐

Er disse målene tatt på legekontoret i dag?

Ja ☐
Nei ☐

MEDISINER I LØPET AV SISTE UKE

Har du brukt P-piller i løpet av siste uke?

Ja ☐
Nei ☐

Hvis ja:
Angi dato for siste tablett — dag ☐☐ mnd ☐☐

Preparatnavn: .... (ikke skriv her)

Har du i løpet av siste uke brukt hormontabletter/-plaster (østrogen, gestagen) for overgangsalderen?

Ja ☐
Nei ☐

Hvis ja:
Angi dato for siste tablett — dag ☐☐ mnd ☐☐

Preparatnavn: .... (ikke skriv her)

Preparatnavn: .... (ikke skriv her)

Preparatnavn: .... (ikke skriv her)

Har du brukt andre medisiner i løpet av siste uke?

Ja ☐
Nei ☐

Hvis ja:
Angi dato for siste tablett — dag ☐☐ mnd ☐☐

Preparatnavn: .... (ikke skriv her)

Preparat navn: .... (ikke skriv her)

Preparat navn: .... (ikke skriv her)

BRUK AV KOSTTILSKUDD I LØPET AV SISTE UKE

Har du brukt tran (flytende) i løpet av siste uke?

Ja ☐
Nei ☐

Hvis ja:
Angi dato du sist tok tran — dag ☐☐ mnd ☐☐

Hvor mye tran tok du da?

☐ 1 ts ☐ 1/2 ss ☐ 1+ ss

Har du brukt trankapsler/Omega-3/fiskeolje i løpet av siste uke?

Ja ☐
Nei ☐

Hvis ja:
Angi dato du sist tok trankapsel/ Omega-3/fiskeolje — dag ☐☐ mnd ☐☐

Hvor mange tok du da?

☐ 1 ☐ 2 ☐ 3+

Navn på preparatet du tok sist:

.... (ikke skriv her)

Har du brukt soya i løpet av siste uke?

Ja ☐
Nei ☐

Preparatnavn: .... (ikke skriv her)

Preparatnavn: .... (ikke skriv her)

Har du brukt andre kosttilskudd (vitaminer/mineraler) i løpet av siste uke?

Ja ☐
Nei ☐

Hvis ja:
Angi dato for siste tablett — dag ☐☐ mnd ☐☐

Preparatnavn: .... (ikke skriv her)

Preparatnavn: .... (ikke skriv her)

Takk for hjelpen!

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KVINNER OG KREFT
Blodprøve

Påminnelse!

Du har tidligere mottatt en forespørsel om å gi en blodprøve.

Dersom du ikke har rukket å sende tilbakemelding ennå, vil vi sette stor pris på om du tar deg tid til det.

Hvis du nylig har svart, ber vi deg se bort fra denne henvendelsen. Vi takker for verdifull bistand.

Alle opplysninger fra undersøkelsen behandles konfidensielt og etter Datatilsynets regler.

Har du spørsmål om undersøkelsen, kan du kontakte:
Institutt for samfunnsmedisin, Det medisinske fakultet,
Universitetet i Tromsø, 9037 Tromsø,
v/Bente Augdal, tlf. 77 64 66 38

Ansvarlig for undersøkelsen er professor dr. med. Eiliv Lund.

Med vennlig hilsen

Eiliv Lund
professor dr.med.

Bente A. Augdal
prosjektmedarbeider

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