Contraceptive Use and the Risk of Ovarian Cancer Among Women with a *BRCA1* or *BRCA2* Mutation

by

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Abstract

BRCA mutation carriers face a high lifetime risk of developing ovarian cancer. Oral contraceptives are protective in this high-risk population; however, the impact of other types of contraception (e.g. intrauterine devices (IUDs), implants, injections) is unknown. This study evaluated the relationships between type of contraception and risk of developing ovarian cancer among women with a *BRCA* mutation. Ever use of any contraceptive was significantly associated with reduced risk of ovarian cancer, which was driven by significant inverse associations with oral contraceptives and implants. We observed a similar albeit non-significant effect with use of injections and IUDs. No significant associations were observed between patterns of IUD use and risk of ovarian cancer. These findings support a protective effect of oral contraceptives and implants on risk of ovarian cancer among women with a *BRCA1* or *BRCA2* mutation. The possible reduced risk with use of injections and IUDs requires further evaluation.

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List of Abbreviations

BARD1	BRCA1 associated RING domain protein 1
BMI	Body mass index
<i>BRCA1</i>	Breast Cancer Susceptibility Gene-1
<i>BRCA2</i>	Breast Cancer Susceptibility Gene-2
BRCT	BRCA1 carboxy-terminal repeats
BRIP1	BRCA1-interacting protein C-terminal helicase 1
CA125	Cancer antigen 125
CDK	Cyclin-dependent kinase
CI	Confidence interval
CtIP	CtBP interactive protein
DMPA	Depot medroxyprogesterone acetate
DSB	Double-stranded break
EE	Ethinyl estradiol
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin-releasing hormone
HGSC	High-grade serous ovarian carcinoma
HR	Homologous recombination
HR	Hazard ratio
HRT	Hormonal replacement therapy
IARC	International Agency for Research on Cancer
IUD	Intrauterine device
LARC	Long-acting reversible contraception
LH	Luteinizing hormone
LNG	Levonorgestrel
LOH	Loss of heterozygosity
NECC	New England Case Control Study
NHEJ	Non-homologous end joining
NHS	Nurses' Health Study
OC	Oral contraceptive

OCCR	Ovarian cancer cluster regions
OR	Odds ratio
PALB2	Partner and localizer of BRCA2 protein
PRA	Progesterone receptor A
PRB	Progesterone receptor B
RANK	Receptor activator of nuclear factor κ B
RING	Really Interesting New Gene
RR	Relative risk
RRBSO	Risk-reducing bilateral salpingo-oophorectomy
STIC	Serous tubal intraepithelial carcinoma

Chapter 1. Introduction

1.1. Breast Cancer Susceptibility Gene-1 (*BRCA1*) and Breast Cancer Susceptibility Gene-2 (*BRCA2*) and Ovarian Cancer

1.1.1. Overview of *BRCA*-associated Ovarian Cancer

In 2020, ovarian cancer ranked as the eighth most common cancer diagnosis in women worldwide but the leading cause of gynecological cancer death¹. According to the International Agency for Research on Cancer (IARC), an estimated 313,959 new cases of ovarian cancer are diagnosed annually and 207,252 women die of it¹. Despite advancements in the understanding and management of ovarian cancer, the majority of patients present with late-stage disease (stage III/IV) and have a five-year survival rate of only 30%². Data from the World Health Organization have shown minimal improvements in ovarian cancer mortality in recent decades³. The greatest declines were observed among developed countries. In the United States, rates dropped approximately 16% from 5.8 deaths per 100,000 women in 2002 to 4.9 deaths per 100,000 women in 2012. These trends paralleled declines in ovarian cancer incidence rather than improvements in early detection or treatment⁴. To a large extent, the reduced incidence of ovarian cancer has been attributed to changes in reproductive risk factors such as oral contraceptive use, parity, breastfeeding and tubal ligation⁴.

Although the majority of ovarian cancers are sporadic, approximately 25% of affected women have a hereditary predisposition⁵. Pathogenic variants in the breast cancer susceptibility genes 1 and 2 (*BRCA1* and *BRCA2*) are the strongest known genetic risk factors and account for almost 45% of cases with a family history of the disease and 15% of all ovarian cancers⁶⁻⁸.

BRCA1 and *BRCA2* are tumour-suppressor genes located on chromosome 17q21 and 13q12, respectively. After the initial identification of *BRCA1* in 1994 and *BRCA2* in 1995, their nuclear protein products have been implicated in several cellular processes required for the maintenance of genome integrity, including DNA repair, cell cycle control, transcriptional regulation, protein ubiquitylation and chromatin remodelling⁹. The role of *BRCA1* and *BRCA2* in homologous recombination (HR), a high-fidelity pathway of DNA double-stranded break (DSB) repair,

accounts for their major tumour suppressor activity. In the context of a deleterious germline mutation in *BRCA1* or *BRCA2*, a somatic mutation in the corresponding normal allele can lead to loss of *BRCA* function, interruption of DNA repair function and initiation of tumourigenesis, according to the two-hit paradigm of tumour development.

Globally, an estimated 1 in 400 to 1 in 800 people are heterozygous carriers of a *BRCA1* or *BRCA2* mutation¹⁰. Although relatively uncommon, inherited pathogenic mutations in these genes are highly penetrant and predispose individuals to high lifetime risks of breast and ovarian cancer. Compared to an average cumulative risk of 12% in the general population, the risk of breast cancer by age 80 has been estimated at 72% in *BRCA1* mutation carriers and 69% in *BRCA2* mutation carriers¹¹. Women who inherit a deleterious *BRCA* mutation also face an increased lifetime risk of developing ovarian cancer, compared to a 1.2% risk in the general population. This is estimated at 44% for *BRCA1* and 17% for *BRCA2*, corresponding to average annual risk estimates of 0.86% and 0.37%, respectively^{11,12}. Lesser elevated risks have also been reported for cancers of other sites such as the prostate, pancreas and colon¹³.

Germline *BRCA* mutations are associated with ovarian cancers of distinct clinicopathological features. On average, mutation carriers are diagnosed at an earlier age compared to sporadic cases of ovarian cancer. The median age at diagnosis of ovarian cancer in the general population is 63 years, whereas the median age at diagnosis for *BRCA1* and *BRCA2* mutation carriers is estimated at 54 and 59.5 years, respectively¹¹. The majority of *BRCA*-associated ovarian cancers are of high-grade serous histology (67%) although endometrioid (12%) and clear cell carcinomas (2%) have also been reported¹⁴. Most carriers present at advanced stage, with over 80% of cases diagnosed at stage III/IV, by which point the disease has spread to peritoneal surfaces^{15,16}. Short-term survival outcomes are more favourable for *BRCA*-positive women compared to mutation-negative women due to greater tumour sensitivity to platinum therapy^{17,18}; however, this response does not predict long-term survival. In a study of 1,421 ovarian cancer cases, 10-year survival estimates were 43% for noncarriers, 57% for *BRCA1* carriers and 69% for *BRCA2* carriers, suggesting significantly worse long-term survival among carriers¹⁹.

1.1.2. Roles of BRCA Proteins within the Cell

BRCA1 encodes a large multi-domain protein spanning 1,864 amino acids in length²⁰ (Figure 1). The BRCA1 protein interacts with multiple protein binding partners through three major domains: the Really Interesting New Gene (RING) finger domain at the N-terminus, a central coiled-coil domain, and tandem BRCA1 carboxy-terminal repeats (BRCTs) at the C-terminus. BRCA1 interacts with its obligatory partner, BRCA1-associated RING domain protein 1 (BARD1) through common RING domains. The BRCA1-BARD1 heterodimer has E3 ubiquitin ligase activity, which is involved in DNA repair and tumour suppression¹²¹. The BRCT repeats mediate interactions between BRCA1 and several DNA damage response proteins including abraxas CtBP interactive protein (CtIP) and BRCA1-interacting protein C-terminal helicase 1 (BRIP1) through binding of phosphorylated serine at the pSXXF motif²². The coiled-coil domain of BRCA1 binds a homologous domain in the partner and localizer of BRCA2 protein (PALB2), which facilitates recruitment of BRCA2 during HR. These structural domains flank an intrinsically disordered central region encoded by exon 11 that contains several DNA-damage-induced phosphorylation sites to facilitate the formation of various BRCA1 protein complexes²³.

BRCA2 does not share a high degree of sequence homology with *BRCA1*, but both proteins are functionally related through involvement in the HR pathway (Figure 1)²². *BRCA2* encodes a 3,418 amino acid product that associates with the HR-related proteins, PALB2 and RAD51. At the N-terminus, BRCA2 executes PALB2 binding between amino acids 21-30. Centrally, eight BRC repeats mediate binding and recruitment of the recombinase, RAD51, which is essential for the initiation of DNA repair. Downstream, BRCA2 contains a DNA-binding domain as well as a cyclin-dependent kinase (CDK) phosphorylation site at S3291 of the C-terminus, which is also important for RAD51 binding²².

Figure 1. Functional domains of the human BRCA1 and BRCA2 proteins

Footnote to Figure 1: *BRCA1* is an 1,863-amino acid protein composed of 23 exons. Functional domains depicted as violet boxes in *BRCA1* include the Really Interesting New Gene (RING) finger domain, two nuclear localization signals (NLS), a coiled-coil domain and two BRCA1 C-terminal (BRCT) domains. *BRCA2* is a 3,418-amino acid protein comprised of 27 exons. Functional domains depicted as green boxes in *BRCA2* include the transcriptional activation domain, eight BRC motifs, a conserved helical (H) domain, three oligonucleotide-binding (OB) folds, a tower (T) domain and two NLS'. Domain names are denoted above.

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1.1.2.1. Role of BRCA1 and BRCA2 in Homologous Recombination

BRCA1 and *BRCA2* are considered ‘caretaker’ genes that serve to maintain genome integrity and tumour suppression in the cell. Through its various functional domains, *BRCA1* has pleiotropic roles in multiple DNA repair pathways, including HR, non-homologous end joining (NHEJ) and single-strand annealing, as well as cell cycle control, chromatin remodelling and transcriptional regulation²². Conversely, *BRCA2* is primarily involved in HR in complex with RAD51. The roles of *BRCA1* and *BRCA2* in the repair of DNA DSBs by HR are well-described and thought to underly their tumour suppressor activity. DSBs can arise from exogenous exposure to ionizing radiation or chemotherapeutic agents as well as endogenous sources such as reactive oxygen species or replication error²². Repair of DSBs can occur by HR or NHEJ. Whereas NHEJ is error-prone and may result in insertions or deletions, HR is a high-fidelity mechanism that uses the homologous sister chromatid as a template for repair²⁰. *BRCA1* forms three main complexes that participate in the early stages of the HR pathway. *BRCA2* participates downstream by mediating assembly of RAD51 on single-stranded DNA. The **BRCA1-A complex** consists of

BRCA1, Rap80, Abraxas, NBA1, BRE and BRCC36 and targets BRCA1 to sites of DNA damage by associating with ubiquitinated histones at the DSB²³. BRCA1 also associates with the resection complex MRN and recruits the resection factor, CtIP to form the **BRCA1-C complex**. BRCA1-C catalyzes DNA end resection to yield a 3' single-stranded DNA tail, signalling the earliest commitment step of HR. BRCA1 associates with BRCA2 through the bridging protein, PALB2. The BRCA1-PALB2-BRCA2 holoenzyme complex, otherwise known as **BRCC**, coordinates RAD51-loading of single-stranded overhangs, which guides strand invasion of the intact homologous chromosome and formation of a homologous duplex²⁴. DNA synthesis proceeds from the 3' end and restores the strand's original sequence. The displacement loop is subsequently resolved to generate crossover or non-crossover products.

1.1.3. Mutation Classification

To date, over 1,600 unique mutations have been identified in *BRCA1* and over 1,800 unique mutations have been reported in *BRCA2*¹⁰. Approximately 80% of known pathogenic mutations in these genes generate a premature stop codon resulting in a truncated and inactive form of the protein^{25–27}. These include nonsense mutations, frameshift mutations, splicing site mutations and large deletions²⁸. While most truncating mutations are dispersed across the lengths of both genes, deleterious missense mutations have been observed to cluster in specific regions of *BRCA1* and *BRCA2*. The most frequently reported mutations in *BRCA1* occur at the BRCT and RING finger domains, whereas mutations in *BRCA2* tend to surround the BRC repeats and OB folds²⁹.

Mutation-specific cancer risks have also been linked to key regions of *BRCA1* and *BRCA2*. Several ovarian cancer cluster regions (OCCR) have been identified in or near exon 11 of both genes, bounded by c.1380 to c.4062 in *BRCA1* as well as c.3249 to c.5681 and c.6645 to c.7471 in *BRCA2*³⁰. Mutations within the OCCR are associated with a significant relative increase in ovarian cancer risk to breast cancer risk³⁰.

Several founder mutations in *BRCA1* and *BRCA2* have reported at high frequencies in specific populations. The most striking examples are observed among families of European (Ashkenazi) Jewish ancestry, in which three founder mutations (185delAG and 5382insC in *BRCA1*; 6174delT in *BRCA2*) account for nearly 80% of all *BRCA* mutations identified in this population³¹. Other founder mutations have been established for European (Polish, Icelandic, Norwegian, Swedish, Italian) and non-European (French Canadian, Hispanic, Chinese, Japanese, Malaysian) populations³².

1.1.4. Fallopian Tube as the Site of Origin for Ovarian Cancer

Ovarian cancer was traditionally thought to arise *de novo* from the ovarian surface epithelium or from cortical inclusion cysts within the ovarian parenchyma³³. However, several limitations have emerged to challenge this theory of cellular origin. Despite decades of effort, no ovarian precursor lesions had been identified at these sites³⁴. Moreover, the majority of ovarian tumours are composed of Müllerian-type epithelium that is not present in the ovary³⁵. The major histologic subtypes of ovarian cancer (serous, endometrioid and mucinous) do not resemble cell types of the ovary. Rather, they are morphologically similar to epithelia of the fallopian tube, endometrium and cervix, respectively³⁵.

In 1999, Dubeau was the first to argue that what seemed to be primary ovarian epithelial tumours may actually originate from derivatives of the Müllerian tract including the fallopian tube and endometrium³⁵. Recent morphological and molecular findings have added support to a dualistic model of ovarian carcinogenesis in which ovarian cancers predominantly arise from the endometrial or fallopian tube epithelium and spread to the ovary secondarily³⁶. According to this model, the various histologic subtypes of ovarian cancer may be classified as Type I or Type II with distinct clinicopathological profiles, molecular features and tissues of origin. Type I carcinomas, such as clear cell, endometrioid, mucinous and low-grade serous carcinomas, arise from borderline serous tumours or endometriosis³⁷. Type II carcinomas mainly comprise high-grade serous ovarian carcinoma (HGSC), the most common type of epithelial ovarian cancer, and develop from the secretory epithelium of the distal fallopian tube as well as the ovary³⁷.

Support for the tubal origin of HGSC is based on a wealth of clinical, epidemiological, histological and molecular evidence that has emerged in the last two decades. Beginning in the early 2000s, studies of asymptomatic *BRCA* mutation carriers undergoing prophylactic salpingo-oophorectomy have identified a high frequency of early serous carcinomas localized to the distal fallopian tube. Between 57% and 100% of occult malignancies exhibited fallopian tube involvement at the fimbriated end^{38–42}. Similar lesions were later discovered in the fallopian tubes of 50% to 60% of women with sporadic HSGC but not among other histologic subtypes^{43,44}. Termed serous tubal intraepithelial carcinomas (STICs), these non-invasive

carcinomas of the fallopian tube represent the immediate precursor of HGSC for both *BRCA* mutation-positive and *BRCA* mutation-negative women. These findings were validated by genetic and molecular studies that further confirmed the link between STICs and HGSC. The detection of identical *TP53* mutations between STICs and concomitant ovarian HGSCs support a clonal relationship and direct evolutionary descent from STICs^{45,46}. Molecular profiling studies have demonstrated that the gene expression signature of HGSC resembles that of the fallopian tube epithelium more than the ovarian surface epithelium⁴⁷. Moreover, a premalignant precursor of STIC has been identified in the fallopian tube that shares a common fimbrial location, cell type, evidence of DNA damage and *TP53* mutations with STICs. Comprised of short stretches of benign fallopian tube epithelium that strongly express p53, ‘p53 signature’ lesions represent the earliest known molecular event in HGSC development.

Based on these observations, the proposed pathogenic sequence initiates with genotoxic stress at the fallopian tube epithelium, leading to DNA damage, induction of *TP53* mutations and clonal expansion of a ‘p53 signature’. The second step involves further malignant transformation, giving rise to a STIC with proliferative activity. STIC has the potential to invade the tubal stroma or exfoliate onto the ovarian surface and develop HGSC⁴⁸.

1.1.5. Molecular Pathogenesis of *BRCA*-associated Ovarian Cancer

1.1.5.1. Two-hit Hypothesis

The molecular pathogenesis of hereditary breast and ovarian cancer is traditionally believed to follow the ‘two-hit’ model proposed by Knudson. *BRCA1* and *BRCA2* represent canonical tumour suppressor genes characterized by an autosomal dominant pattern of inheritance⁴⁹. *BRCA* mutation carriers typically inherit a single mutant copy in the germline and exhibit somatic loss of the remaining normal (wild-type) *BRCA* allele in familial breast and ovarian tumours^{50–52}. Loss of heterozygosity (LOH) is considered the rate-limiting step in *BRCA*-related tumour development and occurs through genetic or epigenetic inactivation of *BRCA* activity⁵³. An inherited *BRCA* defect may accelerate *BRCA* LOH and contribute to the earlier onset of disease seen in carriers.

1.1.5.2. *BRCA* Haploinsufficiency

Accumulating evidence also supports a mechanism of *BRCA* haploinsufficiency as a driver of hereditary breast and ovarian tumour predisposition. Retention of the normal allele has been observed in a subset of germline *BRCA1* and *BRCA2* ovarian tumours⁵⁴. Work in breast cancer cell lines reveal phenotypic effects of *BRCA* heterozygosity that include spontaneous recombination events and impaired HR, which increase the risk of additional cancer-promoting mutations^{55–57}. In animal studies, *Brca1* heterozygous mice had a higher incidence of ovarian tumour formation upon exposure to ionizing radiation, indicating a specific effect on ovarian cells⁵⁸. Thus, *BRCA* haploinsufficiency may cause genomic instability and promote loss of the wild-type allele.

1.1.5.3. *TP53* Loss in Early *BRCA*-associated Tumourigenesis

Biallelic inactivation of *Brca1* or *Brca2* is embryonically lethal in mouse models, and *BRCA* deficiency in human cells is similarly thought to result in cell cycle arrest or cell death due to induction of DNA-damage dependent cell cycle checkpoints^{22,59}. This raises the question of how *BRCA*-deficient cells may escape apoptosis and accumulate chromosomal abnormalities that promote tumourigenesis. Emerging evidence indicates that somatic inactivation of checkpoint

control genes such as *TP53* may be a pre-requisite for *BRCA* gene inactivation in early neoplastic development. Consistent with this hypothesis, 92% of HGSCs of the ovary arising in *BRCA* mutation carriers harbour mutations in *TP53*⁶⁰. Additionally, *TP53* mutations were detected in the absence of *BRCA* LOH in the non-neoplastic fallopian tube epithelium of carriers, whereas all early tubal neoplasias demonstrated loss of the wild-type *BRCA* allele⁶¹. Taken together, the data suggest that *TP53* loss is an essential early event in *BRCA*-associated ovarian cancer progression, allowing for the propagation of *BRCA*-deficient cells.

1.1.6. Proposed Mechanisms of Ovarian Cancer Pathogenesis

The *BRCA* genes are ubiquitously expressed and participate in general cellular mechanisms, thus the predisposition toward site-specific cancers remains unclear. Several hypotheses have been put forward to account for the particular increased risk of ovarian cancer seen among *BRCA1* and *BRCA2* mutation carriers. In these models, the ovary and fallopian tube accumulate specific hormonal and inflammatory exposures through ovulation that may encourage the development of ovarian cancer in a *BRCA*-deficient backdrop; however, these etiologic pathways overlap as physiologically interrelated events during ovulation.

1.1.6.1. Incessant Ovulation Hypothesis

In 1971, Fathalla postulated that ovulation contributes to the etiology of ovarian cancer through repeated minor trauma to the ovarian surface epithelium⁶². Rupture of the ovarian follicle is followed by rapid proliferation of ovarian epithelial cells during postovulatory wound repair. According to the “incessant ovulation” hypothesis, repeated exposure of the ovarian surface epithelium to follicular rupture and repair through menstrual cycles predisposes ovarian epithelial cells to DNA damage and malignant transformation⁶³. The incessant ovulation hypothesis gained further support from studies of comparative oncology demonstrating the rarity of epithelial ovarian carcinomas in animals that limit ovulation to breeding seasons interspersed by non-ovulatory rest periods⁶². In humans, conditions associated with a greater lifetime number of ovulatory episodes, such as early menarche, late menopause and nulliparity, correspond to greater risk of developing ovarian cancer⁶⁴. On the other hand, conditions in which ovulation is suppressed, such as multiple pregnancies, oral contraceptive use and breastfeeding have been reported to lower risk of developing ovarian cancer (described in Section 1.1.7)⁶⁴.

The incessant ovulation hypothesis still holds in light of a tubal origin of ovarian cancer (see Section 1.1.4). Emphasis shifts to the impact of follicular fluid, which is released alongside the cumulus-oocyte complex and bathes the ovarian surface epithelium and distal fallopian tube immediately after ovulation⁶⁵. Mature follicular fluid is abundant in inflammatory cytokines, chemokines, free radicals, growth factors and reactive oxygen species⁶⁶. *In vitro* studies

demonstrate that exposure of fimbrial epithelial cells to follicular fluid upregulates inflammatory signalling, genotoxic stress and DNA damage^{67,68}.

1.1.6.2. Inflammation Hypothesis

Many features of the ovulatory process resemble that of an inflammatory process⁶⁹. Ovulation is initiated by a midcycle luteinizing hormone (LH) surge, which stimulates granulosa and theca cells to produce cytokines, chemokines, prostaglandins, steroid hormones and other inflammatory mediators. In turn, there is increased migration of immune cells (e.g. neutrophils, monocytes, macrophages, mast cells) to the follicle that potentiates local apoptosis, follicular rupture and oocyte release⁷⁰. Exposure of tubal and ovarian epithelial cells to follicular fluid can also be carcinogenic, as described above. In the luteal phase, local macrophages secrete prostaglandins, reactive oxygen species and TNF- α to stimulate apoptosis of corpus luteum cells⁷⁰. Thus, ovulation is considered an inflammatory risk factor that results in cyclic exposure of the fallopian tubes and ovaries to a pro-inflammatory milieu. Local elevation of reactive oxygen species, prostaglandins, cytokines and other inflammatory substances during ovulation enhances the risk of mutagenesis. Lending further support, increased ovarian cancer risk has been observed with other inflammatory factors, such as use of talcum powder and pelvic inflammatory conditions, while use of nonsteroidal anti-inflammatory drugs and tubal ligation are related to reduced risk of ovarian cancer⁷¹.

More recently, Vercellini proposed the incessant menstruation hypothesis as an extension of inflammation hypothesis that considers the retrograde flow of menstrual blood through the fallopian tubes collecting in the peritoneal cavity⁷². In this model, retrograde menstruation from the endometrial cavity is the source of fallopian tube inflammation. The abundance of free heme is broken down to catalytic iron, which initiates the formation of free radicals and increases genotoxic oxidative stress⁷⁰. This theory is consistent with the reduced risk of ovarian cancer associated with tubal ligation in the general population and in some high-risk groups as well as the increased risk of ovarian cancer linked to endometriosis^{73–77}.

1.1.6.3. Gonadotropin Hypothesis

The carcinogenic role of key reproductive hormones has also been explored through two hormonal hypotheses of ovarian tumourigenesis.

The gonadotropin hypothesis implicates high circulating levels of pituitary gonadotropins with increased risk of developing epithelial ovarian cancer. Gonadotropins may directly stimulate the ovarian surface epithelium by activating LH- and follicle stimulating hormone (FSH)-responsive genes that promote cellular proliferation and malignant transformation⁷⁸. Gonadotropins also induce ovarian steroidogenesis, which can indirectly influence malignant transformation through increased estrogenic stimulation of the ovarian surface epithelium via a paracrine mechanism. Indeed, protective factors against ovarian cancer such as pregnancy and oral contraceptive use are associated with lower circulating gonadotropins⁷⁹. Nonetheless, experimental data on the direct effect of gonadotropin hormones on the proliferation of normal human ovarian surface epithelium cells are inconclusive with a number of studies showing no effect⁸⁰. Importantly, the gonadotropin theory has not been adapted to account for a fallopian tube site of origin. An *in vitro* study of tubal epithelial cell cultures did not display increased cellular proliferation or DNA damage in response to LH or FSH⁸¹.

1.1.6.4. Androgen/Progesterone Hypothesis

The androgen/progesterone hypothesis was proposed by Risch in 1998 and implicates raised levels of endogenous androgens in the pathogenesis of ovarian cancer, whereas progesterone may be protective against ovarian cancer development⁸². Several experimental lines of evidence indicate that androgens can increase proliferation and migratory activity of both malignant and normal ovarian cell lines^{83,84}. More recent data suggest that the fallopian tube epithelium also expresses androgen receptors and displays increased cellular viability, proliferation and invasion in response to androgen exposure⁸⁵. Epidemiologic associations between conditions characterized by high androgenic exposure and ovarian cancer risk also lend support to this model. Post-menopausal status, high body mass index (BMI) and possibly polycystic ovarian syndrome have been related to modestly increased risk of ovarian cancer in the general population^{86,87}. Conversely, the protective effect of oral contraceptive use may be mediated by suppression of ovarian testosterone synthesis⁸².

The progesterone hypothesis emerged from observations that the protective effect of pregnancy exceeds the extent of ovulation suppression alone, and that progestin-only oral contraceptives, which do not suppress ovulation, were associated with a comparable decrease in ovarian cancer risk to combined oral contraceptives⁸². Risch suggested that eight to nine months of elevated blood progesterone may be responsible for the additional ovarian cancer risk reduction seen with pregnancy. Furthermore, the protection gained for users of the progestin-only pill may be due to long-term exposure to potent synthetic progestins, compensating for the decrease in endogenous progesterone synthesis⁸².

Expression of progesterone receptors has been documented in the normal fallopian tube epithelium and ovarian surface epithelium independent of *BRCA* mutation status^{88,89}. Anti-proliferative and pro-apoptotic effects of progestogens have been demonstrated in preclinical studies⁹⁰. Progesterone is shown to inhibit cell proliferation and induce apoptosis by p53 upregulation in human ovarian carcinoma cell lines⁹¹. Three-year administration of progestin-containing oral contraceptives was associated with a four- to six-fold increase in the number of apoptotic ovarian epithelial cells compared to the control or estrogen-treated primates⁹². *In vitro* experiments also support a necroptotic effect of progesterone in p53-deficient murine fallopian tube epithelial cells⁹³. These data support the hypothesis that high levels of progestogen may lead to ‘clearing’ of genetically damaged cells of the ovary or fallopian tube. This theory was evaluated in a clinical trial of 64 high-risk women randomized to four to six weeks of oral levonorgestrel (LNG) or placebo⁹⁴. Karyometric results revealed that LNG exposure shifted the nuclear signature of both ovarian and fallopian tube epithelial samples toward normal, resembling that of women at baseline risk⁹⁴. Interestingly, there was no difference in the primary endpoints of apoptosis or TGF- β signalling, which conflicts with findings from animal studies. This may indicate that progestin-mediated clearance occurs through non-apoptotic pathways in humans; however, the study was also limited by the heterogeneity of tubal specimens available for examination. Only 50% of fallopian tube sections contained the fimbriated end, which may have impacted the ability to detect a biological effect⁹⁴. Distinct from these studies, other evidence point toward a carcinogenic effect of progestogen exposure⁹⁵. At low concentrations, progesterone treatment may increase proliferation and

decrease apoptosis of ovarian cancer cells⁹⁶. A large clinical study of premenopausal *BRCA* mutation carriers also observed that average luteal phase progesterone levels were 121% higher in carriers compared to noncarriers, which may contribute toward excess ovarian cancer risk⁹⁷. In line with this theory, Kim *et al.* found that progesterone induced the development of ovarian cancer in a mouse model of HGSC, whereas pharmacologic blockade and genetic ablation of the PR inhibited HGSC development and metastasis formation⁹⁸. Certainly more evidence is necessary to ascertain the biological mechanisms of progestogen action in ovarian cancer.

1.1.7. Management of Ovarian Cancer Risk in *BRCA* Mutation Carriers

Various international guidelines and recommendations inform the clinical management of individuals who harbour a pathogenic *BRCA* variant^{99–102}. *BRCA1* and *BRCA2* mutation carriers are characterized by an increased lifetime risk of breast and ovarian cancer and earlier onset of disease compared to the general population. The penetrance and epidemiology of hereditary breast and ovarian cancer syndrome is detailed in Section 1.1.1. Specific recommendations for ovarian cancer risk management including risk-reducing surgery, screening and modifiable risk factors will be reviewed.

1.1.7.1. Surgical Prevention

For unaffected individuals who are identified with a pathogenic *BRCA* mutation, risk-reducing bilateral salpingo-oophorectomy (RRBSO) is the current standard of care for the management of ovarian cancer risk. RRBSO is a minimally invasive, laparoscopic procedure that removes the ovaries and fallopian tubes in their entirety¹⁰³. There is substantial data demonstrating the effectiveness of RRBSO in a high-risk population with estimated ovarian cancer risk reductions ranging from 80% to 96%^{39,104,105}. A meta-analysis of 10 studies among *BRCA* mutation carriers confirmed approximately 80% reduction in risk of ovarian or fallopian tube cancer following RRBSO¹⁰⁶. Furthermore, in a Cochrane meta-analysis of 10 cohort studies of RRBSO in *BRCA* mutation carriers, the procedure was associated with a 94% reduction in ovarian-cancer specific mortality and a 68% reduction in all-cause mortality¹⁰⁷.

Optimal timing of RRBSO must weigh the age-related risk of ovarian cancer against the adverse effects of early surgical menopause. While cumulative ovarian cancer risk increases with age, premature menopause is associated with a host of short-term and long-term morbidities, including cardiovascular, bone and cognitive decline¹⁰³. Recommendations for surgical timing also vary by *BRCA* mutation type. For *BRCA1* mutation carriers, the National Comprehensive Cancer Network Guidelines recommend RRBSO between 35 to 40 years and after the completion of childbearing⁹⁹. Indeed, the risk of ovarian cancer is remote before the age of 30, but approximately 26% of women with a *BRCA1* mutation will develop ovarian cancer by the age of 50 years^{12,108}. Since the onset of ovarian cancer occurs on average eight to 10 years later

in *BRCA2* mutation carriers compared to *BRCA1* mutation carriers, it is reasonable to delay RRBSO until age 40 to 45 in these women¹⁰⁹. Currently, the uptake of RRBSO stands around 70% of women with a *BRCA* mutation aged 35 and above¹¹⁰.

In view of the evidence that most HGSCs arise from the fallopian tube, bilateral salpingectomy with delayed oophorectomy has been proposed as an alternative to RRBSO. This is an appealing strategy for premenopausal women who wish to preserve fertility and avoid premature menopause. The procedure involves removal of the fallopian tubes with completion of oophorectomy closer to the time of natural menopause. Despite preliminary data suggesting safety and feasibility, there is no evidence of risk reduction or mortality benefit achieved after salpingectomy alone in high-risk patients⁹⁹. Based on consensus from multiple international authorities, salpingectomy is not recommended as primary prevention in carriers of a *BRCA* mutation at this time^{99,100,102}. Prospective, multicentre trials to evaluate this question are ongoing (NCT02321228; NCT02760849).

1.1.7.2. Screening

RRBSO is the only proven intervention to reduce ovarian cancer incidence and mortality, and ovarian cancer screening remains of limited value in the management of high-risk women. Proposed routine screening consists of transvaginal ultrasound combined with measures of serum cancer antigen 125 (CA125) every six months beginning at the age of 30⁹⁹. Screening may be considered at clinician's discretion until the target age of RRBSO; however, there is no evidence that current screening modalities and regimens reduce ovarian cancer-specific mortality in high-risk populations. Large population-based studies of high-risk women monitored by transvaginal ultrasonography and CA125 showed improvements in early-stage detection of cases, but the effect on survival is unknown^{111,112}.

1.1.7.3. Modifiers of Ovarian Cancer Risk in *BRCA* Mutation Carriers

Inherited mutations in *BRCA1* and *BRCA2* confer a significantly increased risk of breast and ovarian cancer. Nonetheless, there is observed interindividual variability in cancer risk among multi-generation families with mutations in the same gene, suggesting a role for non-genetic risk factors that modulate cancer penetrance in *BRCA* mutation carriers^{113,114}. Indeed, predisposition

to developing breast or ovarian cancer is multifactorial, and novel risk prediction models incorporate the effects of gene mutation, family history, lifestyle and hormonal factors that modify risk for mutation carriers¹¹⁵. The following section evaluates the role of select non-genetic risk factors of *BRCA*-associated ovarian cancer, including reproductive, hormonal and lifestyle exposures.

Reproductive Factors

The characteristics related to reproduction and menstruation, including breastfeeding, parity and the timing of menarche or menopause are known to influence ovarian carcinogenesis¹¹⁶. The underlying mechanisms by which these factors protect against ovarian cancer development may involve reducing a woman's lifetime number of ovulations with concomitant changes in hormonal and inflammatory exposures (discussed in Section 1.1.6).

Breastfeeding is associated with a decreased risk of sporadic and hereditary ovarian cancer risk. A pooled analysis of 13 case-control studies concluded that breastfeeding reduces the risk of developing ovarian cancer by 24% compared to women who never breastfed (odds ratio [OR]=0.76 95% CI 0.71-0.80)¹¹⁷. These findings have been replicated in several large, high-risk cohorts^{75,79,118}. The largest evaluation of breastfeeding and ovarian cancer risk among 4,352 *BRCA1* and *BRCA2* mutation carriers reported that a history of breastfeeding is associated with 23% risk reduction (OR=0.77; 95% CI 0.66-0.90), which increased with longer durations of breastfeeding and did not vary by *BRCA* gene¹¹⁸.

Parity is an established protective factor for ovarian cancer in the general population, associated with 30% to 40% decreased risk compared to nulliparity¹¹⁹; however, the relationship is controversial among carriers. A 2014 meta-analysis analyzed five studies reporting on the association between timing and number of pregnancies and ovarian cancer risk in *BRCA* mutation carriers¹¹⁶. Compared to nulliparous women, there was a significant decreased risk associated with four or more live births (effect size=0.42; 95% CI 0.20-0.88) among *BRCA1* mutation carriers alone. Pregnancy at older age did not provide stronger protection than pregnancy at younger age, such that null results were seen with age at first live birth for both mutation types¹¹⁶.

For women in the general population, later age at menarche has been associated with a small decrease in ovarian cancer risk (≥ 15 years vs ≤ 11 years: relative risk [RR]=0.88; 95% CI 0.80-0.97)¹²⁰. Later age at menopause has also been associated with increased ovarian cancer risk (>52 years vs ≤ 45 years: hazard ratio [HR]=1.46; 95% CI 1.06-1.99), and these associations may vary by histologic subtype^{120–122}. In contrast, studies among high-risk women report no relationship between age at menarche and ovarian cancer risk^{75,79,123,124}. One matched case-control study investigated the role of age at menopause among women with a *BRCA1* mutation and observed an 18% increased risk of ovarian cancer per year of delayed menopause (OR=1.18; 95% CI 1.03-1.35)⁷⁹.

Hormonal Factors

Several epidemiologic studies have examined the use of exogenous hormone agents such as oral contraception, fertility treatments and hormone replacement therapy in relation to ovarian cancer. These associations support the etiologic role of “incessant ovulation” and endogenous hormone levels in the development of ovarian cancer. Section 1.3 provides a detailed overview of the epidemiologic data regarding contraceptive use and ovarian cancer across observational studies in the general and high-risk population.

Hormonal replacement therapy (HRT) consists of estrogen-only or estrogen-progestin regimens prescribed to treat menopausal symptoms. Evidence from a meta-analysis of 52 epidemiologic studies indicates that HRT is associated with a minimal increase in ovarian cancer risk in the general population (RR=1.14; 95% CI 1.10-1.19)¹²⁵. The data on cancer risk associated with HRT use in high-risk women is limited and inconsistent¹²⁶. Two studies of women with *BRCA* mutations found no relationship between HRT use and ovarian cancer risk^{127,128}. Although one case-control study of 1,073 Jewish Israeli *BRCA* mutation carriers reported a significant increase in risk associated with HRT (OR=2.58; 95% CI 1.64-4.06), the sample size was small and subgroup analyses were not conducted¹²⁴.

Fertility drugs are ovarian-stimulating drugs that temporarily raise circulating levels of gonadotropins and steroid hormones during in vitro fertilization treatment. A Cochrane meta-analysis of 25 studies and 182,972 women established that there is no relationship between fertility drugs and ovarian cancer risk in the general population¹²⁹. Likewise, a matched case-

control study of 941 pairs of *BRCA* mutation carrier showed that the use of any fertility drug did not increase risk of developing ovarian cancer¹³⁰.

Lifestyle Factors

Lifestyle factors such as body weight, smoking and dietary habits have been investigated in relation to ovarian cancer risk, but there is a paucity of data among women with a hereditary predisposition¹³¹.

Studies that have addressed the role of smoking in ovarian cancer risk report a low increased risk of developing ovarian cancer overall, but associations vary widely by histologic subtype. A meta-analysis of 51 epidemiological studies found that smoking was associated with a 79% increased risk of mucinous ovarian cancer (RR=1.79; 95% CI 1.60-2.00) and a 20% decreased risk in endometrioid (RR=0.81; 95% CI 0.72-0.92) and clear-cell ovarian cancers (RR=0.80; 95% CI 0.65-0.97) compared to women who never smoked¹³². There was no relationship between smoking and serous ovarian cancer (RR=0.99; 95% CI 0.93-1.06)¹³². In contrast, Ko *et al.* conducted a prospective cohort study among 15,731 *BRCA* mutation carriers and observed a 1.7-fold increased risk of ovarian cancer overall among ever-smokers compared to never-smokers (HR=1.68; 95% CI 1.06-2.67), which did not vary by *BRCA* gene¹³³.

Systematic reviews of the general population indicate that BMI is associated with modestly increase ovarian cancer risk^{87,134}. The literature is limited among high-risk women. Qian *et al.* conducted the largest study in *BRCA* mutation carriers, applying a Mendelian randomization approach to examine the relationship between BMI and ovarian cancer risk¹³⁵. Higher adult BMI was associated with significantly increased risk in premenopausal women (HR=1.25; 95% CI 1.06-1.48) but not postmenopausal women (HR=0.98; 95% CI 0.88-1.10). A recent prospective evaluation of 4,340 *BRCA* mutation carriers also suggested that adult weight gain of more than 20 kg is associated with a two-fold increased risk of *BRCA*-associated ovarian cancer compared to women who maintained stable weight (HR=2.00; 95% CI 1.13-3.54)¹³⁶.

Dietary factors such as coffee consumption have generated conflicting findings with respect to ovarian cancer risk. A recent meta-analysis of 20 case-control studies found no significant association between total coffee intake, total caffeine intake or caffeinated coffee intake and risk

of ovarian cancer in the general population¹³⁷. Interestingly, there was a significant inverse association between decaffeinated coffee intake and risk (OR=0.72; 95% CI 0.58-0.90). Similarly, data from high-risk populations indicate no association between coffee consumption and risk of ovarian cancer (OR=0.7; 95% CI 0.4-1.3)¹²³.

Table 1. Summary of known and potential modifiers of sporadic and *BRCA*-associated ovarian cancer risk

Risk modifier	Sporadic ovarian cancer risk	<i>BRCA</i>-associated ovarian cancer risk
Reproductive factors		
Breastfeeding	↓	↓
Parity	↓	↓
Later age at menarche	↓	—
Later age at menopause	↑	↑
Hormonal factors		
Oral contraceptives	↓	↓
Fertility treatment	—	—
Hormone replacement therapy	↑	?
Lifestyle factors		
Body weight	↑	?
Smoking	↑	↑
Coffee	—	—

Legend: ↓ = decreased ovarian cancer risk; ↑ = increased ovarian cancer risk; ? = unknown association with ovarian cancer risk; — = no association with ovarian cancer risk

1.2. Contraception

1.2.1. The Hypothalamic-Pituitary-Ovarian Axis and Regulation of the Ovarian Cycle

The hypothalamic-pituitary-ovarian axis refers to the cumulative effects of the hypothalamus, pituitary gland and ovaries in the development and function of the female reproductive system. Low levels of ovarian steroids prior to the onset of menstruation stimulate the pulsatile release of the neuropeptide, gonadotrophin-releasing hormone (GnRH) from the hypothalamus (Figure 2)¹³⁸. GnRH stimulates the synthesis and release of gonadotropins from the anterior pituitary gland, namely luteinizing hormone (LH) and follicle stimulating hormone (FSH). According to the two-cell two-gonadotrophin model, estradiol synthesis requires the LH-dependent production of androgens by theca cells. In response to blood-borne FSH, granulosa cells upregulate expression of the enzyme, aromatase, which is required to convert androgens from adjacent theca cells into estradiol, serving as the primary source of elevated estrogen during the follicular phase of the menstrual cycle. FSH from the pituitary gland is also important for granulosa cell proliferation and follicular development¹³⁸.

Estradiol plays a key role in regulating gonadotropin secretion through both negative and positive feedback loops depending on the time in the cycle. During the mid-follicular phase, low levels of estradiol exert a negative feedback effect at the level of the hypothalamus and pituitary to suppress levels of LH and FSH. During the late follicular phase, high sustained levels of estradiol coming from preovulatory follicles induces a positive feedback effect, increasing the pulsatile release of GnRH and enhanced LH and FSH production. This culminates to the midcycle LH surge, which develops over approximately 50 hours and triggers ovulation of the oocyte from the mature follicle¹³⁸. The remaining granulosa and theca cells of the follicle form the corpus luteum, a transient endocrine structure that produces peak levels of progesterone during the luteal phase of the menstrual cycle. High levels of progesterone exert negative feedback on gonadotropin secretion, evident in low levels of LH and FSH. The corpus luteum eventually degrades to initiate the next follicular phase.

Figure 2. Hormonal regulation of the menstrual cycle

Footnote to

Figure 2: The early follicular phase of the menstrual cycle is characterised by low levels of the steroid hormones, estrogen and progesterone, corresponding to a slight increase in follicle stimulating hormone (FSH). FSH drops by the mid-follicular phase due to rising levels of estradiol and inhibin from the developing follicle. At the peak of follicular development, high levels of blood estradiol positively induce the release of luteinizing hormone (LH). The LH surge triggers ovulation and the formation of the corpus luteum. During the luteal phase, the corpus luteum secretes estrogen and progesterone. If fertilization does not occur, the corpus luteum degenerates and the drop in progesterone initiates menstruation.

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1.2.2. Contraceptive Pharmacology

1.2.2.1. Classification of Estrogens and Progestogens

Estrogens are a family of natural and synthetic hormones with activity at estrogen receptors. There are four endogenous estrogens that differ by the number of hydroxy groups present on the 19-carbon backbone: estrone, estradiol, estriol and estetrol. Progestogens are defined as natural and synthetic hormones that bind the progesterone receptor (PR) and act as agonists. Among various parameters, progestational activity is evaluated *in vivo* by the ability to induce secretory changes in an estrogen-primed endometrium¹³⁹. On the other hand, progestogens can exert agonistic, partial agonistic and antagonistic effects at other steroid receptors, including estrogen receptor, androgen receptor, glucocorticoid receptor and mineralocorticoid receptor¹⁴⁰. Progestational effect is dependent on the synergistic action of endogenous and exogenous estrogens that induce PR¹⁴¹. The 21-carbon compound, progesterone is the only naturally-occurring progestogen that is critical for female reproductive function and capable of sustaining pregnancy.

Natural estrogens and progesterone have low bioavailability when administered orally due to rapid metabolism in the gastrointestinal tract and liver. Synthetic hormones have been developed by chemically modifying the steroid skeleton to sterically hinder the action of metabolizing enzymes. As a result, synthetic estrogens and progestogens have more favourable bioavailability and half-life following oral administration¹⁴².

Ethinyl estradiol (EE) and its prodrug, mestranol are synthetic estrogens used in combined oral contraception. First generation combined oral contraceptives contained mestranol, the 3-methyl ether of EE, which is rapidly demethylated to EE in the gastrointestinal tract and liver following oral administration¹⁴³. Animal studies indicate that mestranol is weaker than EE, such that 50 mg of mestranol is pharmacokinetically bioequivalent to 35 mg of EE. Mestranol is rarely used in combined oral contraceptives today, and nearly all modern combined oral contraceptives contain EE¹⁴⁴. EE is a very potent oral estrogen. Unlike estradiol, the addition of an ethinyl group at the 17-carbon position prevents oxidation by 17 β -estradioldehydrogenase, resulting in increased oral activity¹⁴¹. After ingestion, EE is rapidly absorbed in the gastrointestinal tract. First-pass

metabolism at the gut mucosa and liver renders a bioavailability of 38% to 48%¹⁴¹. Circulating EE predominantly exists as a 3-sulphate that is bound almost exclusively to albumin¹⁴¹. There is large intraindividual and interindividual variability in blood EE levels due to variations in metabolic capacity. Serum concentrations of EE peak one to two hours following oral intake and typically represent 1% of the ingested dose¹⁴¹.

Synthetic progestogens, called progestins, are used therapeutically for contraception and hormone replacement therapy due to their anti-ovulatory and anti-estrogenic effects¹⁴¹.

Progestins can be classified based on their chemical structure, which varies widely between molecules and results in different patterns of hormonal activity (Table 2)¹³⁹. Progestins that are structurally related to progesterone are further divided into the pregnane and 19-norgpregnane derivatives. Progestins that are structurally related to testosterone are classified as estranes or gonanes. The classification scheme and names of progestins in each of the categories is summarized in Table 2.

Table 2. Classification scheme of progestins used in hormonal contraception

Parent hormone	Testosterone		Progesterone		Spironolactone
Derivative hormone	19-Nortestosterone		17 α -hydroxyprogesterone		17 α -spironolactone
Class	Gonanes	Estranes	Pregnanes	Norpregnanes	Spironolactone
Progestin	Norgestrel Norgestimate Desogestrel Gestodene Levonorgestrel	Norethindrone (norethisterone) Norethindrone acetate Ethinodiol diacetate Norethynodrel Lynestrol	Medroxyprogesterone acetate Megestrol acetate Chlormadinone acetate Cyproterone acetate Dydrogesterone Medrogestrone	Demegestone Promegestone Trimegestone Nomegestrol acetate Nesterone	Drospirenone

1.2.2.2. Testosterone Derivatives

The most common contraceptive progestins belong to the 19-nortestosterone class, which are subdivided into estrane and gonane families based on the structure of the carbon ring from which the progestin is derived. All 19-nortestosterones are derivatives of testosterone, but exert major hormonal effect at PRs with minimal androgenic and anabolic properties¹⁴⁵.

The estrane family (C18) is characterised by an 18-carbon parent steroid with a methyl group substitution at the 13-carbon position. These include commonly used progestins such as norethindrone, norethindrone acetate and ethynodiol diacetate. Following oral administration, all estranes are rapidly metabolized to the progestationally active compound, norethindrone. In addition to potent progestational activity, all 19-nortestosterone derivatives have low androgenic and estrogenic activity¹⁴⁵.

13-ethylgonanes, otherwise known as gonanes (C17) possess an ethyl group at the 13-carbon position, which confers greater progestational and less androgenic activity *in vitro*¹³⁸. While all estranes have the same activity as norethindrone, individual gonanes have distinct properties. Among the most commonly used gonanes include LNG, which is the hormonally active isomer of the racemic compound, norgestrel. Synthesized in 1966, LNG is one of the most potent orally active progestogens¹⁴⁶. LNG binds PR with high affinity and strongly inhibits gonadotrophin secretion. LNG also exerts relatively strong androgenic activity and anti-estrogenic activity without glucocorticoid, mineralocorticoid or anti-mineralocorticoid potential. It does not undergo significant hepatic first-pass metabolism, thus bioavailability is virtually 100% after oral administration. LNG is hydrolyzed by the liver and eliminated after conjugation with glucuronic acid¹⁴⁷. Newer, third-generation progestogens in the gonane family include desogestrel, norgestimate and gestodene that offer further reduced androgenic activity¹⁴⁴. Desogestrel and norgestimate are prodrugs that are converted *in vivo* to the active metabolites etonogestrel and levonorgestrel, respectively¹⁴⁶. In addition to low androgenic activity, desogestrel exhibits high antigonadotropic activity¹⁴⁷. Gestodene has inherent progestational activity, slight mineralocorticoid activity and high anti-estrogenic activity¹⁴⁸. Gestodene is characterized by prolonged half-life and 100% oral bioavailability¹⁴⁷. Several combined oral contraceptives containing gestodene and EE are available, including Logest and Minulet.

1.2.2.3. Progesterone Derivatives

Progestins derived from progesterone are categorized into two families that differ at the 10-carbon position. The pregnane class of progestins possesses a methyl group at the 10-carbon location and include medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, cyproterone acetate, dydrogesterone and medogestone. Medroxyprogesterone acetate (MPA)

exhibits relatively high progestational activity and is typically administered as a 150 mg injection of depot MPA (DMPA; Depo-Provera). The 19-norpregnanes (norpregnanes) are progesterone derivatives that lack a methyl group at the carbon 10 position. The hormonal pattern of norpregnanes is similar to that of pregnanes, in terms of selective binding to PR with no androgenic, estrogenic or glucocorticoid activity¹⁴⁹.

1.2.2.4. Spironolactones

Drospirenone is a derivative of 17 α -spironolactone that has been available in combination with EE (Yasmin) for oral contraception since 2001. This progestin has strong anti-mineralocorticoid and anti-androgenic activity without androgenic, estrogenic, glucocorticoid or anti-glucocorticoid effects^{147,150}.

1.2.2.5. Molecular Mechanism of Action of Progestogens

Progestogens exert their biological effects via interaction with steroid receptors, which are intracellular ligand-activated transcription factors that regulate the transcription of downstream target genes. The progestational activity of all progestins is due to their relatively high binding affinity progesterone receptors A and B (PRA and PRB). Binding of progestogen to PR causes a conformational change, such that the receptor-steroid complex dimerizes, translocates to the nucleus and initiates transcription of target genes¹⁵⁰. This interaction to its receptor may be agonistic or antagonistic to increase or decrease transcription of target genes. Generally, PRAs act as transcriptional repressors and PRBs act as transcriptional activators¹⁵¹.

Progestins vary in chemical structure which accounts for differences in their affinity for other members of the steroid receptor family, including androgen receptor, glucocorticoid receptor and mineralocorticoid receptor. Most progestins demonstrate minimal to no activity at estrogen receptors¹⁴⁵. The hormonal activities of various progestogens are summarized in Table 3.

Table 3. Biological activities of various progestogens

Progestogen	Anti-gonadotrophic	Estrogenic		Androgenic		Glucocorticoid	Anti mineralocorticoid
		Pro	Anti	Pro	Anti		
Progesterone	+	–	+	–	+	±	±
<i>Pregnane derivatives: non acetylated</i>							
Dydrogestrone	–	–	+	–	±	–	±
Medrogestrone	+	–	+	–	±		
<i>Pregnane derivatives: acetylated</i>							
Medroxyprogesterone acetate	+	–	+	±	–	+	–
Megestrol acetate	+	–	+	±	+	+	–
Chlormadinone acetate	+	–	+	–	+	+	–
Cyproterone acetate	+	–	+	–	++	+	–
<i>19-Norpregnane derivatives: non acetylated</i>							
Demegestone	+	–	+	–	–	–	–
Promegestone	+	–	+	–	–	–	–
Trimegestone	+	–	+	–	±	–	±
<i>19-Norpregnane derivatives: acetylated</i>							
Nomegestrol acetate	+	–	+	+	±	–	–
Nesterone	+	–	+	–	–	–	–
<i>19-Nortestosterone derivatives: estranes</i>							
Norethisterone (Norethindrone)	+	+	+	+	–	–	–
Norethisterone acetate	+	+	+	+	–	–	–
Norethynodrel	+	+	±	±	–	–	–
Lynestrenol	+	+	+	+	–	–	–
Tibolone (metabolites)	+	+	–	+	–	–	–
Dienogest	+	±	+	–	+	–	–
<i>19-Nortestosterone derivatives: gonanes</i>							
Levonorgestrel	+	–	+	+	–	–	–
Norgestimate	+	–	+	+	–	–	–
Desogestrel (etogestrel)	+	–	+	+	–	–	–
Gestodene	+	–	+	+	–	+	+
<i>Spirolactone derivative</i>							
Drospirenone	+	–	+	–	+	–	+

Legend: ++ = Strongly positive; + = Positive; ± = Weakly positive; – = Negative

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1.2.3. Overview of Contraceptive Methods

1.2.3.1. Combined Oral Contraceptives

Combined oral contraceptives contain two synthetic steroid components, an estrogen and a progestin. The perfect-use and typical-use failure rates are 0.3% and 9% respectively¹⁵². Oral contraceptives are used by at least 150 million women worldwide and account for 16% of contraceptive use among women of reproductive age (15-49 years)¹⁵³. Oral contraceptives were first approved in 1960 by the United States Food and Drug Administration. Early formulations contained upwards of 150 µg of EE per tablet, but mean estrogen doses have declined over time to improve the safety profile without sacrificing contraceptive efficacy¹⁵². Most pills in the 1960s and 1970s contained 50 µg of EE¹⁵⁴. By 1988, 80% of oral contraceptives in the United States contained less than 50 µg of EE, and the majority of formulations available today contain 20 µg to 35 µg of EE^{154,155}. Concentrations of progestins have also decreased five-fold since the 1960s to under than 100 µg today¹⁴⁰. Alongside reductions in hormonal dose, new progestin types have changed over the years (see Section 1.2.2). Data from the National Survey of Family Growth from 2012 recorded nearly 90 unique formulations of oral contraceptives used in the United States alone¹⁵⁶. Although prescribing patterns differ by country, most oral contraceptive users adopted formulations containing EE in combination with LNG, norgestimate, norethindrone, or drospirenone^{155–157}.

The primary mechanism of action of the combined oral contraceptive pill is inhibition of ovulation. The progestational agent primarily inhibits LH secretion via negative regulation at the hypothalamus and anterior pituitary gland, which terminates the preovulatory LH surge. The estrogenic component suppresses FSH secretion and the emergence of a dominant follicle. These effects are reflected in the reduction of plasma pituitary gonadotropins to levels incompatible with ovulation among combined oral contraceptive users^{158,159}. Moreover, when equal doses of EE are orally administered in combination with different progestins, greater suppression of serum LH and FSH is observed with the more potent progestin¹⁶⁰.

The estrogen also potentiates the action of the progestin by increasing the concentration of intracellular PRs, which has allowed the reduction of progestin dose over the past decades¹⁴⁴. It is important to note that follicular growth is seen with low-dose combination pills; however,

these follicles do not mature and the progestin component is sufficient block the LH surge that is necessary for ovulation.

The effect of the progestational agent takes precedence over the estrogen, such that the effects of oral contraception at the lower genital tract reflect progestational stimulation¹³⁸. The progestin in the oral contraceptive pill causes thickening of cervical mucus to be impervious to sperm transport. In addition, endometrial decidualization and atrophy in a progestin-dominant environment impairs implantation of the fertilized ovum. Progestational influence on secretion and peristalsis within the fallopian tubes provides additional contraceptive effect. These secondary mechanisms alone afford effective contraception independent of ovulation inhibition.

1.2.3.2. Long-acting Reversible Contraceptives

In addition to oral administration, various injectable, implantable and intrauterine devices (IUDs) have been developed over the years for effective, long-acting reversible contraception (LARC) that require no maintenance after insertion. These “set and forget” methods guarantee high user compliance, such that that “typical use” failure rates are very similar to failure rates associated with “perfect use”¹⁶¹. In fact, annual failure rates associated with LARC methods in the United States are less than 1%¹⁶². LARC methods can be classified into those that provide systemic versus local delivery of progestin. Contraceptive implants and injections provide a continued release of progestin into the surrounding tissues that is absorbed into systemic circulation. IUDs provide continued local administration of LNG to the uterus to avoid systemic effects¹⁶³. The three LARC methods are described below with an emphasis on historical development, epidemiology, basic pharmacology and mechanism of action.

1.2.3.3. Implants

Contraceptive implants consist of progestin-only subdermal rods or capsules inserted under the skin of the upper arm. Progestin diffuses in a sustained-release fashion from the implant into the interstitial fluid before absorption into systemic circulation. Similar to other LARCs, implants have higher contraceptive efficacy than oral or barrier methods with a 0.5% perfect-use and typical-use failure rate¹⁶⁴.

The Norplant LNG system was the first implant in widespread use. It was approved in Finland in 1983 and marketed in the United States and Canada by 1990 and 1994, respectively¹⁶⁵. Norplant consisted of six capsules, each containing 36 mg of crystalline LNG, effective for up to five years. Norplant has since been discontinued and superseded by Jadelle, capable of delivering the same daily dose of LNG utilizing only two rods (75 mg LNG each). Jadelle was first introduced in Finland in 1997 and now holds regulatory approval in numerous countries. Both Norplant and Jadelle contain 150 mg LNG and are considered bioequivalent in terms of contraceptive efficacy and side-effect profile¹⁶⁶. The LNG release rate is 100 µg/day during the first few months of use, which decreases to 40 µg/day by one year and 20 µg/day by two years. This provides a continuous low dose of LNG that is equivalent to approximately 25% to 50% of the daily dose delivered by low-dose combined oral contraceptives in the first few months of use^{138,167}. Blood levels of LNG peak around 772 pg/mL within two days and decline to 435 pg/mL within one month, 357 pg/mL at six months and 280 pg/mL sustained from three to five years¹⁶⁷.

Implanon and Nexplanon are available as single rod-implant systems containing 68 mg of etonogestrel and a duration of action of three years. Implanon was first marketed in 1998 in Indonesia and is now available in over 30 countries¹⁶⁸. Nexplanon, approved in the United States in 2011, exhibits comparable hormonal release rates to Implanon. This rate declines from 60 µg/day to 40 µg/day of etonogestrel within the first 12 months to 30 µg/day by two years^{138,169}. Serum concentrations reach 266 pg/mL of etonogestrel within eight hours of insertion, which is sufficient to inhibit ovulation¹⁷⁰. A peak plasma level of around 813 pg/mL etonogestrel is achieved by four days and declines to 196 pg/mL by one year and 156 pg/mL by three years^{166,171}.

Implants are highly effective contraceptives and three modes of action have been investigated. In clinical trials, measures of progesterone levels and ovarian ultrasound scanning have determined that etonogestrel contraceptive implants (Implanon, Nexplanon) consistently inhibit ovulation through a three-year period via suppression of the LH surge; however, ovarian follicular activity remains present with periodic peaks of serum estradiol. After one year of use, blood estradiol levels are about 50 pg/mL and increase to 100 pg/mL by the third year¹⁶⁶.

Increased cervical mucus viscosity and endometrial thinning were also documented among users¹⁷².

In contrast, about one-third of all cycles are ovulatory among users of LNG implants (Norplant, Jadelle), which increases from 10% within the first year to over 50% by five years¹³⁸. To maintain high contraceptive efficacy, the progestin agent confers additional local effects including thickening of the cervical mucus, which forms a barrier to sperm penetration. Further, the progestin induces endometrial atrophy, which prevents implantation in the event of a fertilized egg.

1.2.3.4. Intrauterine Devices

An IUD utilizes a polyethylene, T-shaped frame and releases either copper or a synthetic progestin into the uterine cavity for contraceptive action. The first and most widespread hormonal IUD is the 52 mg levonorgestrel-bearing IUD (LNG IUD) with an effective lifespan of five years. After launching in Finland in 1990, the LNG IUD expanded across most European countries by the late 1990s and was approved in the United States in 2001¹⁷³. Now available in over 140 countries worldwide, the 52 mg LNG IUD is commercially marketed under the brand names, Mirena, Levosert and Liletta¹⁷⁴. Until the last decade, the IUD was not recommended for use in nulliparous women due to concerns about complications and difficulty of insertion. In light of mounting evidence supporting the safety and efficacy of IUD use in younger age groups, various health authorities now recommend IUDs as a first-line contraceptive option in nulliparous women and adolescents¹⁷⁵.

Each device contains a progestin reservoir in the vertical stem that slowly releases hormone through a rate-limiting membrane. In the first year, the 52 mg LNG IUD releases 20 µg of LNG per day, which decreases to 10 ug of LNG per day by five years¹⁷⁶. The LNG IUD is also available in 19.5 mg (Kyleena) and 13.5 mg (Skyla/Jaydess) strengths that provide equally reliable contraception over a five-year and three-year lifespan, respectively¹⁷⁷. The 19.5 mg LNG IUD releases 17.5 ug LNG daily during the first year, which declines to 7.4 ug/day at five years¹⁷⁸. The 13.6 mg LNG IUD has an initial release rate of about 14 ug/day over the first year that declines to 5 ug/day by three years¹⁷⁹. Systemic LNG exposure achieved with use of an IUD

is relatively low compared to that of LNG implants and oral contraceptives¹⁸⁰. In a pooled analysis of pharmacokinetic data from phase II and phase III studies, the average serum LNG concentrations of the 13.5 mg, 19.5 mg and 52 mg LNG IUD over three years were 74.3 ng/L, 114 ng/mL and 218 ng/mL, respectively¹⁷⁷. Serum levels are subject to wide interindividual variation depending on body weight and sex hormone-binding globulin levels^{181,182}.

Various inert and metal-coated IUDs have been available historically such as the Lippes Loop and stainless-steel IUDs that are widely used in China¹⁸³. The most widely distributed non-hormonal IUD today is the copper-bearing IUD (ParaGard) that contains 200 mm² to 380 mm² of exposed copper wire to enhance spermicidal effect¹⁸³. It was launched in the United States in 1988 and is approved for use for 10 to 12 years.

Until the 1980s, IUDs were widely misconceived as abortifacients that acted by preventing implantation of the fertilized egg or destroying existing embryos¹⁸⁴. Today it is known that the principle contraceptive mechanism of action of the LNG IUD is inhibition of fertilization by preventing sperm-egg union. This occurs through induction of a weak foreign body reaction, suppression of endometrial growth, and increased viscosity of cervical mucus that inhibits sperm transport. The foreign body effect, induced by the presence of the IUD frame, is a local sterile inflammatory reaction that renders the uterine cavity toxic to sperm and ova¹⁸⁵. It is characterized by the recruitment of leukocytes into the intrauterine space, including neutrophils, mononuclear cells and macrophages¹. In tubal washings of IUD users compared to control cases, no sperm or fertilized ova were recovered, which was attributed to the phagocytic activity of excess white blood cells in the uterus¹⁸⁷. Sperm may also be destroyed through the elevation of cytotoxic peptides that inhibit sperm motility, capacitation and survival¹⁸⁸. Additional local contraceptive effects of the progestin agent include morphological changes at the endometrium that lower receptivity to implantation. Critchley *et al.* reported stromal decidualization and glandular atrophy in endometrial biopsies of women collected up to 12 months following LNG IUD insertion¹⁸⁹. At the level of the cervix, LNG exposure increases the viscosity of cervical mucus to prevent passage of sperm into the uterus. This was supported by the absence of sperm transport through cervical mucus samples collected from LNG IUD users, as evaluated by the sperm cervical mucus penetration test¹⁹⁰.

The majority of women using the LNG IUD will have ovulatory cycles, as assessed by hormonal measurement and follicular ultrasonography^{191,192}. Ovulation is suppressed in only 20% to 55% of users; however, the effect of the LNG IUD on ovarian function will depend on circulating levels of LNG, which can vary widely between individuals. With continued use, the hormonal release rate declines and the incidence of ovulation increases. By one year after insertion, two-thirds of cycles are ovulatory¹⁹³. By four years after insertion, almost 90% of menstrual cycles are ovulatory¹⁹⁴. Early clinical trials of the LNG IUD suggest a daily release rate of 50 ug LNG is necessary to inhibit ovulation, compared to modern devices that release 20 ug LNG daily¹⁹⁵. Because ovarian steroidogenesis is not affected, circulating estradiol levels also remain within the range of normal menstrual cycles (100 pg/mL to 130 pg/mL)¹⁹⁶.

Similar to the hormonal IUD, the copper IUD inhibits fertilization by impeding sperm transport and the capacity of sperm to fertilize an ovum. All IUD types generate a foreign body reaction, but the inflammatory response is enhanced by the continuous release of copper ions into the luminal fluids of the genital tract¹⁹⁷. In animal studies, the addition of copper stimulates greater mobilization of leukocytes into the uterine cavity that exert phagocytic and cytotoxic effects on sperm¹⁹⁸. Additionally, it is recognized that copper has direct spermicidal effects. Sperm incubated in the presence of copper show a reduction in motility, viability and fertilizing capacity^{199,200}. Moreover, sperm transport is impaired by high copper content in the cervical mucus and tubal fluid of copper IUD users, which inhibits sperm penetration and tubal motility, respectively^{201–204}. Indeed, one clinical study recovered significantly more sperm from the fallopian tubes of women using no contraception 15 to 30 minutes after insemination, while no sperm was found in the tubes of copper IUD users at the same post-coital time²⁰⁵.

1.2.3.5. Injections

Contraceptive injections are widely available, highly effective, long-acting reversible methods that became available in 1967. As of 2019, there are 74 million users of contraceptive injections worldwide, which accounts for 8% of global contraceptive use¹⁵³. Since 1994, the prevalence of injectable methods has increased by 5% globally, driven by growth in Latin American, South-eastern Asian and sub-Saharan African countries¹⁵³. Three types of injectable contraceptives have been developed: DMPA, norethindrone enanthate and various combination estrogen plus

progestin formulations¹³⁸. DMPA (Depo-Provera) is the most widely distributed worldwide. A single injection delivers a 150 mg bolus of MPA intramuscularly, resulting in serum drug concentrations above the minimum concentration for ovulation inhibition (0.2 ng/mL of MPA) over a period of three months. In fact, circulating blood levels of MPA exceed the threshold for contraceptive efficacy within 24 hours¹³⁸. As with other hormonal contraceptives, substantial intraindividual and interindividual variability in pharmacokinetic profile is observed, due to differences in drug metabolism.

The contraceptive failure rate associated with DMPA injections is 0.22 per 100 participant-years, similar to that attributed to IUDs and implants¹⁶². The major mode of contraception action is ovulation inhibition. In randomized trial of DMPA users, authors found immediate depression of serum estradiol and progesterone levels within 24 hours post-injection²⁰⁶. Ovulation was suppressed for the entire 91-day treatment period²⁰⁶. In addition to anovulation, DMPA induces thickening of the cervical mucus and endometrial atrophy^{207,208}.

1.3. Contraceptive Use and Ovarian Cancer

1.3.1. Evidence in the General-Risk Population

This section describes the epidemiologic research on various contraceptive methods and ovarian cancer risk in the general population. Given the substantial body of literature on oral contraceptives and risk, emphasis will be placed on large pooled analyses and meta-analyses. Details on individual cohort studies investigating the relationship between oral contraceptive use and ovarian cancer risk are shown in Table 4. Available observational data on long-acting reversible contraceptives and ovarian cancer risk in the general population will also be presented.

1.3.1.1. Combined Oral Contraceptives

In 1999, the IARC reviewed the epidemiologic data on hormonal contraception and the risk of cancer at various sites. It was determined that oral contraceptive agents have a protective effect against cancers of the ovary²⁰⁹. To date, seven pooled analyses and one meta-analysis have quantified the role of oral contraceptives in ovarian cancer (Table 5). Of these, the largest collaborative pooled analysis included 45 epidemiologic studies (32 case-control, 13 prospective) across 21 countries based on 23,257 cases of ovarian cancer and 87,303 controls²¹⁰. In agreement with the IARC, the authors observed a significant inverse relationship between a history of oral contraceptive use and ovarian cancer risk compared to never use (relative risk [RR]=0.73; 95% CI 0.70-0.76). This protective effect was apparent within one to four years of use (RR=0.78; 95% CI 0.73–0.83) and the RR decreased by 20% per five years of use (95% CI 18–23%, $p<0.0001$). When time since last use was examined within categories of duration of use, there was a greater reduction in ovarian cancer risk observed with more recent use and an inverse trend with longer durations of use. Per five years of use, the RR declined by 29% for use ceased less than 10 years previously, 19% for use ceased 10-19 years previously and 15% for use ceased 20-29 years previously (p -heterogeneity = 0.004). Further, risk reduction appeared to persist for at least 30 years after discontinuation of oral contraceptives (RR=0.83; 95% CI 0.73-0.95). In the analysis of age at first use, the authors also reported no significant heterogeneity across women who started oral contraceptives at different ages (p -heterogeneity=0.5). A forest plot summarizing the findings of select case-control and cohort studies investigating the association between oral contraceptive use and ovarian cancer risk is shown in Figure 3.

Similar findings emerged in an earlier pooled re-analysis of oral contraceptive use and ovarian cancer risk based on 2,768 cases and 6,273 controls from six studies conducted in three European countries^{211,212}. In the most recent meta-analysis of 24 studies (17 case-control, seven prospective), 11 of which were published after the report by Beral *et al.*, the multivariate RR was 0.73 (95% CI 0.66-0.81) for ever use²¹³. Additionally, authors observed a significant duration-response relationship, with an ovarian cancer risk reduction of over 50% for women who used oral contraceptives for 10 or more years (OR= 0.43; 95% CI 0.37-0.51). There was a trend toward greater reduction in ovarian cancer incidence with earlier age at first use and more recent use, but analyses were not stratified by duration of use.

Table 4. Summary of all cohort studies investigating the relationship between oral contraceptive use and ovarian cancer risk in the general population

Author, Year	Location	Source data	Cohort details	Ever use	HR, RR ^a (95% CI)	Covariates
Hankinson, 1995	US	Nurses' Health Study	Overall (n=121,700) Exposed (592,056 p-years) Unexposed (599,301 p-years)	Ever use	1.08 (0.83-1.43)	Age, parity, menopausal status, age at menarche, smoking, tubal ligation, Quetelet's Index
Vessey and Painter, 1995	England, Scotland	Oxford Family Planning Association contraceptive study	Overall (n=17,032) Exposed (n=3,520 women ≥ 8 years use) Unexposed (n=5,881)	Ever use	0.3 (0.1-0.7)	Age, parity
Kumle, 2004	Norway, Sweden	Norwegian-Swedish Women's Lifestyle and Health Cohort Study	Overall (n=103,551) Exposed (n=75,533) Unexposed (n=28,019)	Ever use	0.6 (0.5-0.8)	Age, parity, menopausal status, HRT, country
Vessey and Painter, 2006	UK	Oxford Family Planning Association contraceptive study	Overall (n=17,032) Exposed (187,000 p-years) Unexposed (301,000 p-years)	Ever use	0.5 (0.4-0.7)	Age, parity, BMI, smoking, social class, height, age at first term pregnancy, age at first marriage
Hannaford, 2007	UK	Royal College of General Practitioners' Oral Contraception Study	Overall (n=45,950) Exposed (n=28,762) Unexposed: (n=17,188)	Ever use	0.51 (0.33-0.78)	Age, parity, smoking, social status
TwoRoger, 2007	US	Nurses' Health Study	Overall (n=107,900) Exposed (99,479 p-years) Unexposed (1,403,441 p-years)	>10 years of use	0.62 (0.37-1.04)	Age, BMI, parity, history of tubal ligation, smoking history, age at menarche, age at menopause, duration of postmenopausal hormone use
Dorjgochoo, 2009	China	Shanghai Women's Health Study	Overall (n=66,661) Exposed (n=12,957) Unexposed (n=15,557)	Ever use	1.1 (0.66-1.84)	Age, parity, menopausal status, BMI, family history, age at menarche, smoking, breastfeeding, education, physical activity, other contraceptive methods
Rosenblatt, 2009	China	n/a	Overall (n=258,956) Exposed (n=37,319; 349,539 p-years) Unexposed (2,008,845 p-years)	Ever use	1.17 (0.86-1.60)	Age, parity, injectable contraceptive use
Braem, 2010	Netherlands	Netherlands Cohort Study on Diet and Cancer	Overall (n=62,573) Exposed (8,669 p-years) Unexposed (25,917 p-years)	Ever use	0.71 (0.52-0.97)	Age, parity

Tsilidis, 2011	Europe	European Prospective Investigation into Cancer and Nutrition	Overall (n=327,396) Exposed (n=192,836) Unexposed (n=132,923)	Ever use	0.86 (0.73-1.00)	Age, parity, menopausal status, BMI, smoking, center, unilateral oophorectomy, hysterectomy, menopausal hormones, age at menarche
Yang, 2012	US	NIH-AARP Diet and Health Study	Overall (n=169,391) Exposed (n=67,870) Unexposed (n=100,304)	Ever use	0.74 (0.63-0.87)	Age, parity, menopausal hormone therapy
Vessey and Yeates, 2013	UK	Oxford Family Planning Association contraceptive study	Overall (n=17,032)	Ever use	0.5 (0.4-0.7)	Age, parity, social class, smoking, BMI, age at first marriage
Fortner, 2015	Europe	European Prospective Investigation into Cancer and Nutrition	Overall (n=334,126) Exposed (n=192,207) Unexposed (n=133,008)	Ever use	0.84 (0.73-0.96)	Ever full-term pregnancy, menopausal status at recruitment, age at menopause, ever HRT use
Huang, 2015	China	Shanghai Women's Health Study	Overall (n=70,259) Exposed (n=54,113) Unexposed (n=16,146)	Ever use	0.86 (0.60-1.24)	Age, education, years of ovulation, irregular ovulatory cycles, first-degree family history of cancer, regular exercise in past 5 years, other contraceptive methods
Shafirir, 2017	US	Nurses' Health Study II	Overall (n=110,929) Total exposed (n=129,923) Total unexposed (n=18,994)	≥15 years of use	0.43 (0.18-1.04)	Age, calendar time, tubal ligation, parity, breastfeeding duration, and missing breastfeeding duration, endometriosis, BMI
Iversen, 2017	UK	Royal College of General Practitioners' Oral Contraception Study	Overall (n=46,022) Exposed (n=22,920) Unexposed (n=23,102)	Ever use	0.66 (0.58-0.76)	Age, parity, smoking, and social class
Bethea, 2017	US	Black Women's Health Study	Overall (n=53,141) Exposed (343,045 p-years) Unexposed (147,799 p-years)	≥ 10 years of use	0.50 (0.30-0.83)	Age, questionnaire cycle, parity, lactation, age at first birth, age at last birth, hysterectomy, tubal ligation, menopausal status, use of postmenopausal female hormones, educational attainment, BMI
Michels, 2018	US	NIH-AARP Diet and Health Study	Overall (n=196,536) Exposed (n=78,393) Unexposed (n=118,144)	Ever use	0.74 (0.65-0.84)	Age, race, BMI, baseline smoking status, age at menarche
Iversen, 2018	Denmark	Danish Sex Hormone Register Study	Overall (n=1,879,227) Exposed (7,751,904 p-years of current or recent	Ever use	0.53 (0.45-0.64)	Calendar year, parity, age, education, tubal sterilization, hysterectomy, endometriosis,

			use) Unexposed (8,150,250 p-years)			polycystic ovary syndrome, family history of breast or ovarian cancer
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Body mass index (BMI); hormone replacement therapy (HRT)

^a Relative risk (RR) or hazard ratio (HR) for ever vs never oral contraceptive use.

Table 5. Summary of all pooled analyses and meta-analyses investigating the relationship between oral contraceptive use and ovarian cancer risk in the general population

Author, Year	Country	Type of study	Study details	Outcome	OR (95% CI) ^a	Covariates
Franceschi, 1991	Europe	Pooled analysis	3 case-control studies Cases (n=971) Hospital controls (n=2,258)	Epithelial ovarian cancer	0.6 (0.4-0.8)	Study, age, marital status, socioeconomic status, parity, menopause, contraceptive habits
Whittemore, 1992	US	Pooled analysis	12 case-control studies Cases (n=12,197) Controls (8,893)	Epithelial ovarian cancer	0.70 (0.52–0.94) (hospital-based) 0.66 (0.55–0.78) (population-based)	Study, age, parity
Harris, 1992	Europe	Pooled analysis	12 case-control studies Cases (n=327) Controls (n=4,144)	Borderline ovarian cancer	1.1 (0.7–1.9)	Study, age, parity
Horn-Ross, 1992	US	Pooled analysis	4 case-control studies Cases (n=38) Controls (n=1,142)	Germ cell tumours	2.0 (0.77-5.1)	Study, age, year of birth
			Cases (n=45) Controls (n=2,617)	Sex cord-stromal tumours	0.37 (0.16-0.83)	
Bosetti, 2002	Europe	Pooled analysis	6 case-control studies Cases (n=2,768) Hospital controls (n=6,274)	Epithelial ovarian cancer	0.66 (0.56–0.79)	Study, age, year, socioeconomic status, parity, menopause, age at menopause
Beral, 2008	International	Pooled analysis	13 prospective 32 case-control Cases (n=23,257) Controls (n=87,303)	Epithelial or non-epithelial ovarian cancer	0.73 (0.70-0.76)	Study, age, parity, hysterectomy
Havrilesky, 2013	International	Meta-analysis	17 case-control 7 prospective	Any ovarian cancer	0.73 (0.66-0.81)	n/a
Wentzensen, 2016	International	Pooled analysis	21 studies Cases (n=5,584) Controls (n≈1.3 million)	Invasive epithelial ovarian cancer	0.84 (0.79-0.89)	Age, parity

^a Odds ratio for ever vs. never oral contraceptive use

Figure 3. Forest plot summarizing the findings of select case-control and cohort studies investigating oral contraceptive use and ovarian cancer risk in the general population

Reproduced from Beral V, Doll R, Hermon C, Peto R, Reeves G, Schouten LJ, van den Brandt PA. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. Lancet. 2008;371(9609):303-14 by permission from Elsevier.

Over the past five decades, the estrogen and progestin content of oral contraceptive pills have decreased rapidly in effort to reduce undesirable side-effects without compromising contraceptive efficacy^{152,214}. Low-dose formulations are effective at suppressing ovulation, but it is unclear how changes in hormonal dose and potency affect ovarian cancer risk, particularly for those at high risk^{215,216}. Available data in the general population has been mixed. A meta-analysis considered six case-control studies examining the effect of estrogen and progestin formulation on ovarian cancer risk. Authors concluded that there was no relationship between the estrogen or progestin dose of oral contraceptive pills and risk of ovarian cancer in the general population²¹³. In contrast, more recent data from the Nurses' Health Study II suggest that women who used high-dose estrogen formulations had a non-significant increased risk of ovarian cancer

compared to women who used low-dose estrogen formulations (HR=1.34; 95% CI 0.91-1.95 for high). This finding may have been driven by the positive association with short-term use of mestranol (HR = 1.83; 95% CI 1.16-2.88) or first generation progestins (HR=1.72; 95% CI 1.11- 2.65) compared to never users²¹⁷. There was no association between hormone potency and risk. Other studies have found an inverse relationship between progestin potency and risk, such that older, high-progestin formulations confer greater protection than low-progestin formulations^{213,218–223}

1.3.1.2. Contraceptive Injections

The growing uptake of LARC methods has prompted further research into their non-contraceptive benefits, particularly in terms of ovarian cancer prevention. Ovulation inhibition via suppression of pituitary gonadotropins is the chief biological mechanism by which oral contraceptives, implants and injections may impart protection against the development of ovarian cancer (see Section 1.2.3). Indeed, a recent pooled analysis of seven case-control studies in the Ovarian Cancer Association Consortium concluded a reduced risk of ovarian cancer with use of DMPA shots (OR=0.65; 95% CI 0.50-0.85), which is in line with the majority of other retrospective data^{224–226}. Cohort studies that have explored injection use and ovarian cancer risk have showed inconsistent results. In the Shanghai Women's Health Study, injectables were not associated with risk (HR=1.33; 95% CI 0.58-3.04), whereas the Danish Sex Register Hormone Study observed increased risk with use of DMPA shots compared to never use of any hormonal contraceptive (RR=6.56; 95% CI 2.11-20.40)^{227,228}. Both studies were limited by a low prevalence of injection users and imprecise risk estimates.

1.3.1.3. Implants

With respect to subdermal contraceptive implants, only one study estimated risk of ovarian cancer among implant users in the general population²²⁷. The authors reported a nonsignificant inverse association between implant use and ovarian cancer risk compared to never users (RR=0.51; 95% CI 0.07-3.64); however, any protective effect may have been underestimated because history of contraceptive use prior to study entry was not collected.

1.3.1.4. Intrauterine Devices

A greater body of evidence has explored the associations between IUD use and ovarian cancer risk (Table 6). Of note, two recent meta-analyses both demonstrated that use of an IUD decreases risk of ovarian cancer, reporting ORs of 0.68 (95% CI 0.62-0.75) and 0.67 (95% CI 0.60-0.74)^{229,230}. Population-based cohort studies have specifically linked this protective effect to use of the levonorgestrel-bearing, hormonal IUD^{231,232}. The data with respect to nonhormonal IUDs remain controversial. A pooled analysis of the New England Case-Control Study and Nurses' Health Studies reported no association between IUD use and ovarian cancer risk, wherein the majority of IUD use was attributed to nonhormonal methods (RR=0.94; 95% CI 0.81-1.08)²³³. Conversely, findings from the Shanghai Women's Health Study indicated that long-term IUD use greater than 20 years was associated with a 38% reduced ovarian cancer risk in China, where the high prevalence of IUD use was dominated by stainless steel methods (HR=0.62; 95% CI 0.40-0.97)²²⁸. Interestingly, Tworoger and colleagues observed elevated ovarian cancer risk with nonhormonal IUD use in an earlier analysis of Nurses' Health Study, which was attributed to IUD-related peritoneal inflammation (RR=1.76; 95% CI 1.08-2.85)²³⁴.

Table 6. Summary of cohort studies, pooled analyses and meta-analyses investigating the relationship between use of an intrauterine device and ovarian cancer risk in the general population

Author, Year	Location	Type of study	Study details	Exposure	Comparison	Subgroup analysis	OR, HR, RR (95% CI)	Covariates
Franceschi 1991	Europe	Pooled analysis	3 case-control studies Cases (n=971) Hospital controls (n=2,258) Exposed (n=116)	Any IUD	Ever use	All	0.8 (0.5-1.3)	Age, socio-cultural indicators, marital status, menopausal status, parity, contraceptive habits, study centre
Kotsopoulos, 2013	USA	Pooled analysis	NECC, NHSI, NHSII Cases (n=1,771)	Any IUD	Ever use	Dominant Nondominant	0.83 (0.65-1.05) 1.70 (0.68-4.26)	Age, oral contraceptive use, parity, tubal ligation, family history of breast or ovarian cancer, study phase, center for NECC; time period, cohort, histology for NHS
Wheeler, 2019	International	Meta-analysis	9 case-control studies 2 cohort studies Cases (n=5,133) Controls (n=183,035)	Any IUD	Ever use	All	0.68 (0.62-0.75)	n/a
Balayla, 2020	International	Meta-analysis	5 case-control studies 2 cohort studies	Any IUD LNG IUD	Ever use	All	0.67 (0.60–0.74) 0.58 (0.47-0.71)	n/a
Yang, 2021	USA	Pooled analysis	NECC, NHSI, NHSII	Any IUD	Ever use	All	0.96 (0.81-1.14)	Age, body mass index, age at menarche, parity, oral contraceptive use, history of tubal ligation, family history of ovarian cancer, study center, study phase (NECC)
Tworoger, 2007	USA	Cohort	Overall (n=107,900) Cases (n=625) Exposed (64,847 p-years)	Any IUD	Ever use	All	1.76 (1.08-2.85)	Age, age at menarche, age at menopause, BMI, oral contraceptive use, parity, postmenopausal

								hormone use, tubal ligation, smoking
Huang, 2015	China	Cohort	Overall (n=70,529) Cases (n=174) Exposed (n=39,054)	Any IUD	Ever use	All	0.79 (0.55-1.13)	Age, education, years of ovulation, irregular ovulatory cycles, first-degree family history of cancer, BMI, physical activity, other contraceptive methods
Soini, 2016	Finland	Cohort	Overall (n=93,843) Cases (n=77)	LNG IUD	Ever use	All	0.59 (0.47-0.73)	n/a
Jareid, 2018	Norway	Cohort	Overall (n=104,318) Exposed (n=9,146)	LNG IUD	Ever use	All	0.53 (0.32-0.88)	Age, parity, BMI, oral contraceptive use, menopausal status, maternal history of breast cancer, physical activity
Iversen, 2018	Denmark	Cohort	Overall (n=104,318; 21.4 million p-years) Exposed (172,265 p-years)	LNG IUD	Ever use	All	0.84 (0.53-1.35)	Calendar year, parity, age, education, tubal sterilisation, hysterectomy, endometriosis, polycystic ovary syndrome, family history of breast or ovarian cancer

Odds ratio (OR); hazard ratio (HR); relative risk (RR); New England Case Control Study (NECC); Nurses' Health Study (NHS); Oral contraceptive (OC); Body mass index (BMI); levonorgestrel-releasing IUD (LNG IUD)

1.3.2. Evidence in High-Risk Populations

This section reviews the literature regarding oral contraceptive use and ovarian cancer risk conducted among *BRCA* mutation carriers with a focus on robust meta-analyses and large cohort studies. The role of LARC methods, including intrauterine devices, implants and injections, has not been evaluated in the context of *BRCA*-associated ovarian cancer.

Since 1998, three meta-analyses and 12 epidemiologic studies have evaluated the impact of oral contraceptive use on ovarian cancer risk in high-risk populations, supporting a strong protective effect. The key features of these studies are summarized in Table 7 and reviewed in References 116, 126 and 237. A meta-analysis including three case-control studies published through 2009 showed that women who carried a *BRCA1* mutation had a 44% lower risk of ovarian cancer with oral contraceptive use compared to those who never users ($OR=0.56$; 95% CI 0.49-0.69)²³⁵. For women who carried a *BRCA2* mutation, oral contraceptive use conferred a 51% risk reduction compared to never use ($OR=0.49$; 95% CI 0.32-0.77). Similar findings were reported in a larger meta-analysis based on data from four case-control studies and one retrospective cohort study published through 2010²³⁶. Results indicated significantly reduced risks of ovarian cancer for *BRCA* mutation carriers who used oral contraceptives (summary relative risk [SRR]=0.51, 95% CI 0.40-0.65 for *BRCA1*; SRR=0.52, 95% CI 0.31–0.87 for *BRCA2*). Further, a weak protective effect was observed with a duration of use of at least one year (SRR = 0.96; 95% CI 0.94-0.97) and risk reduction increased linearly per year of use (p-trend <0.01). Moorman *et al.* conducted the most recent systematic review and meta-analysis of oral contraceptives and ovarian cancer risk from six studies in high-risk groups published through 2013²³⁷. Authors reported on 1,763 cases (1,353 *BRCA1*, 277 *BRCA2*) and 2,592 controls (2,160 *BRCA1*, 423 *BRCA2*). A significantly decreased risk of ovarian cancer was observed among *BRCA* mutation carrier ever users compared to never users ($OR=0.58$; 95% CI 0.46-0.73). This was primarily driven by the protective effect of oral contraceptives in *BRCA1* mutation carriers separately ($OR=0.55$; 95% CI 0.47-0.66). For women with a *BRCA2* mutation, the inverse relationship did not reach statistical significance due to the smaller sample size ($OR=0.65$; 95% CI 0.34-1.24). Analysis of duration of use and its association with risk was not performed.

Table 7. Summary of epidemiologic studies investigating the relationship between oral contraceptive use and ovarian cancer risk in *BRCA* mutation carriers

Author, Year	Location	Type of study	Study details	Comparison	Subgroup analysis	OR, HR ^a (95% CI)	Covariates
Narod, 1998	International	Case-control	Cases (n=207; 179 <i>BRCA1</i> , 28 <i>BRCA2</i>) Sister-controls (n=161; 50 <i>BRCA1</i> , 3 <i>BRCA2</i>)	Ever use	<i>BRCA1/2</i> <i>BRCA1</i> <i>BRCA2</i>	0.4 (0.2-0.7) 0.5 (0.3-0.9) 0.4 (0.2-1.1)	Year of birth, parity, age at the delivery of a first child, and geographic area of residence
Modan, 2001	Israel	Matched case-only	Carrier cases (n=240) Population controls (n=2,257)	0.1-1.9 years 2.0-4.9 years ≥ 5 years	<i>BRCA1/2</i>	1.14 (0.67-1.94) 0.77 (0.41-1.44) 1.07 (0.63-1.83)	Age, family history of breast or ovarian cancer, personal history of breast cancer, history of gynecologic surgery, ethnic background
Narod, 2001	International	Case-control	Cases (n=232) Controls (n=232)	Ever use	<i>BRCA1/2</i> <i>BRCA1</i> <i>BRCA2</i>	0.44 (0.28-0.68) 0.48 (0.29-0.80) 0.35 (0.15-0.83)	n/a
McGuire, 2004	US	Matched case-control	Carrier cases (n=46) Untested population controls (n=568)	Ever use	<i>BRCA1</i>	0.54 (0.26-1.13)	Age, number of full-term pregnancies, race/ethnicity
Whittemore, 2004	International	Matched case-control	Cases (n=147) Controls (n=304)	Ever use	<i>BRCA1/2</i>	0.85 (0.53-1.4)	Year of birth, country of residence, gene
Gronwald, 2006	Europe	Matched case-control	Cases (n=150) Controls (n=150)	Ever use	<i>BRCA1</i>	0.4 (0.2-1.0)	n/a
McLaughlin, 2007	International	Matched case-control	Cases (n=799) Controls (n=2,424)	Ever use	<i>BRCA1/2</i> <i>BRCA1</i> <i>BRCA2</i>	0.53 (0.43-0.66) 0.56 (0.45-0.71) 0.39 (0.23-0.66)	Parity, breastfeeding, oral contraceptive use, tubal ligation, ethnicity
Vicus, 2009	International	Case-control	Cases (n=154) Controls (560)	Ever use	<i>BRCA1</i>	0.84 (0.49-1.44)	Hormone replacement treatment, parity, year of birth, age at dx of breast cancer, radiotherapy, chemotherapy, type of breast cancer surgery
Antoniou, 2009	International	Retrospective cohort	Cases (n=253) Controls (n=3,066)	Ever use	<i>BRCA1/2</i> <i>BRCA1</i> <i>BRCA2</i>	0.55 (0.40-0.76) 0.52 (0.37-0.73) 1.04 (0.42-2.54)	Number of full-term pregnancies
Kotsopoulos, 2015	International	Matched case-control	Cases (n=1,329) Controls (n=5,267)	Ever use	<i>BRCA1</i> <i>BRCA2</i>	0.60 (0.50-0.71) 0.63 (0.44-0.92)	Age at menarche, parity, breastfeeding, oral

							contraceptive use, tubal ligation, ethnicity
Perri, 2015	Israel	Prospective cohort	Cases (n=175) Controls (n=898)	Ever use	<i>BRCA1/2</i> <i>BRCA1</i> <i>BRCA2</i>	0.21 (0.14-0.31) 0.21 (0.14-0.33) 0.24 (0.09-0.61)	Mutation type, age at menarche, oral contraceptive use, parity, age at first pregnancy, use of hormone replacement therapy
Schrijver, 2021	International	Retrospective cohort	Cases (n=452) Controls (n=5,982)	Ever use	<i>BRCA1</i> <i>BRCA2</i>	0.51 (0.36-0.71) 0.65 (0.35-1.19)	n/a

Odds ratio (OR); hazard ratio (HR)

The only prospective analysis on oral contraceptive use and risk conducted by Perri *et al.* recruited 1,073 Jewish Israeli women undergoing fertility treatment between 1995 and 2013¹²⁴. Authors reported a similar level of risk reduction for carriers of *BRCA1* (HR=0.21; 95% CI 0.14-0.33) and *BRCA2* mutations (HR=0.24; 95% CI 0.09-0.61), in addition to an inverse association between increasing duration of use and risk. Nonetheless, the number of women in this study was low, and they did not exclude for prior oophorectomy.

With respect to patterns of oral contraceptive use, two reports based on data from the International *BRCA1/2* Carrier Cohort study have investigated associations between year of initiation, age of initiation and time since last use and ovarian cancer^{75,238}. The most recent retrospective cohort analysis involved 1,989 *BRCA1* mutation carriers and 2,445 *BRCA2* mutation carriers²³⁸. Authors took a left-truncated approach to minimize survival bias and accounted for related aspects of oral contraceptive use. After mutual adjustment, only duration of oral contraceptive use was significantly related to decreased risk of ovarian cancer among *BRCA1* mutation carriers (P-trend = 0.008). The inverse association between duration of use and risk was stronger for more recent use within the last 15 years (p-trend = 0.0002 for <15 years since last use; p-trend = 0.37 for >15 y since last use). Findings were similar for *BRCA2* mutation carriers, but confidence intervals were wide (data not published). These results agree with a previous retrospective cohort study based on a subset of the same data. Antoniou *et al.* also found greater protective effect with longer duration of use and more recent use among *BRCA1* mutation carriers⁷⁵.

Collectively, these data demonstrate that oral contraceptives reduce the risk of ovarian cancer by 45% to 50% in carriers of a *BRCA1* mutation and 50% in carriers of a *BRCA2* mutation^{236,238,239}. Moreover, these effects are apparent after relatively short-term use, increase with longer durations of use and persist at least 20 years following cessation of use²³⁸.

1.4. The ‘Risk Factor’ Study

Dr. Steven Narod initiated the ‘Risk Factor Analysis of Hereditary Breast and Ovarian Cancer’ study (i.e. the ‘Risk Factor Study’) in 1995 based at Women’s College Hospital (Toronto, Ontario). The purpose of the study is to identify non-genetic modifiers of breast and ovarian cancer risk in carriers of *BRCA1* or *BRCA2* mutations. Exposures of interest include reproductive, hormonal and lifestyle factors. Accrual is ongoing as of 2021 across 85 institutions and 17 countries. Currently, the database consists of 17,893 women for whom baseline questionnaire data is available.

All female relatives of mutation-positive families are eligible, and each family may supply more than one study subject. Specifically, inclusion criteria include women who are 1) currently alive, 2) above the age of 18 years and 3) determined to carry a pathogenic mutation in *BRCA1* and/or *BRCA2* by molecular analysis. Women who test negative for a mutation (including variants of unknown significance) and women below 18 years of age are excluded. Eligible participants are approached to enter the study during a genetic counselling appointment at one of the participating centres.

Chapter 2. Rationale, Objectives and Hypothesis

2.1. Rationale

Women who inherit a deleterious *BRCA1* or *BRCA2* germline mutation face an increased lifetime risk of developing ovarian and fallopian tube cancer (referred to as ovarian cancer hereafter). Based on prospective studies, the cumulative risk of ovarian cancer to age 80 is estimated between 44% to 49% for *BRCA1* mutation carriers and between 17% to 21% for *BRCA2* mutation carriers^{11,12}. Given the lethality of this disease and lack of adequate screening protocols, primary prevention is strongly recommended. Prophylactic bilateral salpingo-oophorectomy (i.e., oophorectomy) is the gold standard for risk reduction, associated with a 80% to 96% decreased risk of cancer and a 77% reduction in all-cause mortality among women with a *BRCA* mutation^{104,105,240,241}.

Oral contraceptive use is a well-established protective factor against ovarian cancer among women at high risk due to a *BRCA* mutation as well as those at baseline population risk^{210,213,237}. In a recent meta-analysis of six studies conducted among women with a *BRCA1* or *BRCA2* mutation, Moorman *et al.* reported that a history of oral contraceptive use was associated with a 42% reduction in risk (OR = 0.58; 95% CI 0.46-0.73) in women with either mutation, consistent with risk estimates observed in the general population²³⁷. Moreover, this protective effect appears to increase with long-term use and persists over 20 years after discontinuation of use²³⁸.

The introduction of non-oral LARC methods, including IUDs, implants and injections has caused a considerable shift in patterns of contraceptive use. Globally, 27% of contraceptive users now rely on LARC methods. The increase in the uptake of IUDs and implants has corresponded to decline in oral contraceptive pill use¹⁵³. LARCs are the most effective and convenient reversible methods of pregnancy prevention with minimal upkeep required by the user. The IUD utilizes a polyethylene, T-shaped frame and releases either copper or a synthetic progestin into the uterine cavity for contraceptive action. The most commonly used hormonal IUD is the five-year 52 mg LNG-bearing IUD, approved in over 140 countries¹⁷⁴. The most widely distributed non-hormonal IUD is the copper-bearing IUD that contains 200 mm² to 380 mm² of exposed copper wire to enhance spermicidal effect. Contraceptive implants consist of progestin-only subdermal

rods inserted under the skin of the upper arm. Currently available models release either etonorgestrel or LNG, effective for up to five years. Another progestin-only LARC, depot medroxyprogesterone acetate (DMPA), is injected intramuscularly every three months. Similar to oral contraceptives, implants and injections yield higher levels of systemic progestin and primarily prevent pregnancy through inhibition of ovulation²⁴². Conversely, IUDs do not consistently suppress ovulation and inhibition of pregnancy is attributed to a foreign body reaction, thickening of the cervical mucus and endometrial atrophy²⁴³.

Given the documented protective effect oral contraceptive use, it is of interest to investigate whether non-oral contraceptive methods similarly decrease risk of ovarian cancer among women with a *BRCA* mutation. Data from studies conducted among women in the general population are conflicting. The evidence regarding IUD use and ovarian cancer risk is equivocal, while a mitigating role has been observed with the use of injections and possibly implants^{224,227,230,233,234}. To our knowledge, there have been no such studies conducted in the context of *BRCA*-associated ovarian cancer. As such, the goal of the current study was to evaluate the association between type of contraception and ovarian cancer risk in a cohort of *BRCA1* and *BRCA2* mutation carriers, accounting for other reproductive and hormonal exposures including prior oral contraceptive use.

2.2. Objectives

1. To describe the prevalence and patterns of use of different types of contraception by birth cohort and *BRCA* mutation type
2. To evaluate the associations between type of contraception (oral contraceptives, IUDs, implants, injections) and risk of ovarian cancer among *BRCA* mutation carriers.
3. To evaluate temporal associations between IUD use and risk of ovarian cancer among *BRCA* mutation carriers.

2.3. Hypotheses

1. It is hypothesized that oral contraceptives will be the most popular form of contraception across birth cohorts, but rates of lifetime use will decline in more recent birth cohorts. It is hypothesized that the proportions of IUD, implant and injection use will increase in more recent cohorts of *BRCA* mutation carriers.
2. It is hypothesized that a history of oral contraceptive, IUD, implant and injection use will be inversely related to ovarian cancer risk among *BRCA* mutation carriers.
3. It is hypothesized that longer duration of IUD use and later age at first IUD use will be inversely related to ovarian cancer risk among *BRCA* mutation carriers.

Chapter 3. Methods

3.1. Analysis of Trends in Contraceptive Use in *BRCA* Mutation Carriers

3.1.1. Study Population

The study population and methods have been previously described in detail²⁴⁴. Briefly, eligible subjects included women with a deleterious germline mutation in the *BRCA1* or *BRCA2* gene who were enrolled in an international, longitudinal study of *BRCA* mutation carriers from 85 participating centres in 17 countries (see Section **Error! Reference source not found.**). All women had sought genetic testing due to a personal or family history of breast and/or ovarian cancer. Mutation detection was performed using a range of techniques, but all nucleotide sequences were confirmed by the direct sequencing of DNA. The study protocol was approved by the institutional ethics review board of each participating centre. All subjects provided written informed consent for genetic testing and study participation upon enrolment (see Appendix).

3.1.2. Data Collection and Storage

Study subjects completed a standardized baseline research questionnaire at the time of enrollment. Questionnaires were administered by the individual centres either through an in-person interview at the time of their clinic appointment, completed over the phone by a genetic counsellor or research assistant, or mailed to each study participant. The baseline questionnaire collected self-reported information on known or suspected risk factors for breast and ovarian cancer, including personal and/or familial history of cancer, medical history, reproductive history, medication use and selected lifestyle factors. With respect to menopausal status, natural, surgical, medication-induced and other causes for menopause were queried. A follow-up questionnaire was administered every two years thereafter to obtain updated information on exposures, incident cancers, treatments received and vital status. Follow-up questionnaires were either mailed to each participant to complete at home or administered over the phone by a research assistant. The 2018 version of the baseline questionnaire and the 2017 version of the follow-up questionnaire can be found in the Appendix.

The collection, storage and retrieval of clinical data pertaining to this study is performed at the individual participating centres and forwarded to the study coordinating centre at Women's College Research Institute (Toronto, Canada). The data is checked and entered into the REDCap online database, which is only accessible to study personnel. Physical copies of the research questionnaires, medical release forms and other confidential study materials are locked securely in cabinets.

The current analysis used detailed information regarding history of contraceptive use. In the baseline questionnaire, subjects were asked if they had ever used contraceptives to prevent pregnancy or for any other reason (yes/no) (Figure 4). If 'yes', subjects were prompted to list all start years, stop years, durations of use (years), methods of administration (pill, implant, injection, Mirena/IUD, other) and whether they were current users (yes/no). Starting from the 2004 questionnaire cycle, contraceptive medication name was also collected. The 2002 to 2007 questionnaire cycles only referenced pills, implants and injections as options for method of administration; "Mirena/IUD" was listed as an option since the 2009 questionnaire and "Other" was an option since the 2016 questionnaire, which allowed women to further specify a method. Updated information on contraceptive use was obtained at each follow-up questionnaire. Specific contraceptive methods that were not included in this analysis include female and male sterilization, withdrawal, natural family planning methods and barrier methods (condom, diaphragm, foam, sponge, suppositories, jelly).

15a. Have you ever used birth control pills, Norplant (implants), Mirena/IUD, or Depo-Provera (injections) to prevent pregnancy or for any other reason?

- ☐ **No** → **Go to question 16.**
☐ **Yes** → Can you describe the times?

	Method	Name of Medication	Starting Year	Ending Year	Length of Time Used in Years
1	☐ Pills ☐ Implants ☐ Mirena/IUD ☐ Injections ☐ Other				
2	☐ Pills ☐ Implants ☐ Mirena/IUD ☐ Injections ☐ Other				
3	☐ Pills ☐ Implants ☐ Mirena/IUD ☐ Injections ☐ Other				
4	☐ Pills ☐ Implants ☐ Mirena/IUD ☐ Injections ☐ Other				

15b. Are you currently using birth control pills, Norplant (implants), Mirena/IUD, or Depo-Provera (injections)?

- ☐ **No**
☐ **Yes**

Figure 4. Contraceptive use questions included in the baseline research questionnaire for the Risk Factor Analysis of Breast and Ovarian Cancer (Version: 22 May 2018)

3.1.3. Study Subjects Available for Analysis

Study subjects were derived from an exploratory, prospective evaluation of contraceptive use and ovarian cancer risk. A total of 17,694 *BRCA* mutation carriers were available for inclusion in this study. Women were excluded if they had a prevalent cancer at baseline or a missing personal history of cancer (n=10,833); no follow up information (n=2,171); no date of birth, baseline date or date of death (n=590); bilateral oophorectomy prior to baseline (n=777); missing information on birth control use over follow-up period (n=4); or missing *BRCA* mutation type (n=36). After exclusions, a total of 3,283 *BRCA* mutation carriers were available for analysis, including 2,254 women who reported a history of contraceptive use (

Figure 5). The results of the prospective analysis were underpowered and will not be presented; however, data from the 2,254 contraceptive users were included in a descriptive study of patterns of contraceptive use by birth cohort and *BRCA* mutation type.

Figure 5. Recruitment statistics for a prospective cohort study of contraceptive use and ovarian cancer risk among *BRCA* mutation carriers

3.1.4. Data Cleaning

Due to limitations of the earlier Microsoft Access database, baseline data on contraceptive method and duration of use, as well as follow-up data on medication name and year of last use were not historically entered. To address missing information, we cleaned the data pertaining to contraceptive use for 2,254 contraceptive users (20.20% of all identified contraceptive users) identified for an exploratory, prospective analysis (data not presented). Participant file folders were retrieved, and the content of the physical baseline and follow-up questionnaires was cross-checked against information on the REDCap database. Given the variety of brand name and generic products that represent therapeutically equivalent formulations, misclassification of the ‘medication name’ variable was another issue during the data cleaning process. Table 8 shows the hormonal contraception identification chart created as a reference for coding patient self-reported medication names against 60 categorical response options available on REDCap. Specific options include 54 brand names and six umbrella terms (“unknown”, “other pill”, “implant”, “injection”, “IUD”, “more than one”). To address inconsistencies in self-reporting, participants who reported never-use of any contraception, but provided details of contraceptive use were corrected as ever-users. Participants who reported ever-use of contraception but failed to identify a medication name or method were categorized as ‘missing’.

Table 8. REDCap hormonal contraception identification chart

REDCap options	Method	Equivalent drug names	Cycle	Days	Estrogen type	Estrogen dose (mg)	Progestin type	Progestin dose (mg)
Demulen 50	Combined oral contraceptive	Kelnor 1/50, Zovia 1/50	Monophasic	1 to 21	Ethinyl estradiol	0.05	Ethinodiol diacetate	1
				22 to 28	-	-	-	-
Norinyl/ Ortho-Novum 1:50	Combined oral contraceptive	Genora 1/50, Necon 1/50, Nelova 1/50, Norethin 1/50, Nortrel 1/50	Monophasic	1 to 21	Mestranol	0.05	Norethindrone	1
				22 to 28	-	-	-	-
Ovral	Combined oral contraceptive		Monophasic	1 to 21	Ethinyl estradiol	0.05	Norgestrel	0.5
				22 to 28	-	-	-	-
Brevicon/ Ortho 0.5:35	Combined oral contraceptive	Cyclafem 0.5/35, Cyonanz, Modicon, Necon 0.5/35, Nortrel 0.5/35, Ortho 0.5/35, Ovysmen, Wera	Monophasic	1 to 21	Ethinyl estradiol	0.035	Norethindrone	0.5
				22 to 28	-	-	-	-
Brevicon/ Ortho/ Select 1:35	Combined oral contraceptive	Alyacen 1/35, Brevicon 1/35, Cyclafem 1/35, Dasetta 1/35, Necon 1/35, Norethin 1/25, Norinyl 1/35, Nortrel 1/35, Nylia 1/35, Ortho-Novum 1/35, Pirmella 1/35, Select 1/35	Monophasic	1 to 21	Ethinyl estradiol	0.035	Norethindrone	1
				22 to 28	-	-	-	-
Cyclen 35	Combined oral contraceptive	Cilest, Estarylla, Mili, Mono-Linyah, MonoNessa, Ortho-Cyclen, Previfem, Sprintec	Monophasic	1 to 21	Ethinyl estradiol	0.035	Norgestimate	0.25
				22 to 28	-	-	-	-
Demulen 30	Combined oral contraceptive		Monophasic	1 to 21	Ethinyl estradiol	0.03	Ethinodiol diacetate	1
				22 to 28	-	-	-	-
Loestrin 30	Combined oral contraceptive	Aurovela 1.5/30, Blisovi Fe 1.5/30, Gildess 1.5/30, Hailey 1.5/30, Junel 1.5/30, Larin 1.5/30, Loestrin 1.5/30, Microgestin 1.5/30	Monophasic	1 to 21	Ethinyl estradiol	0.03	Norethindrone acetate	1.5
				22 to 28	-	-	-	-

Min-Orval 30	Combined oral contraceptive	Altavera, Ayuna, Chateal, Indayo, Jolessa, Kurvelo, Leven, Levora 15/30, Marlissa, Microgynon 30, Nordette, Ovima, Portia	Monophasic	1 to 21	Ethinyl estradiol	0.03	Levonorgestrel	0.15
				22 to 28	-	-	-	-
Marvelon/Ortho-Cept 30	Combined oral contraceptive	Apri, Desogen, Emoquette, Enskyce, Isibloom, Kalliga, Marvelon, Ortho-Cept, Reclipsen, Solia	Monophasic	1 to 21	Ethinyl estradiol	0.03	Desogestrel	0.15
				22 to 28	-	-	-	-
Alesse	Combined oral contraceptive	Afirmelle, Alesse, Alysena, Aubra, Aviane, Balcoltra, Delyla, Falmina, Larissia, Luteria, Levlite, Lessina, Orsythia, Sronynx, Vienva, Minisiston	Monophasic	1 to 21	Ethinyl estradiol	0.02	Levonorgestrel	0.1
				22 to 28	-	-	-	-
Minestrin 20	Combined oral contraceptive	Aurovela (Fe) 1/20, Blisovi Fe 1/20, Gildess (Fe) 1/20, Hailey Fe 1/20, Junel (Fe) 1/20, Larin (Fe) 1/20, Loestrin (Fe) 1/20, Microgestin 1/20, Tarina (Fe) 1/20	Monophasic	1 to 21	Ethinyl estradiol	0.02	Norethindrone acetate	1
				22 to 28	-	-	-	-
		Aurovela 24 FE, Blisovi 24 FE, Finzala, Gildess 24 FE, Larin 24 FE, Loestrin 24 FE, Lomedia 24 FE, Mibelas 24 FE, Minastrin 24 FE, Taytulla®	Monophasic	1 to 24	Ethinyl estradiol	0.02	Norethindrone acetate	1
				25 to 28	-	-	-	
Micronor	Progestin-only (pill)	Aygestin, Camilla, Errin, Jolivette, Nora-BE, Nor-QD	Monophasic	1 to 28	-	-	Norethindrone	0.35
Ortho 10/11	Combined oral contraceptive	Necon 10/11, Ortho-novum 10/11 (Discontinued)	Biphasic	1 to 10	Ethinyl estradiol	0.035	Norethindrone	0.5
				11 to 21	Ethinyl estradiol	0.035	Norethindrone	1
				22 to 28	-	-	-	-
Ortho 7/7/7	Combined oral contraceptive	Alyacen 7/7/7, Cyclofem 7/7/7, Dasetta 7/7/7, Necon 7/7/7, Nortrel 7/7/7,	Triphasic	1 to 7	Ethinyl estradiol	0.035	Norethindrone	0.5
				8 to 14	Ethinyl estradiol	0.035	Norethindrone	0.75

		NyliaTM 7/7/7, Ortho-Novum 7/7/7, Pirmella 7/7/7		15 to 21	Ethinyl estradiol	0.035	Norethindrone	1
				22 to 28	-	-	-	-
Synphasic	Combined oral contraceptive		Biphasic	1 to 9	Ethinyl estradiol	0.035	Norethindrone	1
				10 to 21	Ethinyl estradiol	0.035	Norethindrone	0.5
			Biphasic	1 to 9	Ethinyl estradiol	0.035	Norethindrone	1
				10 to 21	Ethinyl estradiol	0.035	Norethindrone	0.5
				22 to 28	-	-	-	-
Tri-Cyclen	Combined oral contraceptive	Ortho-Tricyclen, TriNessa, Tricyclen Tri-Cyclen Lo, Ortho Tri-cyclen Lo, Tri-Lo-Sprintec	Triphasic	1 to 7	Ethinyl estradiol	0.035	Norgestimate	0.18
				8 to 14	Ethinyl estradiol	0.035	Norgestimate	0.215
				15 to 21	Ethinyl estradiol	0.035	Norgestimate	0.25
			Biphasic	1 to 7	Ethinyl estradiol	0.035	Norgestimate	0.18
				8 to 14	Ethinyl estradiol	0.035	Norgestimate	0.215
				15 to 21	Ethinyl estradiol	0.035	Norgestimate	0.25
				22 to 28	-	-	-	-
Triphasil/ Triquilar	Combined oral contraceptive	Trionetta, Trigynon, Logynon, Trivora, Trilevlen, Elifemme, Enpresse, Levonest, Myzilra	Triphasic	1 to 7	Ethinyl estradiol	0.03	Levonorgestrel	0.05
				8 to 14	Ethinyl estradiol	0.04	Levonorgestrel	0.075
				15 to 21	Ethinyl estradiol	0.03	Levonorgestrel	0.125
			Triphasic	1 to 7	Ethinyl estradiol	0.03	Levonorgestrel	0.05
				8 to 14	Ethinyl estradiol	0.04	Levonorgestrel	0.075
				15 to 21	Ethinyl estradiol	0.03	Levonorgestrel	0.125

				22 to 28	-	-	-	-
Other Pill		Lolo, Lo Loestrin Fe	Biphasic	1 to 24	Ethinyl estradiol	0.01	Norethindrone acetate	1
				25 to 26	Ethinyl estradiol	0.01	-	-
				27 to 28	Fe	75	-	-
		Generess Fe, Kaitlib Fe, Layolis Fe	Monophasic	1 to 24	Ethinyl estradiol	0.025	Norethindrone	0.8
				25 to 28	Fe	75	-	-
		Demulen 1/35, Kelnor 1/35, Zovia 1/35		1 to 21	Ethinyl estradiol	0.035	Ethinodiol diacetate	1
				22 to 28	-	-	-	-
		Tilia Fe	Biphasic	1 to 21	Ethinyl estradiol	0.02	Norethindrone acetate	1
				22 to 28	Fe	75		
		Estrostep Fe, Tri-Legest Fe	Triphasic	1 to 7	Ethinyl estradiol	0.02	Norethindrone acetate	1
				8 to 14	Ethinyl estradiol	0.03	Norethindrone acetate	1
				15 to 21	Ethinyl estradiol	0.035	Norethindrone acetate	1
				22 to 28	Fe	75		
		Lo-Ovral, Chryselle, Elinest, Low-Ogestrel	Monophasic	1 to 21	Ethinyl estradiol	0.03	Norgestrel	0.3
				22 to 28	-	-	-	-
		LoSeasonique	Monophasic	1 to 84	Ethinyl estradiol	0.02	Levonorgestrel	0.1
				85 to 91	Ethinyl estradiol	0.01		
		Ovrette		Daily	-	-	Norgestrel	0.075
		Qlaira	Quadriphasic	1 to 2	Estradiol valerate	3	-	-
				3 to 7	Estradiol valerate	2	Dienogest	2
				8 to 24	Estradiol valerate	2	Dienogest	3

				25 to 26	Estradiol valerate	1	-	-
				27 to 28	-	-	-	-
		Gravistat	Monophasic	1 to 21	Ethinyl estradiol	0.05	Levonorgestrel	0.125
				22 to 28	-	-	-	-
		Angravid			Mestranol	0.05	Ethinodiol diacetate	1
		Azurette, Mircette, Kariva	Biphasic	1 to 21	Ethinyl estradiol	0.02	Desogestrel	0.15
				22 to 23	-	-	-	-
				24 to 28	Ethinyl estradiol	0.01	-	-
		Cyclessa, Cesia, Caziant, Velivet, Linessa	Triphasic	1 to 7	Ethinyl estradiol	0.025	Desogestrel	0.1
				8 to 14	Ethinyl estradiol	0.025	Desogestrel	0.125
				9 to 21	Ethinyl estradiol	0.025	Desogestrel	0.15
				22 to 28	-	-	-	-
Injection	Injectable	Noristerat	2 months		-	-	Norethisterone enantate	200
		Sayana Press	13 weeks		-	-	Medroxyprogesterone acetate	104
		Lunelle	Monthly		Estradiol cypionate	5	Medroxyprogesterone acetate	25
Implants	Progestin-only (implant)	Norplant					Levonorgestrel	216
		Jadelle					Levonorgestrel	150
Logest	Combined oral contraceptive	Meliane, Sylvie 20	Monophasic	1 to 21	Ethinyl estradiol	0.02	Gestodene	0.075
Trionetta	Combined oral contraceptive	Triquilar, Triphasil, Trigynon, Logynon, Trivora, Trilevlen, Elifemme, Enpresse, Levonest, Myzila	Triphasic	1 to 7	Ethinyl estradiol	0.03	Levonorgestrel	0.05
				8 to 14	Ethinyl estradiol	0.04	Levonorgestrel	0.075
				15 to 21	Ethinyl estradiol	0.03	Levonorgestrel	0.125
				1 to 7	Ethinyl estradiol	0.03	Levonorgestrel	0.05

				8 to 14	Ethinyl estradiol	0.04	Levonorgestrel	0.075
				15 to 21	Ethinyl estradiol	0.03	Levonorgestrel	0.125
				22 to 28	-	-	-	-
Diane 35	Combined oral contraceptive	Cleo-35, Cyestra-35	Monophasic	1 to 21	Ethinyl estradiol	0.035	Cyproterone acetate	2
Yasmin	Combined oral contraceptive	Qismette, Zamine, Zarah, Ocella, Yaela, Syeda, Safyral	Monophasic	1 to 21	Ethinyl estradiol	0.03	Drospirenone	3
				22 to 28	-	-	-	-
				1 to 21	Ethinyl estradiol	0.03	Drospirenone	3
Depo-provera	Injectable		Monophasic	Every 3 months	-	-	Medroxyprogesterone acetate	150
Meliane	Combined oral contraceptive	Logest, Sylvie 20, Harmonet	Monophasic	1 to 21	Ethinyl estradiol	0.02	Gestodene	0.075
Orgametril	Progestin-only (pill)	Exluton, Exlutona	Monophasic	1 to 30	-	-	Lynestronol	5
Cilest	Combined oral contraceptive	Cilest 35/250, Cyclen 35, Sprintec	Monophasic	1 to 21	Ethinyl estradiol	0.035	Norgestimate	0.25
Patentex	Vaginal suppository (spermicide)	Patentex Oval	Monophasic	-	Nonoxynol	75	-	-
Harmonet {Europe}	Combined oral contraceptive	Logest, Sylvie 20, Meliane	Monophasic	1 to 21	Ethinyl estradiol	0.02	Gestodene	0.075
Tri-Regol	Combined oral contraceptive	Fironetta, Levordiol, Logynon, Modutrol, Triagynon, Triclor, Triette, Trigoa, Trigynon, Trikvilar, Tri-Levlen, Trinordiol, Trionetta, Triovlar, Triphasil, Triquilar, Trisiston, TriStediril, Trolit	Triphasic	1 to 7	Ethinyl estradiol	0.03	Levonorgestrel	0.05
				8 to 14	Ethinyl estradiol	0.04	Levonorgestrel	0.075
				15 to 21	Ethinyl estradiol	0.03	Levonorgestrel	0.125
Mercilon	Combined oral contraceptive	Novynette, Desmin 20, Femina	Monophasic	1 to 21	Ethinyl estradiol	0.02	Desogestrel	0.15

				1 to 21	Ethinyl estradiol	0.02	Desogestrel	0.15
				22 to 28	-	-	-	-
Desogen	Combined oral contraceptive	Marvelon, Ortho-Cept, Apri, Emoquette, Enskyce, Isibloom, Reclipsen, Solia	Monophasic	1 to 21	Ethinyl estradiol	0.03	Desogestrel	0.15
				1 to 21	Ethinyl estradiol	0.03	Desogestrel	0.15
				22 to 28	-	-	-	-
Tri-Minulet	Combined oral contraceptive	Milvane, Phaeva, Triadene, Tricilomex, TriFemoden, Trigynera, Tri-Gynera, Trigynovin, Triodeen, Trioden, Triodena, Triodene	Triphasic	1 to 7	Ethinyl estradiol	0.03	Gestodene	0.05
				8 to 14	Ethinyl estradiol	0.04	Gestodene	0.07
				15 to 21	Ethinyl estradiol	0.03	Gestodene	0.1
Seasonale	Combined oral contraceptive	Quasense, Iclevia, Introvale, Setlakin, Sylevia Seasonique, Amethia is the next generation of Seasonale that contains 0.01 mg E in the inactive pills to suppress breakthrough bleeding	Extended cycle	1 to 84	Ethinyl estradiol	0.03	Levonorgestrel	0.15
				85 to 91	-	-	-	-
Ortho evra	Combined hormonal contraceptive (patch)	EVRA	Monophasic	Every 7 days	Ethinyl estradiol	0.6	Norelgestromin	6
Mirena	Progestin-only (IUD)	Liletta, Levosert		Every 5 years	-	-	Levonorgestrel	52
IUD-gestagen releasing	Progestin-only (IUD)	Progestasert					Progesterone	38
		Skyla, Jaydess		Every 3 years			Levonorgestrel	13.5
		Kyleena		Every 5 years			Levonorgestrel	19.5
Milvane	Combined oral contraceptive	Phaeva, Triadene, Tricilomex, TriFemoden, Trigynera, Tri-Gynera, Tri-Minulet, Trigynovin, Triminulet, Triodeen, Trioden, Triodena, Triodene"	Triphasic	1 to 7	Ethinyl estradiol	0.03	Gestodene	0.05
				7 to 14	Ethinyl estradiol	0.04	Gestodene	0.07
				15 to 21	Ethinyl estradiol	0.03	Gestodene	0.1

Microgynon	Combined oral contraceptive		Monophasic	1 to 21	Ethinyl estradiol	0.03	Levonorgestrel	0.15
Ovcon	Combined oral contraceptive	Ovcon 35, Ovcon 50, Balziva, Briellyn, Femcon Fe, Gildagia Nexesta Fe, Philith, Vyfemla, Wymzya Fe, Zenchent 0.4/35, Zenchent Fe, Zeosa	Monophasic	1 to 21	Ethinyl estradiol	0.035	Norethindrone	0.4
				22 to 28	-	-	-	-
				1 to 21	Ethinyl estradiol	0.05	Norethindrone	0.4
				22 to 28	-	-	-	-
Minulet	Combined oral contraceptive	Sylvie 30, Femodene	Monophasic	1 to 21	Ethinyl estradiol	0.03	Gestodene	0.075
				22 to 28	-	-	-	-
Novynette	Combined oral contraceptive	Desmin 20, Femina, Mercilon	Monophasic	1 to 21	Ethinyl estradiol	0.02	Desogestrel	0.15
				22 to 28	-	-	-	-
Ovysmen	Combined oral contraceptive	Ovysmen 0.5/35, Ovysmen 1/35, Brevicon 0.5/35, Ortho 0.5/35, Necon 0.5/35, Nortrel 0.5/35, Wera, Modicon, Cyonanz	Monophasic	1 to 21	Ethinyl estradiol	0.035	Norethindrone	0.5
				1 to 21	Ethinyl estradiol	0.035	Norethindrone	1
Gestagen	-	-	-	-	-	-	-	-
Ortho Evra	Combined hormonal contraceptive (patch)	EVRA	Monophasic	Every 7 days	Ethinyl estradiol	0.6	Norelgestromin	6
More than one	-	-	-	-	-	-	-	-
Camilla	Progestin-only (pill)	Aygestin, Micronor, Errin, Jolivette, Nora-BE, Nor-QD	Monophasic	Daily	-	-	Norethindrone	0.35
Provera	Not indicated for contraception				-	-	Medroxyprogesterone acetate	2.5>5>10
Cerazette	Progestin-only (pill)	Cerelle, Zellesta, Azalia, Lactinette	Monophasic	Daily	-	-	Desogestrel	0.075
Evra	Combined hormonal contraceptive (patch)		Monophasic	Every 7 days	Ethinyl estradiol	0.6	Norelgestromin	6

Rigevidon	Combined oral contraceptive		Monophasic	1 to 21	Ethinyl estradiol	0.03	Levonorgestrel	0.15
Nuvaring	Combined hormone contraceptive (ring)		Monophasic	Every 21 days/28 days/35 days	Ethinyl estradiol	2.6	Etonogestrel	11.4
Regulon	Combined oral contraceptive	Desorelle, Desmin 30	Monophasic	1 to 21	Ethinyl estradiol	0.03	Desogestrel	0.15
Yasminelle	Combined oral contraceptive	Yaz, Beyaz, Gianvi	Monophasic	1 to 24	Ethinyl estradiol	0.02	Drospirenone	3
				25 to 28	-	-	-	
Duphaston	Not indicated for contraception		Monophasic		-	-	Dydrogesterone	10
Femodene	Combined oral contraceptive	Sylvie 30, Minulet	Monophasic	1 to 21	Ethinyl estradiol	0.03	Gestodene	0.075
				22 to 28	-	-	-	-
Implanon	Progestin-only (implant)	Nexplanon	Monophasic	Every 3 years	-	-	Etonogestrel	68

3.1.5. Exposure Classification

The following variables were created for this descriptive analysis. Women were stratified into eight birth cohorts corresponding to each decade from 1920 to 2000: 1920-1929, 1930-1939, 1940-1949, 1950-1959, 1960-1969, 1970-1979, 1980-1989, 1990-1999. Specific contraceptive methods defined for this analysis include oral contraceptives, implants, injections, IUDs and other. *Oral contraceptive users* were defined as subjects who reported ever using an estrogen-progestin, progestin-only or nonspecific oral contraceptive pill. *Implant users* were defined as subjects who reported ever using Implanon or a nonspecific contraceptive implant. *Injection users* were defined as subjects who reported ever using Depo-Provera or a nonspecific injection. Type of IUD was classified as hormonal (LNG IUDs) or non-hormonal (metal-bearing or inert IUDs). *IUD users* were defined as subjects who reported ever using a hormonal or non-hormonal IUD. Subjects who indicated IUD use without reporting a specific IUD name were classified as nonhormonal IUD users if the year of initiation occurred prior to the year in which the hormonal IUD was introduced in each country. *Other* pertained to subjects who reported use of less popular contraceptive methods including vaginal ring and transdermal patch. For women who reported multiple methods of contraception, all methods were included.

3.1.6. Statistical Analysis

Descriptive statistics were used to summarize method-specific contraceptive use by birth cohort and mutation type through tabular and graphical means. Analyses were conducted for oral contraceptives, IUDs, implants, injections and other contraceptive methods. All analyses were performed using the SAS statistical package, version 9.1.3 (SAS Institute, Cary, NC).

3.2. Analysis of Contraceptive Use and *BRCA* Ovarian Cancer Risk

Details of the study population, data collection and data storage methodology were previously described in Section 3.1.

3.2.1. Exposure classification

Any contraceptive use was defined as use of any method of hormonal contraception (including transdermal patches and vaginal rings) or a nonhormonal IUD. Specific contraceptive methods defined for this analysis include oral contraceptives, implants, injections and IUDs (defined in Section 3.1).

The following temporal characteristics of IUD use were created for this analysis. Among IUD ever users, the *total duration of IUD use* was calculated from the sum of all periods of IUD use prior to the censor date. *Age at first IUD use* was calculated by subtracting the date of birth from the date of first use. *Age at last IUD use* was calculated by subtracting the date of birth from the date of last use. Total duration of use, age at first use and age at last use were categorized into two groups: less than or equal to and greater than the median distribution. *Recency of IUD use* was categorized as never use, former use (discontinued more than six months prior) or current or recent use (discontinued within the last six months).

Contraceptive users who failed to indicate a medication name or method were classified as oral contraceptive users due to the high proportion of oral contraceptive use among *BRCA* mutation carriers in our cohort.

3.2.2. Assessment of Cancer Outcomes

The primary outcome of interest was a diagnosis of a primary invasive ovarian or fallopian tube cancer, collectively referred to as ovarian cancer henceforth. Diagnoses of ovarian cancer were identified by self-report. For each reported case of incident cancer in the follow-up questionnaire, pathology reports were requested to abstract information regarding histological subtype (serous, endometrioid, mucinous, clear cell), clinical staging (I-IV), primary site of

origin (ovary, fallopian tube, peritoneum, other) and sites of spread (fallopian tubes, peritoneum, uterus, other). When pathology reports could not be obtained, medical records were requested to confirm the diagnosis. Pathology reports were not available for participants diagnosed prior to the baseline questionnaire.

3.2.3. Study Subjects Available for Analysis

A total of 17,893 *BRCA1* or *BRCA2* mutation carriers were available for inclusion in this analysis. Subjects were excluded if they had a previous diagnosis of cancer other than breast or ovarian cancer (n = 2,295), were missing information on personal history of breast or ovarian cancer (n = 407), missing *BRCA* mutation type (n = 197), had an oophorectomy prior to censor date (n = 77), missing information on oophorectomy status or year of surgery (n = 467), or missing date of birth or baseline date (n = 8). Subjects were also excluded if they were missing pertinent information regarding contraceptive use (n = 237). After exclusions, a total of 14,205 *BRCA1* or *BRCA2* mutation carriers were available for analysis, including 2,765 women diagnosed with ovarian cancer (potential case subjects) and 15,128 women with no history of ovarian cancer (potential control subjects).

Case subjects were defined as women with a diagnosis of ovarian cancer in the baseline (prevalent) or follow-up period (incident). Control subjects were defined as women who never had ovarian cancer. A control was eligible to be matched to a case if the date of prophylactic oophorectomy in the control occurred at or after the year of ovarian cancer diagnosis of the matched case. Each case was matched to a single control subject according to year of birth (within one year), year of completion of the baseline questionnaire (within two years), country of residence (Canada, United States, Poland, Other), *BRCA* mutation type (*BRCA1* or *BRCA2*) and personal history of breast cancer (yes/no). Matching variables were selected *a priori* as established confounders that influence risk of *BRCA*-ovarian cancer. In total, 1,732 matched pairs were generated. A flow diagram for the selection of study participants is provided in Figure 6.

Figure 6. Recruitment statistics for a matched case-control study of contraceptive use and ovarian cancer risk among *BRCA* mutation carriers

3.2.4. Statistical Analysis

A matched case-control analysis was conducted to evaluate the associations between methods of contraceptive use and the risk of ovarian cancer. Distributions of continuous and categorical variables were compared between cases and controls using the Student's *t*-test and Chi-square test, respectively. Univariate and multivariate ORs and 95% confidence intervals (CI) of ovarian cancer associated with each contraceptive exposure were estimated by conditional logistic regression. The multivariate model adjusted for the following covariates: parity (parous/nulliparous), history of breastfeeding (ever/never), menopausal status (premenopausal/postmenopausal) and previous oral contraceptive use (ever/never). Selection of covariates was based on the literature. Participants were categorized as postmenopausal if they had stopped menstruating for at least a year prior to censoring/event due to natural causes, radiation, or chemotherapy.

The follow-up period for this analysis is from the date of completion of the baseline questionnaire (i.e. study entry) until March 31st 2021. Cases were followed from study entry until the date of their ovarian cancer diagnosis, prophylactic oophorectomy, death or date of completion of their last follow-up questionnaire. For the controls, data on exposures was censored at the date of diagnosis of the matched case.

The analysis of any hormonal contraceptive included women who never used any hormonal contraception as the referent group. Each contraceptive method was then examined in a separate model with women who never used that respective method (not excluding other methods) before the censor date serving as the referent group. For select analyses with insufficient data, we used unconditional logistic regression to calculate age-adjusted and multivariate estimates (adjusted for aforementioned covariates as well as matching variables).

All analyses were performed using the SAS statistical package, version 9.1.3 (SAS Institute, Cary, NC). *P*-values were based on two-sided tests and were considered statistically significant if $P < 0.05$.

Chapter 4. Results

4.1. Trends in Contraceptive use among *BRCA* Mutation Carriers

4.1.1. Subject Characteristics

Demographic characteristics of the 2,254 women who reported a history of any contraceptive use are shown in Table 9. Of these women, 73% were *BRCA1* mutation carriers and 27% were *BRCA2* mutation carriers. The mean year of birth was 1971 and 1966 for women with a *BRCA1* and *BRCA2* mutation, respectively. There were low proportions of women in the earlier birth cohorts, from 0.04% in the 1920-1929 birth cohort to 14% in the 1950-1959 birth cohort. Only 3% of women belonged to the most recent birth cohort, which will likely increase as the cohort ages. The majority of data was derived from the United States, Canada and Poland. Oral contraceptives were the most popular contraceptive method, reported by 95% of *BRCA1* and *BRCA2* mutation carriers born between 1920 and 1999 who had a history of any contraceptive use (Table 9). Lifetime rates of use for IUDs, implants and injections were lower at 9%, 3% and 4%, respectively. Approximately 4% of women reported use of other hormonal contraceptive methods including the vaginal ring and transdermal patch at one point.

4.1.2. Trends in Use of Various Contraceptive Methods Across Birth Cohorts

The total number of contraceptive users rose from the 1920-1929 birth cohort and peaked at the 1970-1979 birth cohort (Table 10). The declining number of contraceptive users in more recent birth cohorts may be attributed to the older average age of our population. Overall, nearly all contraceptive users (96%) reported a history of oral contraceptive use, which was consistent across birth cohorts (Table 10, Figure 7). Trends in the proportions of oral contraceptive use were broadly similar between *BRCA1* and *BRCA2* mutation carriers (Figure 8, Column totals may exceed 100% as women were allowed to report more than one method

Figure 9); however, only 88% of *BRCA2* mutation carriers in the 1990-1999 birth cohort reported using oral contraceptives at some point in their lives, compared to 96% of *BRCA1* mutation carriers (Table 11,

Table 12). This difference is likely due to low numbers of contraceptive users with a *BRCA2* mutation, which may increase as the cohort ages.

IUDs were the most commonly used LARC method. The highest proportions of lifetime IUD use were reported among women born in 1930-1939 (12%) and 1990-1999 (12%) , although the 1930-1939 birth cohort was contained only eight contraceptive users (Table 10, Figure 7). Rates of IUD use remained fairly constant across birth cohorts, aside from a decline to around 6% in the 1960-1969 birth cohort for both *BRCA1* and *BRCA2* mutation carriers (Table 11,

Table 12). Regarding contraceptive injections, increasing proportions of successive cohorts reported lifetime injection use, 1% of those born in 1940-1949 to 6% of those born in 1970-1979 (Table 10, Column totals may exceed 100% as women were allowed to report more than one method

Figure 7). Implant use was initiated in the 1940-1949 cohort and rates remained stable for *BRCA1* and *BRCA2* mutation carriers born after 1950. The greatest proportion of implant use was observed among *BRCA* mutation carriers born from 1970-1979 (4%). For *BRCA2* mutation carriers, 12% uptake of both implants and injections were observed for the 1990-1999 birth cohort, but likely attributed to low numbers of total contraceptive users (Table 12). Taken together, lifetime rates of LARC use increased across birth cohorts from 12% in the 1930-1939 cohort to 22% in the 1970-1979 cohort. Use of other hormonal contraceptive methods increased steadily from less than 1% of the 1950-1959 cohort to approximately 9% of the 1980-1989 birth cohort, which did not vary by *BRCA* mutation type (Table 10, Column totals may exceed 100% as women were allowed to report more than one method

Figure 7). Rates of injections and other hormonal contraceptive methods were slightly lower among the youngest birth cohort (1990-1999), but this cohort has yet to reach menopause and remain within the period of initiation of use.

Table 9. Demographic characteristics of contraceptive users with a *BRCA1* or *BRCA2* mutation

Characteristic	Total (n=2,254)	Mutation	
		<i>BRCA1</i> (n=1,652)	<i>BRCA2</i> (n=602)
Mean year of birth	1970.12	1971.49	1966.34
Birth cohort, n (%)			
1920-1929	1 (0.04)	0 (0.00)	1 (0.2)
1930-1939	8 (0.35)	4 (0.18)	4 (0.7)
1940-1949	113 (5.01)	59 (3.57)	54 (9.0)
1950-1959	325 (14.42)	203 (12.29)	122 (20.3)
1960-1969	564 (25.02)	396 (23.97)	168 (27.9)
1970-1979	687 (30.48)	533 (32.3)	154 (25.6)
1980-1989	498 (22.09)	407 (24.6)	91 (15.1)
1990-1999	58 (2.57)	50 (3.0)	8 (1.3)
Contraceptive methods, n (%)			
Oral contraceptive	2,142 (95.03)	1,559 (94.37)	583 (96.84)
Intrauterine device	210 (9.32)	145 (8.78)	65 (10.80)
Injection	95 (4.21)	65 (3.93)	30 (4.98)
Implant	68 (3.02)	58 (3.51)	10 (1.66)
Other	102 (4.53)	82 (4.96)	10 (3.32)
Country of residence, n (%)			
United States	536 (23.80)	311 (18.83)	225 (37.38)
Canada	726 (32.20)	395 (23.91)	331 (54.98)
Poland	785 (34.80)	775 (46.91)	10 (1.66)
Other	207 (9.20)	171 (10.35)	36 (5.98)

Table 10. Distribution of *BRCA1* and *BRCA2* mutation carriers who reported ever use of various contraceptive methods by birth cohort

Contraceptive method	Birth cohort							
	1920-1929	1930-1939	1940-1949	1950-1959	1960-1969	1970-1979	1980-1989	1990-1999
Oral contraceptive, n (%)	1 (100.00)	8 (100.00)	110 (97.35)	307 (94.46)	535 (94.86)	649 (94.47)	477 (95.78)	55 (94.83)
Intrauterine device, n (%)	0 (0.00)	1 (12.50)	10 (8.85)	30 (9.23)	31 (5.50)	80 (11.64)	51 (10.24)	7 (12.07)
Injection, n (%)	0 (0.00)	0 (0.00)	1 (0.88)	7 (2.15)	23 (4.08)	41 (5.97)	20 (4.02)	2 (3.45)
Implant, n (%)	0 (0.00)	0 (0.00)	1 (0.88)	10 (3.08)	14 (2.48)	28 (4.08)	13 (2.61)	2 (3.45)
Other, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.62)	7 (1.24)	43 (6.26)	46 (9.24)	4 (6.90)
Total, n (%)	1 (100.00)	8 (100.00)	113 (100.00)	325 (100.00)	564 (100.00)	687 (100.00)	498 (100.00)	58 (100.00)

Column totals may exceed 100% as women were allowed to report more than one method

Figure 7. Trends in proportions of *BRCA1* and *BRCA2* mutation carriers who reported ever use of various contraceptive methods by birth cohort

Table 11. Distribution of *BRCA1* mutation carriers who reported ever use of various contraceptive methods by birth cohort

Contraceptive method	Birth cohort							
	1920-1929	1930-1939	1940-1949	1950-1959	1960-1969	1970-1979	1980-1989	1990-1999
Oral contraceptive, n (%)	0 (0.00)	4 (100.00)	57 (96.60)	188 (92.6)	370 (93.4)	503 (94.4)	389 (95.6)	48 (96.0)
Intrauterine device, n (%)	0 (0.00)	0 (0.00)	5 (8.47)	20 (9.85)	21 (5.30)	60 (11.26)	36 (8.85)	3 (6.00)
Injection, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	5 (2.46)	16 (4.04)	28 (5.25)	15 (3.69)	1 (2.00)
Implant, n (%)	0 (0.00)	0 (0.00)	1 (1.69)	10 (4.93)	13 (3.28)	23 (4.32)	10 (2.46)	1 (2.00)
Other, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.99)	6 (1.52)	34 (6.38)	36 (8.85)	4 (8.00)
Total, n (%)	0 (100.00)	4 (100.00)	59 (100.00)	203 (100.00)	396 (100.00)	533 (100.00)	407 (100.00)	50 (100.00)

Column totals may exceed 100% as women were allowed to report more than one method

Figure 8. Trends in proportions of *BRCA1* mutation carriers who reported ever use of various contraceptive methods by birth cohort

Table 12. Distribution of *BRCA2* mutation carriers who reported ever use of various contraceptive methods by birth cohort

Contraceptive method	Birth cohort							
	1920-1929	1930-1939	1940-1949	1950-1959	1960-1969	1970-1979	1980-1989	1990-1999
Oral contraceptive, n (%)	1 (100.0)	4 (100.00)	53 (98.15)	119 (97.54)	165 (98.21)	146 (94.81)	88 (96.70)	7 (87.50)
Intrauterine device, n (%)	0 (0.00)	1 (25.00)	5 (9.26)	10 (8.20)	10 (5.95)	20 (12.99)	15 (16.48)	4 (50.0)
Injection, n (%)	0 (0.00)	0 (0.00)	1 (1.85)	2 (1.64)	8 (4.76)	13 (8.44)	5 (5.49)	1 (12.50)
Implant, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.60)	5 (3.25)	3 (3.30)	1 (12.50)
Other, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.60)	9 (5.84)	10 (10.99)	0 (0.00)
Total, n (%)	1 (100.00)	4 (100.00)	54 (100.00)	122 (100.00)	168 (100.00)	154 (100.00)	91 (100.00)	8 (100.00)

Column totals may exceed 100% as women were allowed to report more than one method

Figure 9. Trends in proportions of *BRCA2* mutation carriers who reported ever use of various contraceptive methods by birth cohort

4.2. Associations between Contraceptive Method and *BRCA* Ovarian Cancer Risk

4.2.1. Subject Characteristics

Table 13 describes the demographic, reproductive, hormonal and other clinical characteristics of the 1,732 cases of ovarian cancer and 1,732 matched controls that were included in the final analysis. Cases and controls did not differ with respect to year of birth, country of residence, *BRCA* gene mutation type, and personal history of breast cancer. On average, *BRCA1* mutation carriers were diagnosed with ovarian cancer at a younger age than *BRCA2* mutation carriers (48.94 years vs. 53.60 years). Fewer cases than controls reported a history of breastfeeding (63.14% vs. 67.68%; $P=0.008$). Mean parity and mean months of breastfeeding were slightly lower in cases than controls (2.69 vs. 2.82, $P=0.008$ for parity; 13.19 months vs. 14.76 months, $P=0.02$ for breastfeeding). Cases were more likely to have a history of HRT use (20.64% vs. 13.45%; $P<0.0001$).

Table 14 shows the characteristics of women who reported ever use of each contraceptive method. Oral contraceptives were the most popular contraceptive method adopted by 49.22% of the cohort. The mean duration of oral contraceptive use was 7.41 years (range 0.08-32 years). The mean age at first use was 24 years (range 6-53 years) and the mean age at last use was 31 years (range 6-61 years). Overall, only 56 women reported use of an IUD (1.62%). Of those, the nonhormonal IUD was the most common method ($n=35$; 62%) with only 13 women reporting use of a hormonal IUD (23%). The mean duration of IUD use was 6.85 years (range 0.08-24 years). On average, women reported a longer duration of use for the nonhormonal IUD (8.0 years; range 1-24 years) than the hormonal IUD (4.85 years; 0.08-19 years). The mean age at first use of any IUD was 30.53 years (range 19-50 years) and the mean age at last use of any IUD was 36.98 years (range 20-52 years). Women who used a hormonal IUD reported a later average age at first use (34.15 years; range 20-48 years) and last use (39 years; range 26-49 years) than women who used a nonhormonal IUD (28.50 years, range 19-42 years for age at first use; 35.85 years, range 20-52 years for age at last use). There were 25 women who had an implant (0.72%) for a mean duration of 6.67 years (range 1-20 years). The mean age at initiation was 29.12 years (range 20-42 years) and the mean age at last use was 35.73 years (range 22-48 years). Injections

were the least common contraceptive method reported by 0.23% of the cohort. Among injection users, the average age at first use is 29.25 years (range 22-37 years) over a mean duration of 3.77 years (range 0.42-19 years). The mean age at last use of an injection was 33.75 (range 24-44 years). On average, women who used oral contraceptives were older than women who used other methods (mean year of birth, 1953).

4.2.2. Associations between Methods of Contraceptive Use and Ovarian Cancer Risk

The associations between various methods of contraceptive use and the risk of developing ovarian cancer are presented in Table 15. Cases were less likely to have a history of oral contraceptive use (45.09% vs. 53.35%; $P<0.0001$) and implant use (0.35% vs. 1.10%; $P<0.001$) than controls. Compared to never users of any hormonal contraceptive, women who used any type of hormonal contraceptive had a significant 38% reduction in risk of developing ovarian cancer according to the univariate model (OR=0.62; 95% CI 0.53-0.73). The relationship was similar after adjusting for previous oral contraceptive use, history of breastfeeding, menopausal status and parity (OR=0.58; 95% CI 0.48-0.70).

Oral contraceptive use was associated with a significant 41% reduction in the risk of ovarian cancer (OR=0.59; 95% CI 0.49-0.71). Use of contraceptive implants and injections were associated with a 62% to 68% reduction in risk respectively; however, analyses were based on a small number of women and should be interpreted with caution (OR=0.38, 95% CI 0.15-0.97 for implants; OR=0.32, 95% CI 0.063-1.66 for injections). Only six cases and 19 controls reported ever use of an implant. Only two cases and six controls reported ever use of an injection, and results did not achieve statistical significance (Table 15).

Women who used an IUD had a nonsignificant decreased risk of ovarian cancer compared to women who never used an IUD (OR=0.81; 95% CI 0.46-1.45). Although limited by a small number of users, the OR for hormonal IUDs was 0.91 (95% CI 0.28-2.91) and the OR for nonhormonal IUDs was 0.66 (95% CI 0.31-1.39) (Table 15). Temporal characteristics of IUD use including duration of use, recency of use, age at first use and age at last use were not significantly associated with risk of ovarian cancer ($P\geq 0.15$) (Table 16).

Although based on a small number of exposed cases, the associations did not vary substantially by menopausal status (Table 15). In the analysis stratified by history of oral contraceptive use, implant use was associated with a greatly decreased risk of ovarian cancer among women who never used contraceptives (OR=0.23; 95% CI 0.01-0.80) (Table 17).

Table 13. Comparison of ovarian cancer cases and controls with a *BRCA1* or *BRCA2* mutation

Characteristic	Cases (n=1,732)	Controls (n=1,732)	P
Year of birth, mean (range)	1952.50 (1917-1986)	1952.60 (1917-1987)	Matched
Age at diagnosis, mean (range)			n/a
<i>BRCA1</i>	48.94 (17-84)	n/a	
<i>BRCA2</i>	53.60 (22-82)	n/a	
Country of residence, n (%)			Matched
United States	466 (26.91)	466 (26.91)	
Canada	382 (22.06)	382 (22.06)	
Poland	645 (37.24)	645 (37.24)	
Other	239 (13.80)	239 (13.80)	
Mutation type, n (%)			Matched
<i>BRCA1</i>	1,443 (83.31)	1,443 (83.31)	
<i>BRCA2</i>	289 (16.69)	289 (16.69)	
Personal history of breast cancer, n (%)			Matched
No	1,131 (65.30)	1,131 (65.30)	
Yes	601 (34.70)	601 (34.70)	
Parity, n (%)			0.05
Nulliparous	264 (15.30)	225 (13.00)	
Parous	1,462 (84.70)	1,506 (87.00)	
Missing	6	1	
Number of pregnancies ^a , mean (range)	2.69 (1-8)	2.82 (1-10)	0.008
Breastfeeding history, n (%)			0.008
Never	656 (36.86)	491 (32.32)	
Ever	942 (63.14)	1028 (67.68)	
Missing	180	213	
Months of breastfeeding, mean (range)	13.19 (0.04-137)	14.76 (0.10-159)	0.02
Age at menarche (year), mean (SD)	13.11 (4-28)	13.17 (8-18)	0.24
Age at menopause (year), mean (SD)	46.66 (23-59)	46.50 (26-68)	0.52
Menopausal status ^b , n (%)			0.12
Premenopausal	855 (51.35)	905 (54.03)	
Postmenopausal	810 (48.65)	770 (45.97)	
Missing	67	57	
HRT use, n (%)			<0.0001
Never	1373 (79.36)	1493 (86.55)	
Ever	357 (20.64)	232 (13.45)	
Missing	2	7	
Tubal ligation, n (%)			0.10
Never	1393 (86.68)	1314 (84.60)	
Ever	214 (13.32)	239 (15.39)	
Missing	125	179	
Hysterectomy, n (%)			0.40
Never	1508 (92.97)	1416 (92.19)	
Ever	114 (7.03)	120 (7.81)	
Missing	110	196	
Oral contraceptive use, n (%)			<0.0001
Never	951 (54.91)	808 (46.65)	
Ever	781 (45.09)	924 (53.35)	
Implant use, n (%)			0.009
Never	1,726 (99.65)	1,713 (98.90)	
Ever	6 (0.35)	19 (1.10)	
Injection use, n (%)			0.16
Never	1,730 (99.88)	1,726 (99.65)	

Ever	2 (0.12)	6 (0.35)	
IUD use, n (%)			0.42
Never	1,707 (98.56)	1,701 (98.21)	
Ever	25 (1.44)	31 (1.79)	

Abbreviations: SD, standard deviation; HRT, hormone replacement therapy; IUD, intrauterine device

^aAmong parous

^bControl subjects with age at menopause less than their age at censor date were classified as postmenopausal. Case subjects with age at menopause less than their age at diagnosis were classified as postmenopausal. Case subjects with age of menopause equivalent to their age at diagnosis due to cancer-related surgery were classified as premenopausal.

Table 14. Characteristics of users of various contraceptive methods with a *BRCA1* or *BRCA2* mutation

Characteristic	Any IUD (n=56)	Hormonal IUD (n=13, 23%) ^a	Nonhormonal IUD (n=35) ^a	Implant (n=25)	Injection (n=8)	Oral contraceptive (n=1,705)
Year of birth, mean (range)	1957 (1941-1983)	1967 (1956-1983)	1953 (1941-1972)	1955 (1945-1972)	1962 (1955-1973)	1953 (1920-1987)
Recency of use, n (%)						
Current or recent	14 (25)	9 (69.23)	4 (11.43)	2 (8)	0 (0)	51 (10.30)
Former	42 (75)	4 (30.77)	31 (88.57)	23 (92)	8 (100)	444 (89.70)
Years of use, mean (range)	6.85 (0.08-24) ^b	4.85 (0.08-19)	8.00 (1-24) ^c	6.67 (1-20) ^h	3.77 (0.42-19) ⁱ	7.41 (0.08-32) ^j
Age at last use, mean (range)	36.98 (20-52) ^c	39.00 (26-49)	35.85 (20-52) ^g	35.75 (22-48) ^h	33.75 (24-44)	31.04 (6-61) ^l
Age at first use, mean (range)	30.53 (19-50) ^d	34.15 (20-48)	28.50 (19-42) ^f	29.12 (20-42) ^h	29.25 (22-37)	23.60 (6-53) ^k

Abbreviations: IUD, intrauterine device.

^aIUD users who reported use of both hormonal and nonhormonal IUDs (n=1). IUD users who were missing IUD type (n=9).

^bIUD users excluded due to missing start year or stop year (n=4).

^cIUD users excluded due to missing stop year (n=3).

^dIUD users excluded due to missing start year (n=3).

^eIUD users excluded due to missing start year or stop year (n=2).

^fIUD users excluded due to missing start year (n=1).

^gIUD users excluded due to missing stop year (n=1).

^hImplant users excluded due to missing start and stop year (n=1).

ⁱInjection users excluded due to missing duration of use (n=1).

^jOral contraceptive users excluded due to missing duration of use (n=23).

^kOral contraceptive users excluded due to missing start and stop year (n=16).

^lOral contraceptive users excluded due to missing stop and/or stop year (n=19)

Table 15. Associations between use of various contraceptive methods and ovarian cancer risk among women with *BRCA1* or *BRCA2* mutation, stratified by menopausal status.

Variable	Cases/controls	Univariate OR (95% CI)	P	Multivariate OR (95% CI)	P
<u>Overall</u>					
Any contraceptive					
Never ^a	931/785	Ref		Ref	
Ever	801/947	0.62 (0.53-0.73)	<0.0001	0.58 (0.48-0.70) ^b	<0.0001
Oral contraceptive					
Never	951/808	Ref		Ref	
Ever	781/924	0.62 (0.52-0.73)	<0.0001	0.59 (0.49-0.71) ^b	<0.0001
Implant					
Never	1726/1713	Ref		Ref	
Ever	6/19	0.32 (0.13-0.79)	0.01	0.38 (0.15-0.97) ^c	0.04
Injection					
Never	1730/1726	Ref		Ref	
Ever	2/6	0.33 (0.07-1.65)	0.18	0.32 (0.06-1.66) ^c	0.18
IUD use					
Never	1707/1701	Ref		Ref	
Ever	25/31	0.81 (0.48-1.37)	0.42	0.81 (0.46-1.45) ^c	0.48
Type of IUD					
Never	1707/1701	Ref		Ref	
Hormonal	6/7	0.86 (0.29-2.55)	0.78	0.91 (0.28-2.91) ^c	0.87
Non-hormonal	14/21	0.67 (0.34-1.31)	0.24	0.66 (0.31-1.39) ^c	0.27
<u>Premenopausal</u>					
Any contraceptive					
Never ^a	423/368	Ref		Ref	
Ever	432/532	0.63 (0.49-0.81)	0.0002	0.57 (0.43-0.75) ^b	<0.0001
Oral contraceptive					
Never	432/380	Ref		Ref	
Ever	423/520	0.63 (0.49-0.81)	0.0003	0.57 (0.43-0.75) ^b	<0.0001
Implant					
Never	851/891	Ref		Ref	
Ever	4/9	0.43 (0.11-1.66)	0.22	0.60 (0.15-2.37) ^c	0.47
Injection					
Never	854/895	Ref		Ref	
Ever	1/5	0.25 (0.03-2.24)	0.22	0.24 (0.03-2.18) ^c	0.20
IUD use					
Never	840/890	Ref		Ref	
Ever	15/10	1.50 (0.67-3.34)	0.32	1.53 (0.65-3.60) ^c	0.33
<u>Postmenopausal</u>					
Any contraceptive					
Never ^a	508/417	Ref		Ref	
Ever	369/415	0.63 (0.48-0.83)	0.0010	0.57 (0.42-0.79) ^b	0.0005
Oral contraceptive					
Never	519/428	Ref		Ref	
Ever	358/404	0.62 (0.47-0.81)	0.0006	0.57 (0.41-0.79) ^b	0.0007
Implant					
Never	875/822	Ref		Ref	
Ever	2/10	0.33 (0.07-1.65)	0.18	0.44 (0.09-2.24) ^c	0.33

Injection						
Never	876/831	Ref			Ref	
Ever	1/1	0.84 (0.05-13.50) ^d	0.90		0.89 (0.05-15.30) ^e	0.94
IUD use						
Never	867/811	Ref			Ref	
Ever	10/21	0.60 (0.26-1.37)	0.23		0.59 (0.23-1.46) ^c	0.25

Abbreviations: IUD, intrauterine device; CI, confidence interval.

^aReferent group includes women who never reported use of any hormonal contraceptive.

^bAdjusted for history of breastfeeding (ever/never), menopausal status (premenopausal/ postmenopausal), parity (ever/never).

^cAdjusted for previous oral contraceptive use (ever/never), history of breastfeeding (ever/never), menopausal status (premenopausal/postmenopausal), parity (ever/never).

^dAnalyzed by unconditional logistic regression due to insufficient data. Adjusted for age (continuous)

^eAnalyzed by unconditional logistic regression due to insufficient data. Adjusted for history of breastfeeding (ever/never), menopausal status (premenopausal/postmenopausal), parity (ever/never), age (continuous), year of baseline questionnaire (continuous), country of residence (United States, Canada, Poland, Other), history of breast cancer (ever/never), *BRCA* mutation type (*BRCA1/BRCA2*)

Table 16. Temporal associations between IUD use and ovarian cancer risk among *BRCA1* or *BRCA2* mutation carriers

Variable	Cases/controls	Univariate OR (95% CI)	P	Multivariate OR (95% CI) ^a	P
Duration of IUD use ^b					
Never ^c	1707/1701	Ref		Ref	
< 5 years	9/13	0.69 (0.30-1.62)	0.40	0.75 (0.31-1.82)	0.52
≥ 5 years	15/15	1.00 (0.49-2.05)	1.00	0.91 (0.41-2.03)	0.82
p-trend		0.98 (0.92-1.04)	0.44	0.97 (0.91-1.04)	0.41
Recency of IUD use					
Never	1707/1701	Ref		Ref	
Current or recent	4/10	0.40 (0.12-1.28)	0.12	0.38 (0.10-1.41)	0.15
Former	21/21	1.00 (0.55-1.83)	1.00	1.00 (0.53-1.97)	0.96
Age at first IUD use ^d					
Never	1707/1701	Ref		Ref	
≤ 29 years	14/14	1.00 (0.48-2.10)	1.00	0.89 (0.40-1.98)	0.77
> 29 years	10/15	0.67 (0.30-1.48)	0.32	0.73 (0.30-1.76)	0.48
Age at last IUD use ^e					
Never	1707/1701	Ref		Ref	
< 37 years	14/15	0.93 (0.45-1.93)	0.85	0.92 (0.42-1.98)	0.82
≥ 37 years	11/16	0.69 (0.32-1.48)	0.34	0.70 (0.29-1.67)	0.42

Abbreviations: IUD, intrauterine device; CI, confidence interval.

^aAdjusted for previous oral contraceptive use (ever/never), history of breastfeeding (ever/never), menopausal status (premenopausal/postmenopausal), parity (ever/never).

^bIUD users excluded due to missing start year or stop year (n=4).

^cReferent group includes women who never reported use of any IUD.

^dIUD users excluded due to missing start year (n=3).

^eIUD users excluded due to missing stop year (n=3).

Table 17. Associations between use of various LARC methods and ovarian cancer risk among women with *BRCA1* or *BRCA2* mutation, stratified by history of oral contraceptive use

Variable	Cases/controls	Univariate OR (95% CI)	<i>P</i>	Multivariate OR ^a (95% CI)	<i>P</i>
<u>No history of oral contraceptive use</u>					
Implant					
Never	950/798	1.00	Ref	1.00	Ref
Ever	1/10	0.11 (0.01-0.88)	0.04	0.10 (0.01-0.80)	0.03
Injection					
Never	950/805	1.00	Ref	1.00	Ref
Ever	1/3	0.27 (0.03-2.63) ^b	0.26	0.23 (0.02-2.26) ^c	0.21
IUD use					
Never	946/800	1.00	Ref	1.00	Ref
Ever	5/8	0.71 (0.23-2.25)	0.57	0.42 (0.11-1.61)	0.20
<u>History of oral contraceptive use</u>					
Implant					
Never	776/915	1.00	Ref	1.00	Ref
Ever	5/9	0.66 (0.22-1.96) ^b	0.45	0.60 (0.20-1.83) ^c	0.37
Injection					
Never	780/921	1.00		1.00	Ref
Ever	1/3	0.39 (0.04-3.80) ^b	0.42	0.44 (0.05-4.31) ^c	0.48
IUD use					
Never	761/901	1.00	Ref	1.00	Ref
Ever	20/23	1.56 (0.67-3.59)	0.30	1.43 (0.59-3.45)	0.42

Abbreviations: IUD, intrauterine device; CI, confidence interval.

^aAdjusted for history of breastfeeding (ever/never), menopausal status (premenopausal/postmenopausal), parity (ever/never)

^bAnalyzed by unconditional logistic regression due to insufficient data. Adjusted for age (continuous)

^cAnalyzed by unconditional logistic regression due to insufficient data. Adjusted for history of breastfeeding (ever/never), menopausal status (premenopausal/postmenopausal), parity (ever/never), age (continuous), year of baseline questionnaire (continuous), country of residence (United States, Canada, Poland, Other), history of breast cancer (ever/never), *BRCA* mutation type (*BRCA1/BRCA2*)

Chapter 5. Discussion

5.1. General Discussion

Trends in contraceptive use across birth cohorts for *BRCA1* mutation carriers largely parallels that of *BRCA2* mutation carriers, despite fluctuations among the fewer women with a *BRCA2* mutation. The lifetime rates of oral contraceptive use did not vary between birth cohorts and remained high among all women born after 1920; however, use of LARCs, including implants, IUDs and injections increased successively across birth cohorts. To our knowledge, this is the first study to investigate trends in method-specific contraceptive use among *BRCA* mutation carriers by birth cohort.

These patterns are consistent with findings from nationally representative cross-sectional studies of the United States, Canada and Europe that report significant increases in the use of LARC methods over time. From 2006 to 2017, the annual increase in IUD and implant use was 6.2% and 12.4% respectively among premenopausal women in the United States, while oral contraceptive use decreased by 2% annually²⁴⁵. During the same period, rates of IUD use increased significantly from 4.3% to 8.8% among Canadian women aged 15 to 50²⁴⁶. Data from the National Health Service indicate a similar trend of increasing LARC uptake from 26% in 2009 to 46% in 2019, while oral contraceptives declined from 45% in 2014 to 38% in 2019²⁴⁷.

Of note, the low prevalence of lifetime IUD use in the 1960-1969 birth cohort corresponds to a rapid decline in the popularity of IUDs in the 1990s. By 1995, IUDs were used by less than 1% of the United States population due to controversies of the Dalkon Shield and related complications of pelvic inflammatory disease and infertility²⁴⁸. The introduction of hormonal IUDs in North America by the early 2000s prompted a resurgence of IUD use in the ensuing decades. In an analysis of the 2012 National Surveys of Family Growth data, Mosher *et al.* reported that the highest proportion of IUD users were aged 25 to 34²⁴⁹. This corresponds to the 1970-1979 and 1980-1989 birth cohorts that saw an increase in rates of IUD use in our study.

Implants and injections played a minor role in the overall use of hormonal contraception in our cohort. Nonetheless, results suggest that the highest proportion of injection and implant use

occurred in the 1970-1979 birth cohort, which aligns with the timing of approval of these methods in Canada and the United States^{165,250}. Women born between 1970 and 1979 would be of reproductive age during the 1990s and ideal candidates for the introduction of these modes of contraception.

As the landscape of contraceptive usage changes, it is of interest to evaluate the relationships between various types of contraception and ovarian cancer risk in women with *BRCA* mutations who possess increased predisposition for developing ovarian cancer. Oral contraceptives represent an established protective factor among high-risk women, but the potential benefit of other contraceptive methods is not clear. As such, the goal of the present study was to investigate the associations between different modes of contraception and the risk of developing ovarian cancer among women with an inherited *BRCA1* or *BRCA2* mutation

Results from our case-control study of 1,732 finely matched pairs suggest a significantly reduced risk of ovarian cancer with use of any contraceptive method, which was driven by the significant inverse association with use of implants or oral contraceptives. There was suggestion of a reduced risk with use of contraceptive injections and IUDs, although this was based on a small number of exposed cases and confidence intervals were wide. To our knowledge, this represents the first report of the association between contemporary contraceptive methods and the risk of ovarian cancer in high-risk women.

We showed an inverse but nonsignificant association with use of any type of IUD compared to never use among carriers. Consistent with our results, a recent meta-analysis of nine case-control and two cohort studies observed an inverse association between ever use of an IUD and ovarian cancer risk in the general population (OR=0.68; 95% CI 0.62-0.75)²²⁹. We further examined risk of ovarian cancer by types of IUD and detailed patterns of IUD use. Although not statistically significant, we observed cancer-protective effects with use of both hormonal and nonhormonal IUD types. Several population-based cohort studies have corroborated these findings, demonstrating significant decreases in risk of ovarian cancer for users of LNG IUDs^{231,232}. These studies were combined in a meta-analysis by Balayla *et al.* that reported a standardized incidence ratio of 0.58 (95% CI 0.47-0.71) for use of the LNG IUD specifically²³⁰.

As rates of hormonal IUD use now surpass that of nonhormonal devices, future epidemiologic studies are needed to confirm the consistency and magnitude of risk reductions associated with this IUD type²⁴⁵.

With respect to nonhormonal IUDs, a recent pooled analysis of data from the New England Case-Control Study and Nurses' Health Studies reported no association between IUD use and ovarian cancer risk, wherein the majority of IUD use was attributed to nonhormonal methods²³³. Conversely, findings from the Shanghai Women's Health Study indicated that long-term IUD use (≥ 20 years) was associated with reduced ovarian cancer risk in China, where the high prevalence of IUD use was dominated by the stainless steel ring²²⁷. Although the average duration of nonhormonal IUD use in our cohort was only eight years, our results agree with that of the latter report and suggest a nonsignificant decrease in risk of ovarian cancer with use of the nonhormonal IUD in *BRCA* mutation carriers.

Interestingly, Tworoger and colleagues observed a significantly elevated risk of ovarian cancer with IUD use in the Nurses' Health Study, which was attributed to possible IUD-related inflammation in the peritoneal cavity (RR=1.76; 95% CI 1.08-2.85)²³⁴. Both hormonal and nonhormonal IUDs elicit a foreign body reaction, a local sterile inflammatory response characterized by the influx of immune cells to the intrauterine environment²⁵¹. The cellular and humoral components of the foreign body reaction extend to the extrauterine space and represent a mechanism by which IUDs promote the elimination of premalignant cells at the ovary and fallopian tube²⁰⁴. Nonetheless, inflammatory factors are also associated with ovarian carcinogenesis by generating genotoxic stress⁷⁰. One hypothesis to reconcile these contradicting theories considers the timing of IUD use²⁵². IUDs stimulate an inflammatory milieu that may be more protective for older women, who are more likely to have accumulated a greater number of precancerous cells than younger women. This hypothesis is consistent with our finding of an inverse (albeit nonsignificant) trend with increasing age at last IUD use.

Few studies in the general population have assessed the relationship between duration of IUD use and ovarian cancer risk to mixed results. Huang and colleagues found that long-term IUD use of at least 20 years was associated with a 38% reduction in risk (HR= 0.62; 95% CI 0.40-

0.97)²²⁸. Ness and colleagues observed the opposite relationship, such that a shorter duration of IUD use (≤ 4 years) was associated with reduced risk of ovarian cancer (OR=0.53; 95% CI 0.39-0.72) and longer duration of IUD use (≥ 10 years) trended toward increased risk (OR=1.40; 95% CI 0.82-2.39)²⁵³. In the present study, protection against ovarian cancer was not associated with duration of use in high-risk women, but only 52 IUD users informed the analysis.

Our finding of a 62% to 68% reduction in ovarian cancer risk with use of contraceptive implants or injections was based on a total of eight exposed cases; however, these results are consistent with the one other study that estimated risk of ovarian cancer among implant users in the general population²²⁷. The authors reported a nonsignificant inverse association between implant use and ovarian cancer risk compared to never users (RR=0.51; 95% CI 0.07-3.64). Any protective effect may have been underestimated because history of contraceptive use prior to study entry was not collected. Regarding contraceptive injections, a recent pooled analysis of seven case-control studies in the Ovarian Cancer Association Consortium concluded a reduced risk of ovarian cancer with use of DMPA injections, which is in line with the majority of other retrospective data^{224–226}. The few cohort studies that explored injection use and ovarian cancer risk have showed inconsistent results. In the Shanghai Women's Health Study of 70,259 Chinese women and 174 cases, injectables were not associated with ovarian cancer risk²²⁸. On the other hand, the Danish Sex Register Hormone Study observed increased risk with DMPA shots in a subgroup analysis of exclusive use until first switch (RR=6.56; 95% CI 6.56-20.40)²²⁷. Both studies were limited by a low prevalence of injection users and imprecise risk estimates.

Oral contraceptive use is an established protective factor for ovarian cancer in the general and carrier population alike^{116,210,213,254}. In the current study, we found that oral contraceptive use was associated with a 41% decreased risk of developing ovarian cancer among *BRCA* mutation carriers. This result is in agreement with our two earlier reports on this topic, which were based on a subset of carriers also included in the current investigation^{79,244}. Findings from the most recent analysis indicated a similar level of risk reduction with oral contraceptive use for *BRCA1* mutation carriers (OR=0.60; 95% CI 0.50-0.71) and *BRCA2* mutation carriers (OR=0.63; 95% CI 0.39-0.80)⁷⁹. Compared to our previous publications, the results here include an additional 403 cases and represent the most updated analysis of oral contraceptive use and ovarian cancer

risk among women with a *BRCA* mutation. The protective effects of oral contraceptives have been observed across all tumour histologic subtypes, but there is some evidence to suggest that the associations between oral contraceptive use may be stronger for serous and clear cell ovarian cancers^{120,255,256}. Although we were not able to stratify by histotype, *BRCA*-associated ovarian cancers tend to be of endometrioid or high-grade serous subtype¹⁴.

Several mechanisms may explain the inverse relationships between various contraceptive methods and ovarian cancer risk. According to the ‘incessant ovulation’ hypothesis, the proliferative behaviour of the ovarian surface epithelium and the acute inflammatory environment at the distal fallopian tube following ovulation may predispose cells to DNA damage and malignant transformation^{62,65}. Under this paradigm, ovulation inhibition via suppression of pituitary gonadotropins may be the primary biological mechanism by which oral contraceptives and injections impart protection against the development of ovarian cancer; however, the protective effects of these methods varied by magnitude, which implies additional mechanisms at play.

Alternate theories highlight a potential protective effect of high progestogen exposure⁸². In experimental studies, progestogens have exhibited pro-apoptotic and antiproliferative effects on transformed cells of the ovary as well as a necroptotic effect on abnormal cells of the fallopian tube epithelium^{92,93,257,258}. Likewise, epidemiologic data reveal protective associations with pregnancy and breastfeeding, physiologic states characterized by elevated levels of progesterone⁷⁹. Use of oral contraceptives, implants, IUDs and injections that contain highly potent synthetic progestins reinforce this theory by increasing exogenous progestogenic exposure compared to levels experienced by regularly cycling women²⁵⁹. This mechanism may be especially relevant among Mirena IUD users, for whom LNG has been measured at significant levels in the peritoneal fluid²⁶⁰. Further, the 68% risk reduction observed with injection use in our population is consistent with the high serum concentrations of DMPA attained, five-fold that of equivalent oral contraceptive use²²⁴. On the other hand, the majority of implant users in our cohort likely used LNG implants that do not consistently inhibit ovulation and sustain low serum concentrations of LNG, 25% to 50% that of oral contraceptives¹³⁸. Although LNG is a highly

potent progestin, the mechanism by which implants confer significant protection against ovarian cancer requires further investigation.

Lastly, inhibition of retrograde menstruation may explain the protective effect observed with hormonal IUD use. Hormonal IUDs are associated with sparse menstruations or amenorrhea in over 20% of users²⁶¹. This is thought to reduce retrograde flow of menstrual blood, thereby minimizing local inflammation in the peritoneal cavity and malignant transformation at the site of the ovarian surface epithelium and distal fallopian tube⁷². Retrograde transport of endometrial cells can further implant as endometriosis, which has been linked to ovarian cancers of endometrioid, clear cell and low-grade serous histology⁷⁷. Although data on tumour histology was not available in the present study, approximately 12% of *BRCA*-associated ovarian cancers have an endometrioid histotype¹⁴. Other studies in the general population have shown that women who use a LNG IUD have a lower risk of ovarian carcinomas of endometrial origin, supporting a partial preventive effect through of inhibition of menstruation^{79,232}.

5.2. Strengths and Limitations

This represents the first study to investigate the effects of different contraceptive methods on ovarian cancer risk in women with *BRCA1* or *BRCA2* mutation. Although only very small proportions of women used methods other than oral contraceptives, our analysis was strengthened by the large number of mutation carriers and the strict matching criteria to account for differences between cases and controls. We also obtained information about contraceptive use at baseline and biennially through follow-up questionnaires. Therefore, we were able to account for changes in exposure status, including method switches, cease of use or initiation of use during the follow-up period.

We also acknowledge important limitations to this research. First, given the small number of women who used implants, injections and IUDs, use of progestin-only contraceptives generated imprecise estimates and associations must be interpreted with caution. As a result, we had limited power to examine associations by *BRCA* mutation type, ovarian cancer histologic subtype or site of origin. Nonetheless, there has not been significant variability by histology in prior analyses of injectables or IUDs, and previous studies of oral contraceptive use among high-risk women have reported similar protective effects in carriers of either mutation type^{79,124,224,232,239}. Secondly, there were too few women who reported use of a transdermal patch or vaginal ring to analyze these methods separately. When these women were excluded, the results did not change substantially. Thirdly, we were not sufficiently powered in our analysis stratified by history of oral contraceptive use and additional studies are necessary to confirm if the other modes of contraceptives independently impact risk. Fourth, it is important to note that the results of this study apply exclusively to premenopausal hormone use. The LNG IUD is additionally indicated for endometrial protection in HRT regimens. Further study is necessary to determine if postmenopausal IUD use provides ovarian cancer risk reduction for carriers who undergo natural menopause. Furthermore, hormonal IUDs were first launched in Finland in 1990 and only approved in North America in the early 2000s¹⁸⁰. More follow-up time may be required to capture changes in the direction or magnitude of association, considering the long latency period of ovarian cancer. Exposure misclassification may have affected our results because this analysis was based in self-reported contraceptive data. Despite this, previous studies have demonstrated fairly accurate recall of contraceptive history obtained through self-

administered questionnaires²⁶². Survivorship bias may influence our findings because prevalent cases comprised 87% of ovarian cancer cases included in our study, with an average of four years elapsed between the time of diagnosis to enrollment. Therefore, our results may be less representative for women with aggressive cases of ovarian cancer who died before enrolment. Nonetheless, the characteristics of the prevalent cases were similar to that of the incident cases (data not shown). Lastly, although there was a significant difference in the proportion of cases and controls who used hormone replacement therapy (21% vs. 13%; $P<0.0001$), we have previously demonstrated that hormone replacement therapy does not influence the risk of ovarian cancer in this cohort¹²⁷.

5.3. Future Directions and Conclusion

Concerns have been raised regarding the progestin content of hormonal contraception as a possible risk factor for breast cancer. We previously reported an increased risk of breast cancer with oral contraceptive use at an early age in *BRCA1* mutation carriers²⁶³. Moreover, we found a possible increased risk of breast cancer with estrogen-progesterone hormone replacement therapy among women with a *BRCA1* or *BRCA2* mutation, but not for estrogen therapy alone²⁶⁴. This is in line with an emerging role for the progesterone-mediated receptor activator of nuclear factor κ B (RANK) signalling pathway in *BRCA1* breast carcinogenesis^{265–267}. Shamseddin *et al.* used *ex vivo* and xenograft models to demonstrate that androgenic progestins including LNG stimulate proliferation of breast epithelial cells via upregulation of the RANK ligand pathway²⁶⁸. These findings are also consistent with the epidemiologic association between hormonal IUD use and breast cancer risk. A recent meta-analysis of five case-control studies and two cohort studies concluded that women who use the LNG IUD have a 16% increased risk of breast cancer (OR=1.16; 95% CI 1.06-1.28)²⁶⁹. Given growing uptake of the hormonal IUD, the role of IUD use on the risk of *BRCA1*-associated breast cancer would be an important avenue of investigation.

For premenopausal women electing for permanent contraception, it is important to acknowledge surgical sterilization methods with potential ovarian cancer-protective effects. Tubal ligation has been associated with lower risk of developing ovarian cancer in previous studies of high-risk women; however, no relationship was observed in the most recent cohort study of 5,038 *BRCA* mutation carriers (HR=1.09; 95% CI 0.83-1.45)^{73,76,244,270,271}. Bilateral salpingectomy with delayed oophorectomy is also the subject of ongoing clinical trials to determine the effect on ovarian cancer incidence. Although beyond the scope of this project, tubal ligation and salpingectomy are gaining attention as highly effective contraceptive methods with potential roles in ovarian cancer prevention for high-risk women.

In conclusion, our findings confirm the well-established protective role of oral contraceptive use on the risk of developing ovarian cancer specifically among women with a *BRCA1* or *BRCA2* mutation. We also demonstrated a decreased risk with implant use and a potentially protective role of IUDs and injections. In agreement with our previous recommendations, oral

contraceptives should be discussed as an effective contraceptive option and a likely cancer-protective factor during the risk assessment and counseling of this high risk population⁷⁹. At this time, injections, implants and IUDs should not be contraindicated for *BRCA* mutation carriers who are considering these highly effective methods of contraception, although whether the level of risk reduction is equivalent to combined oral contraceptives warrants additional study. As non-oral, LARC methods continue to gain popularity, studies with larger numbers of users will further clarify the influence of these contraceptive methods on ovarian cancer development. It will be of interest to evaluate the differential impact of contraceptives on risk of cancers originating in the ovaries versus the fallopian tubes and whether contraceptives may improve survival outcomes for *BRCA* mutation carriers.

Chapter 6. References

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Appendices

Appendix 1. Research Questionnaire for the Risk Factor Analysis of Breast and Ovarian Cancer

RESEARCH QUESTIONNAIRE FOR A STUDY OF BREAST AND OVARIAN CANCER IN HIGH RISK FAMILIES

This questionnaire is part of a research study to improve our understanding of the prevention and treatment of hereditary breast and ovarian cancers.

Family/Individual #: _____

Date of Birth: ____/____/____ **Age:** ____
mm yyyy

Date Completed: ____/____/____
mm dd yyyy

SECTION I –Reproductive History

1. Have you ever been pregnant?

☐ **No** → **Go to question 5.**

☐ **Yes**

2. Please consider all pregnancies in order from first to last. Give year of pregnancy. Indicate the outcome of each pregnancy. For live-born children, try to recall how long you breast-fed each child and indicate months of breast-feeding (if not breast-fed, enter '0').

PREGNANCY							
#	Year of Pregnancy	Length of Pregnancy (In Weeks)	Pregnancy Outcome (Live, Still, Miscarriage, Abortion, Twins, etc.)	Live Birth Caesarean Section? (Yes, No)	Live Born (Month/Year of Birth)	Birth Weight (lbs.)	Months of Breast-Feeding
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

3. Did you ever have difficulty breast-feeding?

☐ **No**

☐ **Yes** → Reason: ___ poor milk production ___ pain
 ___ premature infant ___ mastitis (breast inflammation)
 ___ other (please specify): _____

Circle which child (1-10 in chart above) you experienced this with:

1 2 3 4 5 6 7 8 9 10

4. Have you ever taken medication to stop milk production?

☐ **No**

☐ **Yes** → Name of medication (if known): _____

Method: ___ injections or ___ pills

Circle with which child (1-10 in preceding chart) you took this medication.

1 2 3 4 5 6 7 8 9 10

5. How old were you when you had your first menstrual period? _____ YEARS OLD
6. How would you describe your menstrual cycle? By regular we mean that the start of your period was predictable within 5 days. (*Please check one*)
- ☐ **My periods are/were always regular.**
- ☐ **My periods are/were usually regular.**
- ☐ **My periods are/were never regular.**
7. How many days apart are your menstrual periods? That is, from the start of one period to the start of the next period. For example, many women have cycles of 28 days. (*Please check one*)
- ___ 28 days ___ 29 days ___ 30 days ___ 31 days ___ 32 days **Other**→ ___ days
8. Do you currently have menstrual periods? That is, have you had a menstrual period within the last year? Please answer “Yes” if you are currently pregnant, breast-feeding or taking hormones which temporarily stop your menses (Mirena IUD or birth control pills).
- ☐ **No**
- ☐ **Yes**
9. Have your periods stopped completely?
- ☐ **No** → **Go to question 12.**
- ☐ **Yes**
10. How old were you when your periods stopped completely? _____ YEARS OLD
11. What was the reason your periods stopped? (*Select one only*)
- ☐ **Natural Menopause** (change of life)
- ☐ **Hysterectomy** (uterus removed only, ovaries not removed)
- ☐ **One ovary removed only**
- ☐ **Both ovaries removed only**
- ☐ **Fallopian tubes and ovaries removed**
- ☐ **Uterus, fallopian tubes, and ovaries removed**
- ☐ **Medication / Chemotherapy**
- ☐ **Other** (please specify): _____
12. Have you ever seen a doctor for a problem of difficulty in getting pregnant or in carrying a pregnancy, such as several miscarriages?
- ☐ **No** → **Go to question 15.**
- ☐ **Yes** → What reason did the doctor give to explain why you had trouble getting or staying pregnant? (*Please check all that apply.*)
___ no problem was found	___ problem with cervix
___ problem with ovaries	___ partner has fertility problem
___ problem with fallopian tubes	___ endometriosis
___ other (<i>please specify</i>): _____	

13. Have you ever taken medication to increase your chances of becoming pregnant?

☐ **No**

☐ **Yes** → Name of medication(s): _____

For how many months did you take this medication? _____ months

What years did you take this medication? ____ __ __ __, ____ __ __ __

14. Have you ever received fertility treatment such as *in vitro* fertilization/Embryo Transfer (IVF/ET) to help you get pregnant?

☐ **No**

☐ **Yes** → What type of treatment did you receive? _____

15a. Have you ever used birth control pills, Norplant (implants), Mirena/IUD, or Depo-Provera (injections) to prevent pregnancy or for any other reason?

☐ **No** → **Go to question 16.**

☐ **Yes** → Can you describe the times?

	Method	Name of Medication	Starting Year	Ending Year	Length of Time Used in Years
1	☐ Pills ☐ Implants ☐ Mirena/IUD ☐ Injections ☐ Other				
2	☐ Pills ☐ Implants ☐ Mirena/IUD ☐ Injections ☐ Other				
3	☐ Pills ☐ Implants ☐ Mirena/IUD ☐ Injections ☐ Other				
4	☐ Pills ☐ Implants ☐ Mirena/IUD ☐ Injections ☐ Other				

15b. Are you currently using birth control pills, Norplant (implants), Mirena/IUD, or Depo-Provera (injections)?

☐ **No**

☐ **Yes**

16a. Have you ever taken hormone replacement therapy for menopause (i.e. estrogen, progesterone)?

☐ **No** → Go to question 17.

☐ **Yes** → Complete table below:

	Method	Name of Medication	Starting Year	Ending Year	Length of Time Used in Years
1	☐ Pills ☐ Skin Patches ☐ Vaginal Suppositories ☐ Gel ☐ Cream ☐ Ring ☐ Injections ☐ Other				
2	☐ Pills ☐ Skin Patches ☐ Vaginal Suppositories ☐ Gel ☐ Cream ☐ Ring ☐ Injections ☐ Other				
3	☐ Pills ☐ Skin Patches ☐ Vaginal Suppositories ☐ Gel ☐ Cream ☐ Ring ☐ Injections ☐ Other				
4	☐ Pills ☐ Skin Patches ☐ Vaginal Suppositories ☐ Gel ☐ Cream ☐ Ring ☐ Injections ☐ Other				

16b. Are you currently taking hormone replacement therapy?

☐ **No**

☐ **Yes** → Name of medication: _____ dose? _____

SECTION II – Breast Cancer Screening/Prevention

17. Have you ever taken any drugs for the prevention of breast cancer (i.e. drugs taken before any diagnosis of breast cancer)?

☐ **No**

☐ **I'm not sure**

☐ **Yes** → Name of Drug: ____ Tamoxifen (Nolvadex) ____ Raloxifene (Evista)
 ____ Aromasin (Exemestane) ____ Femara (Letrozole)
 ____ Arimidex (Anastrozole)
 ____ Other (please name): _____

Number of pills per day: _____

Date started: ____ / ____ / ____ (mm / yyyy) to ____ / ____ / ____ (mm / yyyy)

18. Approximately how many mammograms have you had in your lifetime?
 I have had _____ mammograms. If you do not recall the exact number, please provide a range.
 Age at first mammogram? _____ years old.
 Age at last mammogram? _____ years old.
19. Have you ever had any abnormalities detected by mammogram?
☐ **No**
☐ **Yes** → Please describe the abnormality: _____
 Month/Year of abnormality: ____ / ____
20. Have you ever had MRI screening of your breasts?
☐ **No**
☐ **Yes** → Age at first MRI: _____
 Month/Year of first MRI: ____ / ____
 How many MRIs have you had? _____
 Where were they done: _____
 Were there any abnormalities detected by MRI? ☐ **Yes** ☐ **No**
If yes, please describe: _____
 Year of abnormality: ____
 Month/Year of your last MRI: ____ / ____
21. Have you ever had a breast biopsy?
☐ **No**
☐ **Yes** → Number of biopsies: _____
 Month/Year of last biopsy: ____ / ____
 What was the result of the biopsy? (*Check all that apply*)
 ____ normal ____ DCIS ____ atypical hyperplasia
 ____ cancer ____ LCIS ____ fibroadenoma
 ____ other (please specify): _____
22. Have you undergone surgery at any time in order to prevent breast cancer (e.g. preventive removal of the breasts)? **NOTE: The surgery is only preventive if you've never previously been diagnosed with cancer in that breast.**
☐ **No**
☐ **Yes** → What year? ____ Month (if known)? ____
 Which breast? ____ **Left** ____ **Right** ____ **Both**
 Procedure: ____ **Total mastectomy** (nipple and areola removed)
 ____ **Subcutaneous mastectomy** (nipple and areola preserved)
23. Have you ever had breast implants or breast reconstruction?
☐ **No**
☐ **Yes** → Type: ____ Saline ____ Silicone ____ TRAM-flap
 ____ Other _____
 Year of surgery: ____
24. Have you ever had breast reduction?
☐ **No**
☐ **Yes** → Year of surgery: ____

SECTION III – Breast Cancer Diagnosis

25. Have you **ever** been diagnosed with breast cancer?

- ☐ **No** → Go to question 39 (Section IV).
☐ **Yes**

Check one → I have had cancer in ☐ one breast
OR ☐ both breasts

We would like to discuss your **first** diagnosis.

What year was it? ____ ____ ____ Month (if known)? _____

Which breast? ☐ **Left** ☐ **Right**

Type of Cancer (check one):

- ☐ It was an invasive cancer.
☐ It was a non-invasive cancer (early stage breast cancer).
→ Was it Ductal Carcinoma In-situ (DCIS)? ☐ **Yes** ☐ **No**
→ Was it Lobular Carinoma In-situ (LCIS)? ☐ **Yes** ☐ **No**
☐ It was both invasive and non-invasive.
☐ I'm not sure.

Treatment

Did you have surgery for the **first** breast cancer?

- ☐ **No**
☐ **Yes** → Year of surgery: ____ ____ ____ Month (if known)? _____

What type of surgery did you have?

Check one → ____ **Lumpectomy** (tumour removed, breast remains)
____ **Mastectomy** (one breast removed)
____ **Bilateral mastectomy** (both breasts removed)

Detection

How was the lump first detected?

- Check one → ____ Only by mammography screening
____ Did you feel a lump?
____ Did the doctor feel a lump?
____ MRI screening
____ Other: _____

26. Did you receive chemotherapy for the first breast cancer?

- ☐ **No**
☐ **Yes**

27. Did you receive radiation therapy for the first breast cancer?

- ☐ **No**
☐ **Yes**

28. Did you receive Tamoxifen (Nolvadex) for the first breast cancer?

- ☐ **No**
☐ **Yes** → Number of pills per day: _____
 From: ____ ____ ____ (year) to ____ ____ ____ (year)

29. Did you receive any other drugs for the first breast cancer?

- ☐ **No**
☐ **Yes** → Which drug? ☐ Femara (letrozole)
☐ Aromasin (exemestane)
☐ Arimidex (anastrozole)
☐ Other: _____
 Number of pills per day: _____
 From: ____ ____ ____ (year) to ____ ____ ____ (year)

30. Has your breast cancer come back in the same breast (local recurrence) or spread elsewhere (distant recurrence/metastasis)?

- ☐ **No**
☐ **Yes** → Date: ____ / ____ ____ (mm / yyyy)
 Site of recurrence: _____
 Treatment for recurrence: _____

31. Have you had further surgery in the affected breast (such as complete mastectomy or reconstruction)?

- ☐ **No**
☐ **Yes** → Date: ____ / ____ ____ (mm / yyyy)
 Type of surgery: _____

**IF YOU DID NOT HAVE A SECOND BREAST CANCER,
GO TO SECTION IV QUESTION 39.**

32. We would like to discuss your **second** diagnosis (This does NOT include a recurrence, i.e. a cancer in the same breast as the previous breast cancer).

What year was it? ____ ____ ____ Month (if known)? _____
 Which breast? ☐ **Left** ☐ **Right**

Type of Cancer (check all that apply):

- ☐ It was an invasive cancer.
☐ It was a non-invasive cancer (early stage breast cancer).
 → Was it Ductal Carcinoma In-situ (DCIS)? ☐ **Yes** ☐ **No**
 → Was it Lobular Carinoma In-situ (LCIS)? ☐ **Yes** ☐ **No**
☐ It was both invasive and non-invasive.
☐ I'm not sure.

Treatment

Did you have surgery for the **second** breast cancer?

☐ **No**

☐ **Yes** → Year of surgery: ____ ____ ____ ____ Month (if known)? ____

What type of surgery did you have?

Check one → ____ **Lumpectomy** (tumour removed, breast remains)

____ **Mastectomy** (one breast removed)

____ **Bilateral mastectomy** (both breasts removed)

Detection

How was the lump first detected?

Check one → ____ Only by mammography screening

____ Did you feel a lump?

____ Did the doctor feel a lump?

____ MRI screening

____ Other: _____

33. Did you receive chemotherapy for the second breast cancer?

☐ **No**

☐ **Yes**

34. Did you receive radiation therapy for the second breast cancer?

☐ **No**

☐ **Yes**

35. Did you receive Tamoxifen (Nolvadex) for the second breast cancer?

☐ **No**

☐ **Yes** → Number of pills per day: _____

From: ____ ____ ____ ____ (year) to ____ ____ ____ ____ (year)

36. Did you receive any other drugs for the second breast cancer?

☐ **No**

☐ **Yes** → Which drug?

☐ Femara (letrozole)

☐ Aromasin (exemestane)

☐ Arimidex (anastrozole)

☐ Other: _____

Number of pills per day: _____

From: ____ ____ ____ ____ (year) to ____ ____ ____ ____ (year)

37. Has your breast cancer come back in the same breast (local recurrence) or spread elsewhere (distant recurrence/metastasis)?

☐ **No**

☐ **Yes** → Date: ____ / ____ ____ ____ (mm / yyyy)

Site of recurrence: _____

Treatment for recurrence: _____

38. Have you had further surgery in the affected breast (such as complete mastectomy or reconstruction)?

☐ **No**

☐ **Yes** → Date: ____ / ____ ____ ____ (mm / yyyy)

Type of surgery: _____

SECTION IV – Ovarian Cancer Screening/Prevention

39. Have you ever had a trans-vaginal ultrasound (internal ultrasound done through the vagina)?

☐ **No**

☐ **Yes** → Age at first ultrasound: _____

Number of ultrasounds: _____

Abnormalities detected by ultrasound: ☐ **Yes** ☐ **No**

If Yes: Describe: _____

Year of abnormality: ____ _

Year of last trans-vaginal ultrasound: ____ _

40. Have you ever had a blood test for CA125 (marker for ovarian cancer)?

☐ **No**

☐ **Yes** → Age at first CA125: _____

Number of CA125 tests: _____

Abnormalities detected: ____ **No** ____ **Elevated** ____ **Don't Know**

Year of abnormality: ____ _

Year of the last CA125: ____ _

41. Have you had one or both of your ovaries removed (oophorectomy) for reasons **other than ovarian cancer**? (e.g. preventative measures, fibroids, cyst, scar tissue, or pain)

☐ **No**

☐ **Yes** → Year of surgery: ____ _ Month (if known)? ____ _

Reason for the surgery: _____

Number of ovaries removed: ____ **One** ____ **Two**

42. Have you ever had your fallopian tubes removed?

☐ **No**

☐ **Yes** → Year: ____ _

43. Have you ever had a tubal ligation (fallopian tubes tied)?

☐ **No**

☐ **Yes** → Year: ____ _

44. Have you had any other surgery performed on your reproductive organs: (e.g. hysterectomy, cervix removed, myomectomy, D & C)

☐ **No**

☐ **Yes** → Year: ____ _

What operation was performed? _____

Reason for the surgery: _____

SECTION V – Ovarian Cancer Diagnosis

45. Have you ever had ovarian cancer?

☐ **No** → **Go to question 51 (Section VI).**

☐ **Yes** → Year of diagnosis: ____ ____ ____ ____ Month (if known)? _____

46. How was the cancer first detected?

Check one → ____ Screening (CA125, imaging)

____ Symptoms presented (bloating, abdominal pain, etc.)

____ At the time of preventative surgery for ovarian cancer (removal of ovaries, fallopian tubes, etc.)

____ At the time of other abdominal surgery

____ Other: _____

47. Did you have surgery for your ovarian cancer?

☐ **No**

☐ **Yes** → Check one → ____ One ovary was removed

____ Both ovaries were removed

48. Did you receive chemotherapy for your ovarian cancer?

☐ **No**

☐ **Yes** → Was chemotherapy given before or after surgery? _____

Method of chemotherapy?

Check one → ____ Intravenous (through the vein)

____ Intraperitoneal (through the peritoneum)

____ Both

49. Did you receive radiation therapy for your ovarian cancer?

☐ **No**

☐ **Yes**

50. Has your ovarian cancer come back or spread elsewhere (distant recurrence/metastasis)?

☐ **No**

☐ **Yes** → Date: ____ / ____ ____ (mm /yyyy)

Site of recurrence: _____

Treatment for recurrence: _____

SECTION VI – Personal Information

Today's Date: _____
Month – Day – Year

Date of Birth: _____
Month – Year

Place of Birth: _____
City Province/State

Current Residence: _____
City Province/State

Ethnic Background: _____

51a. What is the major ancestry of your father? (*Please circle one option.*)

African or African American (country of origin: _____)	Irish
Ashkenazi Jewish	Italian
Asian/Pacific Islander (country of origin: _____)	Native American (Amer. Indian)
Dutch	Polish/Slavic/Eastern
English	Russian
European Bloc countries	Scandinavian (Swedish/Finnish/ Norwegian/Dane)
French Canadian	Scot-Irish or Scottish
German	Sephardic Jewish
Hispanic (country of origin: _____)	Other (specify: _____)
	Unknown

51b. What is the major ancestry of your mother? (*Please circle one option.*)

African or African American (country of origin: _____)	Irish
Ashkenazi Jewish	Italian
Asian/Pacific Islander (country of origin: _____)	Native American (Amer. Indian)
Dutch	Polish/Slavic/Eastern
English	Russian
European Bloc countries	Scandinavian (Swedish/Finnish/ Norwegian/Dane)
French Canadian	Scot-Irish or Scottish
German	Sephardic Jewish
Hispanic (country of origin: _____)	Other (specify: _____)
	Unknown

52. What is your: current weight? _____ pounds
current height? _____ feet _____ inches

Think back to when you were 18 years old, about the time you graduated from high school.

How much did you weigh then? _____ pounds

at age 30? _____ pounds

at age 40? _____ pounds

What is the most you have ever weighed (exclude pregnancy)? _____ pounds

How old were you when you weighed the most? _____ years old

53. Do you know how much you weighed when you were born?

☐ **No**

☐ **Yes** → _____ pounds _____ ounces **OR** _____ grams

54. What is your mother's year of birth? _ _ _ _

55. What is your birth order (i.e. first-born, second-born, third-born etc.)? _____

56. Were you part of a multiple birth (i.e. twin, triplet)?

☐ **No**

☐ **Yes** → *(Please check one)*

___ twin → Are you an identical twin? ☐ **Yes** ☐ **No**

___ triplet

___ other (please specify): _____

SECTION VII – Lifestyle

57. Have you ever smoked cigarettes regularly?

☐ **No**

☐ **Yes** → From: _____ (age first started) to _____ (age last used)

On average, how many packs do/did you smoke per week? _____

Do you still smoke? ☐ **Yes** ☐ **No**

58. Do you or did you ever drink coffee regularly?

☐ **No**

☐ **Yes** → From: _____ (age first started) to _____ (age last used)

On average, how many cups do/did you drink a day?

Caffeinated: _____ cups **Decaffeinated:** _____ cups **Total:** _____ cups

Do you still drink coffee? ☐ **Yes** ☐ **No**

59. Do you drink alcoholic beverages?

☐ No

☐ Yes → From: _____ (age first started) to _____ (age last used)

On average, how many alcoholic drinks do/did you have per week? (*Please check one*)

_____ 0-3 _____ 4-9 _____ 10-20 _____ 20 or more

What type of alcoholic beverages do/did you drink? (*Check all that apply*)

_____ beer _____ wine _____ hard liquor

60. Have you ever been a regular user of talcum powder?

☐ No

☐ Yes → Did you apply it directly to the vaginal area? ☐ Yes ☐ No

Did you apply it to sanitary napkins? ☐ Yes ☐ No

Other use of talcum powder? (describe) _____

What age did you start using talcum powder? _____ years old

What age did you stop using talcum powder? _____ years old

Do you still use talcum powder? ☐ Yes ☐ No

61. DURING THE PAST YEAR, what was your average time PER WEEK spent at each of the following recreational activities?

TIME PER WEEK

	Zero	1-4 min	5-19 min	20-59 min	One hour	1-1.5 hrs	2-3 hrs	4-6 hrs	7-10 hrs	11+ hrs
Walking for exercise or walking to work	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Jogging (slower than 6 minutes/km)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Running (6 minutes/km or faster)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bicycling (include stationary machine)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tennis, squash, racquetball	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lap swimming	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other aerobic exercise (aerobic, dance, ski or stair machine, etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lower intensity exercise (yoga, stretching, toning)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other vigorous activities (e.g., lawn mowing)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Weight training or resistance exercises (include free weights or machines)	Arms	☐	☐	☐	☐	☐	☐	☐	☐	☐
	Legs	☐	☐	☐	☐	☐	☐	☐	☐	☐

62. DURING THE PAST YEAR, on average, how many hours PER WEEK did you spend:

TIME PER WEEK

	None	1	2-5	6-10	11-20	21-40	41-60	61-90	91+
Standing or walking around work or away from home	☐	☐	☐	☐	☐	☐	☐	☐	☐
Standing or walking around at home	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sitting at work or away from home or while driving	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sitting at home while watching TV	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other sitting at home (eg, reading, meal times, at desk)	☐	☐	☐	☐	☐	☐	☐	☐	☐

SECTION VIII – Other Medical History

63. Have you been diagnosed with any cancer **other** than breast or ovarian (not including a recurrence/metastasis of a breast or ovarian cancer)?

☐ **No**

☐ **Yes** → What type? _____ Year of Diagnosis? ____ _

64. Please describe briefly any medical problems that you have had in the past which are listed below. Check all that apply.

Medical Condition	Age of Diagnosis	Medication Taken for Condition	Medication Start Year	Medication Stop Year
☐ Osteoporosis				
Thyroid Problems: ☐ Hyperthyroidism ☐ Hypothyroidism				
☐ High Cholesterol				
☐ High Blood Pressure				
Diabetes: ☐ Type I ☐ Type II ☐ Gestational				
Heart Disease: ☐ Cardiomyopathy ☐ Angina ☐ Heart Attack				
☐ Blood Clot				
☐ Stroke				
☐ Crohn's Disease/Ulcerative Colitis				
Urinary/Genital Problems: ☐ Urinary Tract Infections ☐ Fibroids ☐ Endometriosis				
Mental illness: ☐ Depression ☐ Anxiety ☐ Dementia				
☐ Other:				

65a. Are you taking, or have you taken, any prescription medications on a regular basis which were NOT previously listed?

☐ **No** ☐ **Yes** → **Complete below:**

1. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
2. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
3. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
4. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
5. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____

65b. Are you taking, or have you taken, any over-the-counter medications (i.e. Aspirin, Tylenol, Advil, Aleve, etc.,) on a regular basis which were NOT previously listed?

☐ **No** ☐ **Yes** → **Complete below:**

1. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
2. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
3. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
4. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
5. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____

66. Do you take dietary supplements or vitamins (i.e. multi-vitamins, calcium supplement etc.)?

☐

No

☐

Yes

→

___ Multi-vitamins for ___ years ___ months

___ Supplements (including herbal supplements)

→ Type (e.g. Evening primrose oil, calcium etc):

1. _____ for _____ years _____ months

2. _____ for _____ years _____ months

3. _____ for _____ years _____ months

67. Have you ever had another operation on your abdomen? (e.g. gall bladder, appendix, hernia, etc)

☐

No

☐

Yes

→

1. Type of surgery: _____ Year: ____ _

2. Type of surgery: _____ Year: ____ _

3. Type of surgery: _____ Year: ____ _

68. Have you received your genetic test results?

☐

No

☐

Yes

→ Date you received your results: ____ / ____ / ____ (mm / dd / yyyy)

FOR OFFICE USE (to be completed by Genetic Counsellor):

Participating centre: _____

Contact person: _____

Interviewer (if by phone): _____

Family Number: _____

Individual Identification No: _____

Date questionnaire sent: _____
Month – Day – Year

Date questionnaire received: _____
Month – Day – Year

Information received by mail _____ **or by telephone** _____ **or in clinic** _____

Genetic Test Result Disclosed to Patient? ☐ Yes ☐ No

Date Test Result Disclosed: _____
Month – Day – Year

Mutation: BRCA____: _____ **Exon**_____

PALB2 : _____ **Exon**_____

Appendix 2. Follow-up Questionnaire for the Risk Factor Analysis of Breast and Ovarian Cancer

FOLLOW-UP QUESTIONNAIRE FOR A STUDY OF BREAST AND OVARIAN CANCER IN HIGH RISK FAMILIES

Date Completed: _____(mm / dd / yyyy)

Name: _____

Date of Birth: _____(mm / dd / yyyy)

Fam/Ind#: _____

Telephone #: _____

Date of Previous Questionnaire: _____(mm / dd / yyyy)

Centre: _____

SECTION I - Reproductive Update

1. Have you had any pregnancies since ____ / ____ (mm / yyyy) (including still born, miscarriage, abortion)?

☐ **No** → **Go to question 4.**

☐ **Yes** → Please consider any NEW pregnancies since the date above in order from first to last. Give year of pregnancy. Indicate the outcome of each pregnancy. For live-born children, try to recall how long you breast-fed each child and indicate months of breast-feeding (if not breast-fed, enter '0').

PREGNANCY							
#	Year of Pregnancy	Length of Pregnancy (In Weeks)	Pregnancy Outcome (Live, Still, Miscarriage, Abortion, Twins, etc.)	Live Birth Caesarean Section? (Yes, No)	Live Born (Month/Year of Birth)	Birth Weight (lbs.)	Months of Breast-Feeding
1							
2							

2. Did you ever have difficulty breast-feeding?

☐ **No**

☐ **Yes** → Reason: ____ poor milk production ____ pain
____ premature infant ____ mastitis (breast inflammation)
____ other (please specify): _____

3. Have you ever taken medication to stop milk production?

☐ **No**

☐ **Yes** → Name of medication (if known): _____
Method: ____ injections or ____ pills

4. Are you still having menstrual periods? That is, have you had a menstrual period within the last year? Please answer "Yes" if you are currently pregnant, breast-feeding or taking hormones which temporarily stop your menses (Mirena IUD or birth control pills.)

☐ **Yes**

☐ **No** → At what age did they stop? _____

Reason they stopped? (*Please check only one*)

☐ **Natural Menopause** (change of life)

☐ **Hysterectomy** (uterus removed only, ovaries not removed)

☐ **One ovary removed only**

☐ **Both ovaries removed only**

☐ **Fallopian tubes and ovaries removed**

- ☐ **Uterus, fallopian tubes, and ovaries removed**
☐ **Medication / Chemotherapy**
☐ **Other** (please specify): _____

5. Have you ever taken birth control medication including birth control pills, Norplant (implants), Mirena/IUD, or Depo-Provera (injections) to prevent pregnancy or for any other reason?

- ☐ **No**
☐ **Yes** → Please complete the table below:

	Method	Name of Medication	Starting Year	Ending Year	Length of Time Used in Years
1	☐ Pills ☐ Implants ☐ Mirena/IUD ☐ Injections ☐ Other				
2	☐ Pills ☐ Implants ☐ Mirena/IUD ☐ Injections ☐ Other				

Are you still using birth control medication? ☐ **Yes** ☐ **No**

6. Have you ever taken hormone replacement therapy (HRT)?

- ☐ **No**
☐ **Yes** → Please complete the table below:

	Method	Name of Medication	Starting Year	Ending Year	Length of Time Used in Years
1	☐ Pills ☐ Skin Patches ☐ Vaginal Suppositories ☐ Gel ☐ Cream ☐ Ring ☐ Injections ☐ Other				
2	☐ Pills ☐ Skin Patches ☐ Vaginal Suppositories ☐ Gel ☐ Cream ☐ Ring ☐ Injections ☐ Other				

Are you still using HRT? ☐ **Yes** ☐ **No**

Other aerobic exercise (aerobic, dance, ski or stair machine, etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lower intensity exercise (yoga, stretching, toning)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other vigorous activities (e.g., lawn mowing)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Weight training or resistance exercises (include free weights or machines)	Arms	☐	☐	☐	☐	☐	☐	☐	☐	☐
	Legs	☐	☐	☐	☐	☐	☐	☐	☐	☐

13. DURING THE PAST YEAR, on average, how many hours PER WEEK did you spend:

	TIME PER WEEK								
	None	1	2-5	6-10	11-20	21-40	41-60	61-90	91+
Standing or walking around work or away from home	☐	☐	☐	☐	☐	☐	☐	☐	☐
Standing or walking around at home	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sitting at work or away from home or while driving	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sitting at home while watching TV	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other sitting at home (eg, reading, meal times, at desk)	☐	☐	☐	☐	☐	☐	☐	☐	☐

SECTION III – Breast Cancer Screening/Prevention

14. Have you ever taken any drugs for the prevention of breast cancer (i.e. drugs taken before any diagnosis of breast cancer)?

☐ No

☐ Yes → Name of Drug: ___ Tamoxifen (Nolvadex) ___ Raloxifene (Evista)
 ___ Aromasin (Exemestane) ___ Femara (Letrozole)
 ___ Arimidex (Anastrozole)
 ___ Other (please name): _____

Dosage (mg/day): _____

Date started: ___ / ___ / ___ (mm/yyyy) to ___ / ___ / ___ (mm/yyyy)

15. Have you ever had a mammogram?

☐ No

☐ Yes → Age at first mammogram: _____

How many mammograms have you had? _____

Were there any abnormalities detected by mammogram? ☐ Yes ☐ No

If yes, please describe: _____

Month/Year of abnormality: ___ / ___ / ___

What year was your last mammogram done? _____

16. Have you ever had MRI screening of your breasts?

☐ **No**

☐ **Yes** → Age at first MRI: _____

Month/Year of first MRI: ____ / ____

How many MRIs have you had? _____

Where were they done: _____

Were there any abnormalities detected by MRI? ☐ **Yes** ☐ **No**

If yes, please describe: _____

Year of abnormality: ____

Month/year of your last MRI ? ____ / ____

17. Have you ever had a breast biopsy (this includes needle and core biopsies)?

☐ **No**

☐ **Yes** → Number of biopsies: _____

Date of first biopsy: ____ / ____ (mm / yyyy)

Date of last biopsy: ____ / ____ (mm / yyyy)

What was the result of the biopsy? (*Check all that apply*)

____ normal ____ DCIS ____ atypical hyperplasia

____ cancer ____ LCIS ____ fibroadenoma

____ other (please specify): _____

18. Have you undergone preventive removal of your breasts? **NOTE: The surgery is only preventive if you've never previously been diagnosed with cancer in that breast.**

☐ **No**

☐ **Yes** → Date: ____ / ____ / ____ (mm / dd / yyyy)

Which breast was removed? ____ **Left** ____ **Right** ____ **Both**

Procedure: ____ **Subcutaneous Mastectomy** (nipple and areola preserved)

____ **Total Mastectomy** (nipple and areola removed)

____ **Other:** _____

19. Have you ever had breast implants or breast reconstruction?

☐ **No**

☐ **Yes** → Type: ____ Saline ____ Silicone ____ TRAM-flap

____ Other _____

Year of surgery: ____

20. Have you ever had breast reduction?

☐ **No**

☐ **Yes** → Year of surgery: ____

SECTION IV – Breast Cancer (Previous Diagnosis)

Did you have breast cancer at the time of or prior to the date of the last questionnaire?

☐ **NO** → Go to Question 24 (Section V).

☐ **YES** → Please complete this section

Year of previous cancer: ____/____/____ (mm / yyyy)

Side: ____ Left ____ Right ____ Both

Type (check all that apply):

____ Invasive

____ Non-invasive → ____ Ductal Carcinoma In-situ (DCIS)

____ Lobular Carcinoma In-situ (LCIS)

Treatment: ____ Surgery: _____ ____ Radiation

____ Chemotherapy ____ Hormone Therapy

21. Have you ever taken any drugs for the treatment (and to prevent a recurrence) of your breast cancer?

☐ **No**

☐ **Yes** → Name of Drug: ____ Tamoxifen (Nolvadex) ____ Raloxifene (Evista)

____ Aromasin (Exemestane) ____ Femara (Letrozole)

____ Arimidex (Anastrozole)

____ Other (please name): _____

Dosage (mg/day): _____

Date started: ____/____/____ (mm/yyyy) to ____/____/____ (mm/yyyy)

22. Has your breast cancer come back in the same breast (local recurrence) or spread elsewhere (distant recurrence/metastasis)?

☐ **No**

☐ **Yes** → Date: ____/____/____ (mm / dd / yyyy)

Site: _____

Mode of detection: ____ Mammogram ____ X-ray ____ Doctor

____ Self-exam ____ Other: _____

Treatment: _____

23. Have you had any further surgery on the affected breast(s)?

☐ **No**

☐ **Yes** → Type of surgery: ____ **Lumpectomy** (part of breast removed)

Which breast? ____ Left ____ Right ____ Both

Date of lumpectomy: ____/____/____ (mm/dd/yyyy)

____ **Mastectomy** (whole breast removed)

Which breast? ____ Left ____ Right ____ Both

Date of mastectomy: ____ / ____ / ____ (mm/dd/yyyy)

____ **Reconstruction**

Which breast? ____ Left ____ Right ____ Both

Date of reconstruction: ____ / ____ / ____ (mm/dd/yyyy)

SECTION V – Ovarian Cancer Screening/Prevention

24. Have you ever had a trans-vaginal ultrasound (internal ultrasound done through the vagina)?

☐ **No**

☐ **Yes** → Age at first ultrasound: _____

Number of ultrasounds: _____

Abnormalities detected by ultrasound: ☐ **Yes** ☐ **No**

If Yes: Describe: _____

Year of abnormality: ____

Year of last trans-vaginal ultrasound: ____

25. Have you ever had a blood test for CA125 (marker for ovarian cancer)?

☐ **No**

☐ **Yes** → Age at first CA125: _____

Number of CA125 tests: _____

Abnormalities detected: ____ **No** ____ **Elevated** ____ **Don't Know**

Year of abnormality: ____

Year of the last CA125: ____

26. Have you had your ovaries removed (oophorectomy) for reasons **other than ovarian cancer**? (e.g. preventative measures, fibroids, cyst, scar tissue, or pain)

☐ **No**

☐ **Yes** → Number of ovaries removed: ☐ **1** ☐ **2**

Year of surgery: ____ Month (if known)? ____

If ovaries removed in separate surgeries:

Month/Year of second surgery ____ / ____

Reason: ____ Abnormal CA125 test ____ Preventative

____ Cyst

____ Other _____

27. Have you ever had your fallopian tubes removed?

☐ **No**

☐ **Yes** → Year: ____

28. Have you ever had a tubal ligation (fallopian tubes tied)?

☐ **No**

☐ **Yes** → Year: ____

29. Have you had any other surgery performed on your reproductive organs: (e.g. hysterectomy, cervix removed, myomectomy, D & C)

☐

No

☐

Yes → Year: _ _ _ _

What operation was performed? _____

Reason for the surgery: _____

SECTION VI – Ovarian Cancer (Previous Diagnosis)

Did you have ovarian cancer at the time of or prior to the date of the last questionnaire?

☐

NO → **Go to Question 32 (Section VII).**

☐

YES → Please complete this section

Year of previous cancer: _ _ / _ _ _ _ (mm / yyyy)

30. Has your ovarian cancer come back or spread elsewhere (distant recurrence/metastasis)?

☐

No

☐

Yes → Date: _ _ / _ _ / _ _ _ _ (mm / dd / yyyy)

Site: _____

Mode of detection: _ _ CA125 test _ _ Trans-vaginal ultrasound

 _ _ Doctor _ _ Other _____

Treatment: _____

31. Do you still have your ovaries?

☐

No

☐

Yes → How many?

☐

1

☐

2

SECTION VII – New Breast Cancer Diagnosis

32. Have you been diagnosed with breast cancer since _ _ / _ _ _ _ (mm / yyyy)?

☐

No → **Go to Question 34 (Section VIII).**

☐

Yes → Date of diagnosis: _ _ / _ _ / _ _ _ _ (mm / dd / yyyy)

Which breast? _ _ Left _ _ Right _ _ Both

Type of Cancer (check all that apply):

☐

It was an invasive cancer.

☐

It was a non-invasive cancer (early stage breast cancer).

→ Was it Ductal Carcinoma In-situ (DCIS)? ☐ **Yes** ☐ **No**

→ Was it Lobular Carinoma In-situ (LCIS)? ☐ **Yes** ☐ **No**

☐

I'm not sure.

Treatment: (i) Surgery? ☐ **Yes** ☐ **No**

If Yes: ____ **Mastectomy** (whole breast removed)

Which breast? ____ Left ____ Right ____ Both

Date of mastectomy: ____ / ____ / ____ (mm/dd/yyyy)

____ **Lumpectomy** (part of breast removed)

Which breast? ____ Left ____ Right ____ Both

Date of lumpectomy: ____ / ____ / ____ (mm/dd/yyyy)

____ **Axillary Node Dissection** (lymph nodes removed)

Number removed: ____

Were any found to have cancer? ☐ **Yes** ☐ **No**

How many? ____

(ii) Chemotherapy? ☐ **Yes** ☐ **No**

If yes, name of drug: _____

(iii) Radiation therapy? ☐ **Yes** ☐ **No**

(iv) Other treatment drugs (e.g. Tamoxifen etc.)? ☐ **Yes** ☐ **No**

If yes, name of drug: _____ Dosage (mg/day): ____

Date started: ____ / ____ / ____ to ____ / ____ / ____ (mm/yyyy)

Name of hospital: _____ City: _____

How was the cancer first detected? _____

If a lump was felt, was a mammogram done to confirm the cancer? ☐ **Yes** ☐ **No**

If yes, was an abnormality detected? ☐ **Yes** ☐ **No**

33. Has your breast cancer come back in the same breast (local recurrence) or spread elsewhere (distant recurrence/metastasis)?

☐ **No**

☐ **Yes** → Date: ____ / ____ / ____ (mm / dd / yyyy)

Site of recurrence: _____

Treatment: _____

SECTION VIII – New Ovarian Cancer Diagnosis

34. Have you been diagnosed with ovarian cancer since ____ / ____ / ____ (mm / yyyy)?

☐ **No** → **Go to Question 36 (Section IX).**

☐ **Yes** → Date of diagnosis: ____ / ____ / ____ (mm / dd / yyyy)

Treatment: (i) Ovaries removed? ☐ **Yes** ☐ **No**

If Yes: How many? **1** **2**

Date of surgery: ____ / ____ / ____ (mm/dd / yyyy)

(ii) Chemotherapy? ☐ **Yes** ☐ **No**

If Yes: Name of drug: _____

(iii) Radiation therapy? ☐ **Yes** ☐ **No**

Name of hospital: _____ City: _____

How was the cancer first detected? _____

Was a trans-vaginal ultrasound done? ☐ **Yes** ☐ **No**

If yes, were any abnormalities detected? ☐ **Yes** ☐ **No**

Was a CA125 test done? ☐ **Yes** ☐ **No**

If yes, were any abnormalities detected? ☐ **Yes** ☐ **No**

35. Has your ovarian cancer come back or spread elsewhere (distant recurrence/metastasis)?

☐ **No**

☐ **Yes** → Date: ____ / ____ / ____ (mm / dd / yyyy)

Site of recurrence: _____

Treatment: _____

SECTION IX – Other Medical History

36. Have you been diagnosed with any cancer **other** than breast or ovarian (not including a recurrence/metastasis of a breast or ovarian cancer)?

☐ **No**

☐ **Yes** → Date of diagnosis: ____ / ____ / ____ (mm / dd / yyyy)

Type: _____

Treatment: _____

37. Please describe briefly any other medical problems that you have had in the past which are listed below. Check all that apply.

Medical Condition	Age of Diagnosis	Medication Taken for Condition	Medication Start Year	Medication Stop Year
☐ Osteoporosis				
Thyroid Problems: ☐ Hyperthyroidism ☐ Hypothyroidism				
☐ High Cholesterol				
☐ High Blood Pressure				
Diabetes: ☐ Type I ☐ Type II ☐ Gestational				
Heart Disease: ☐ Cardiomyopathy ☐ Angina ☐ Heart Attack				
☐ Blood Clot				
☐ Stroke				
☐ Crohn's Disease/Ulcerative Colitis				
Urinary/Genital Problems: ☐ Urinary Tract Infections ☐ Fibroids ☐ Endometriosis				
Mental illness: ☐ Depression ☐ Anxiety ☐ Dementia				
☐ Other:				

38a. Have you taken any other prescription medications on a regular basis since ____ / ____ (mm/yyyy) which were NOT previously mentioned?

☐ **No** ☐ **Yes** → **Complete:**

1. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
2. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
3. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
4. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
5. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____

38b. Have you taken any new over-the-counter medications (i.e. Aspirin, Tylenol, Advil, Aleve, etc.,) on a regular basis since ____ / ____ (mm / yyyy) which were NOT previously mentioned?

☐ **No** ☐ **Yes** → **Complete:**

1. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
2. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
3. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)
 Reason: _____
4. Drug: _____
 Frequency (days/week): _____ From: _____ (year) to _____ (year)

Reason: _____

5. Drug: _____

Frequency (days/week): _____ From: _____ (year) to _____ (year)

Reason: _____

39. Do you take dietary supplements or vitamins (i.e. multi-vitamins, calcium supplement etc.)?

☐ **No**

☐ **Yes** → _____ Multi-vitamins for _____ years _____ months

_____ Supplements (including herbal supplements)

→ Type (e.g. Evening primrose oil, calcium etc):

1. _____ for _____ years _____ months

2. _____ for _____ years _____ months

3. _____ for _____ years _____ months

40. Have you had any abdominal surgery since ____ / ____ / ____ (mm / yyyy) not previously mentioned (e.g. gall bladder, appendix, hernia, etc)?

☐ **No**

☐ **Yes** → 1. Type of surgery: _____ Year: ____ / ____ / ____

2. Type of surgery: _____ Year: ____ / ____ / ____

3. Type of surgery: _____ Year: ____ / ____ / ____

41. Have you received your genetic test results?

☐ **No**

☐ **Yes** → Date you received your results: ____ / ____ / ____ (mm / dd / yyyy)

SECTION XII – PEDIGREE UPDATE

42. Have there been any new cancers diagnosed in your family since ____ / ____ (mm / yyyy)?

☐

No

☐

Yes (Complete table below)

Name			
Relationship			
Type of Cancer			
Year of Diagnosis			
Age at Diagnosis			

43. Have there been any births or deaths in your family since ____ / ____ (mm / yyyy)?

☐

No

☐

Yes (Complete table below)

Name			
Relationship			
Date of Birth			
Date of Death			
Cause of Death			

Thank you very much for taking the time to complete this questionnaire.

Should the need arise, may we contact you again? ☐ No ☐ Yes

Telephone number: _____ **Alternate contact:** _____

E-mail address: _____

Mailing address: _____

CITY: _____ **PROVINCE:** _____ **POSTAL CODE:** _____

Appendix 3. Informed Consent Form for the Risk Factor Analysis of Breast and Ovarian Cancer

Dr. Steven Narod, MD, FRCPC
Canada Research Chair in Breast Cancer
76 Grenville Street, 6th Floor, Toronto, Ontario M5S 1B2
Tel: 416-351-3765 Fax: 416-351-3767 E-mail: steven.narod@wchospital.ca

INFORMED CONSENT FORM **Risk Factor Analysis of Hereditary Breast and Ovarian Cancer**

PRINCIPAL INVESTIGATOR: Dr. Steven Narod
SITE: Women's College Research Institute
SPONSOR: The Prevention Research Grant of the Canadian Cancer Society and the Canadian Institutes of Health Research-Institute of Cancer Research

We would like to invite you to take part in a multi-site research study called the Risk Factor Analysis of Hereditary Breast and Ovarian Cancer.

BACKGROUND:

Women with a *BRCA1/2* mutation have a high lifetime risk of developing breast cancer (80% vs. 12% for women without the mutation) and/or ovarian cancer (30% vs. 3.5%). Women who have a *PALB2* mutation can also participate in this study. Prevention in these women at high risk is an important part of their care. This project began in 1996 studying women with any of these mutations. As of 2014, this study has upwards of 15,000 participants from around the world.

PURPOSE OF RESEARCH:

The purpose of this study is to identify hormonal, reproductive and lifestyle factors that are associated with the risk of developing breast and ovarian cancer among women at high risk. It is expected that around 20,000 individuals will participate in this project.

WHO CAN PARTICIPATE IN THE STUDY?

- Individuals who fully understand the study and give informed consent to participate as demonstrated by signing the provided consent form.
- Women with *BRCA1* and/or *BRCA2* and/or *PALB2* mutations.
- Women who are at least 18 years of age.

WHO CANNOT PARTICIPATE IN THE STUDY?

- Women who do not meet the criteria above.
- Individuals who are unable to give voluntary, informed consent.

PROCEDURE:

You will be asked to complete a baseline questionnaire asking about your medical history, family history of cancer and factors related to your lifestyle. You will be asked to update us every two years by completing a new questionnaire. As a result, members of the research team may contact you by telephone or by mail every two years to obtain follow-up questionnaires as part of this research study. Providing correct information about your medical history is your responsibility. You authorize members of the research team to access your pathology and medical records for research purposes. In the event that additional health information is required by the research team and you no longer have the capacity to provide health information, the research team has consent to contact your next of kin to obtain the information.

Participants will be asked to contribute to the research for a maximum of twenty years. The questionnaire data, in an anonymous form (a form that does not identify you), may be sent to other academic institutions for additional studies on the hereditary basis of cancer. Data from the questionnaire will not be used for purposes other than the study of familial cancer.

CONFIDENTIALITY:

You understand that medical and all other information produced by this study will be strictly confidential and will be held securely at and will be subject to the confidentiality and privacy regulations of Women's College Hospital. This information will not become part of your personal medical record. This information will be available to the study research team, and not released to any other party, except upon your expressed written consent. Information regarding your medical history or test results will not be disclosed to any other member of your family. Information pertaining to the medical history or test results of your relatives will not be disclosed to you.

You have the right to have any information about you and your health that is collected, used or disclosed for this study to be handled in a confidential manner.

If you decide to participate in this study, the investigator(s) and study staff will look at your personal health information and collect only the information they need for this study. It includes information such as your

- name,
- address,
- telephone number,
- date of birth,
- new and existing medical records, or
- the types, dates and results of various tests and procedures.

You have the right to access, review and request changes to your personal health information.

BENEFITS:

You may or may not benefit directly from participating in the study. Genetic counselling will be available to you and members of your family. The research team will be available to provide the most current information regarding genetic risk assessment and will provide referral to screening centres for cancer if requested. You understand that you may have access to any information regarding your personal risk of developing cancer upon request. You understand that there is no compensation available for your participation in this research study. You understand that representatives of the National Cancer Institute of Canada, (the research granting agency) may inspect the research records.

RISKS:

There are no medical risks to you from participating in this study but taking part in this study may make you feel uncomfortable or feel distressed answering some of the questions in the questionnaire. If you do not feel comfortable responding to a question, you may feel free to skip it. If you require additional support, please talk to the investigator who will discuss additional support options with you.

WITHDRAWAL FROM STUDY PARTICIPATION

You understand that your participation is voluntary and if you agree to participate you may withdraw your consent and discontinue your participation at any time without prejudice to or loss of your medical care or the benefits to which you, or your family, are otherwise entitled. If you withdraw your consent, the information about you that was/were collected before you left the study will still be used. No new information about you will be collected (and no further testing of you will be done) without your permission. You understand that your participation will not affect your choice of, or access to, treatment or screening. You understand that your participation may be terminated with or without your consent.

REQUEST FOR MORE INFORMATION:

You understand that you may ask more questions about the study. Dr. Narod is available to answer your questions and concerns (Tel. (1)-416-351-3765). If you have any questions about your rights as a research participant, please call Melissa Sidhu, Women's College Research Ethics Board Manager, at (416) 351-3732 Ext 2723. This person is not involved with the research project in any way and calling her will not affect your participation in the study.

CONFIRMATION OF PARTICIPATION:

DOCUMENTATION OF INFORMED CONSENT

You will be given a copy of this informed consent form after it has been signed and dated by you and the study staff.

Full Study Title: Risk Factor Analysis of Hereditary Breast and Ovarian Cancer

Name of Participant: _____

Participant/Substitute decision-maker

By signing this form, I confirm that:

- This research study has been fully explained to me and all of my questions answered to my satisfaction
- I understand the requirements of participating in this research study
- I have been informed of the risks and benefits, if any, of participating in this research study
- I have been informed of any alternatives to participating in this research study
- I have been informed of the rights of research participants
- I have read each page of this form
- I authorize access to my personal health information, medical record and research study data as explained in this form
- I have agreed, or agree to allow the person I am responsible for, to participate in this research study
- I understand that my family doctor may be informed of my participation in this research study

I give permission to Dr. Narod and the study team to contact me by telephone if additional information is needed.

☐ Yes ☐ No

I give permission to Dr. Narod and the study team to contact me by telephone/email/mail about future studies.

☐ Yes ☐ No

Name of Participant/Substitute decision-maker

Signature of Participant/Substitute

Date

WOMEN'S COLLEGE HOSPITAL
Health care for women | REVOLUTIONIZED

Dr. Steven Narod, MD, FRCPC

Canada Research Chair in Breast Cancer
76 Grenville Street, 6th Floor, Toronto, Ontario M5S1B2
Tel: 416-351-3765 Fax: 416-351-3767 E-mail: steven.narod@wchospital.ca

By signing this form, I confirm that:

- This study and its purpose has been explained to the participant above
- All questions asked by the participant have been answered
- I will give a copy of this signed and dated document to the participant

_____ Name of person (investigator) obtaining consent	_____ Signature	_____ Date
--	--------------------	---------------

ASSISTANCE DECLARATION

The Assistance Declaration provides a mechanism for potential participants who are unable to read the informed consent form (i.e. illiterate, blind or for whom English is their second language) to participate in research studies.

Was the participant assisted during the consent process? Yes _____ No _____

The consent form was read to the participant/substitute decision-maker, and the person signing below attests that the study was accurately explained to, and apparently understood by, the participant/substitute decision-maker. The person signing below acted as a translator for the participant/substitute decision-maker during the consent process.

He/she attests that they have accurately translated the information for the participant/substitute decision-maker, and believe that that participant/substitute decision-maker has understood the information translated.

_____ Name of Person Assisting (Print)	_____ Signature	_____ Date
---	--------------------	---------------

Person obtaining consent

By signing this form, I confirm that:

- This study and its purpose has been explained to the participant named above
- All questions asked by the participant have been answered
- I will give a copy of this signed and dated document to the participant

_____ Name of person (investigator) obtaining consent	_____ Signature	_____ Date
--	--------------------	---------------

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