Chapter 1

General introduction
The introduction to this thesis will explore several aspects of Hereditary Ovarian Cancer. First, a brief overview of the epidemiology of ovarian cancer is outlined. Subsequently, the function of the tumor suppressor genes *BRCA1* and *BRCA2* on protecting cells against cancer is discussed, followed by a description of the ovarian cancer risks associated with *BRCA1/2* mutations. Next, the current clinical practice regarding genetic testing in ovarian cancer patients and the management of women with *BRCA1/2* mutations is discussed, with special considerations on the sequellae of preventive surgery in *BRCA1/2* mutation carriers and differences in access to health care across the world. Finally, the aim and contents of this thesis are summarized.

**EPIDEMIOLOGY OF OVARIAN CANCER**

Ovarian cancer is the seventh most prevalent cancer in women worldwide, with approximately 240,000 new cases diagnosed every year.\(^1\) The age standardized incidence rates are higher in developed countries than in developing countries, but due to a decreasing incidence in the highest incidence countries and a concomitant increase of rates in the lowest incidence countries, this difference is becoming less pronounced.\(^2\) Ovarian cancer is most frequently diagnosed at older ages, with over 80% of cases being diagnosed in women over the age of 50.\(^3\) The cumulative risk of ovarian cancer by age 75 is estimated to be approximately 0.7%, with estimates ranging from 0.4% in central Africa to 1.3% in Eastern Europe.\(^1\) Due to the lack of specific symptoms in early stages of the disease and the absence of an effective screening strategy, most women are diagnosed at advanced stages, which are associated with poor prognosis. With five-year survival rates below 50%,\(^4\) ovarian cancer is the most lethal of gynecologic malignancies, causing the death of 150,000 women annually.\(^5\)

Ovarian cancer is a heterogeneous disease with many different histological subtypes. The vast majority of cases (90%) are of epithelial origin, which are classified according to histological presentation and molecular biology as type I or type II. Type I tumors include low-grade serous, clear cell, endometrioid, or mucinous carcinomas and mostly arise from endometriosis or from serous borderline tumors, having usually a more indolent clinical course and a more stable genomic profile.\(^6\) Type II tumors include high-grade serous carcinomas, which is the most common and most lethal histological type among epithelial ovarian cancers.\(^3\) Recently, accumulating evidence indicates that the majority of high-grade serous ovarian cancers actually originate in the fimbrial portion of the fallopian tube, where presumed precursor lesions (serous tubal intraepithelial carcinomas, STIC) have been identified.\(^7,8\) Although different subtypes of ovarian cancer present different clinical behavior, they had historically been treated as one single entity.\(^9\) However, distinct molecular pathways have been recently identified, which are characteristic of individual subtypes of ovarian cancer, and very important for the development of potential targeted therapies.\(^10\)

Although the majority of epithelial ovarian cancer is considered to be sporadic, i.e. not hereditary, more than 20% of all cases are associated with a genetic predisposition.\(^11\) The vast majority of hereditary cases can be attributed to mutations in the tumor suppressor genes *BRCA1* and *BRCA2*, with the remaining hereditary cases being associated with mutations in...
the mismatch repair genes (Lynch syndrome), STK11 (Peutz-Jeghers Syndrome) and other genes involved in DNA repair (RAD51, BRIP1).\textsuperscript{12,13} Women with germ-line mutations in \textit{BRCA1} or \textit{BRCA2} present markedly increased ovarian cancer risks,\textsuperscript{14,15} with life time risk estimates ranging between 30-60\% in \textit{BRCA1} and between 5-20\% in \textit{BRCA2} mutation carriers.\textsuperscript{15-18} The majority of \textit{BRCA1}/2-associated ovarian cancers is of high-grade serous type (type II tumors) and commonly occurs at earlier age than sporadic ovarian cancer, especially in \textit{BRCA1} mutation carriers.\textsuperscript{15,19,20} Furthermore, it has been reported that \textit{BRCA1}/2-associated ovarian cancers are more often at an advanced stage upon diagnosis than sporadic tumors or ovarian cancers in Lynch syndrome.\textsuperscript{21,22}

\textbf{BRCA1 AND BRCA2}

Cells are constantly exposed to numerous stressing stimuli that may lead to DNA damage. The genome is especially vulnerable during replication, when damage on a single strand can be converted into a double-strand break (DSB). DNA DSBs, when both strands of DNA are compromised simultaneously, are one of the most threatening forms of DNA damage and its repair is essential for maintenance of genome integrity. When DSBs occur, the damage is detected by DNA-damage binding proteins, which trigger the activation of cellular signal transduction cascades that activate DNA repair pathways. The two main mechanisms of DSB repair identified so far are non-homologous end joining (NHEJ) and homologous recombination (HR), with HR being less error prone than NHEJ.\textsuperscript{23}

\textit{BRCA1} and \textit{BRCA2} are tumor suppressor genes that encode large proteins, which have multiple biological functions. \textit{BRCA1} has several functional domains through which it interacts with other tumor suppressors and DNA repair proteins, playing an important role in cell cycle control (checkpoint regulation) and response to DNA damage (particularly DNA DSB repair by HR), while \textit{BRCA2}'s primary function is in HR.\textsuperscript{24-28} Loss of \textit{BRCA1}/2 protein function may result in unrepaired damage or in repair of DSB by other more error-prone mechanisms, resulting in accumulation of genetic damage.\textsuperscript{23,28} This genetic alteration can induce cell growth or proliferation defects, which can culminate in tumor development.\textsuperscript{28}

\textbf{GENETIC TESTING}

The frequency of \textit{BRCA1}/2 mutations in the general population has been estimated to range from 1 in 400 to 1 in 800.\textsuperscript{29,30} It is important to notice that the prevalence of mutations and their spectrum vary widely according to ethnicity and genetic background.\textsuperscript{20,31} For example, Ashkenazi Jews are 10 times more likely to have a \textit{BRCA1}/2 mutation than people from a non-Jewish population, with an estimated frequency of \textit{BRCA1}/2 mutations of 1 in 40.\textsuperscript{32} Higher rates of \textit{BRCA1}/2 mutations have also been reported among people originating from Norway, Iceland and The Netherlands.\textsuperscript{33} This variation in prevalence is associated with the presence of founder mutations in certain populations: mutations with shared polymorphic markers, consistent with a common ancestor.\textsuperscript{34-36} So far, over 3500 different sequence variants scattered over the coding sequence of these two genes have been described and are listed at the Breast Cancer Information Core database.\textsuperscript{37} New mutations in \textit{BRCA1}/2 appear
to be very rare.

Most developed countries have established their own guidelines for referral of patients for genetic testing. The most recent North American and European guidelines recommend that genetic risk evaluation is offered to all women diagnosed with invasive ovarian cancer or to those with a higher than 10% probability of having a BRCA1/2 mutation. 38-44 Several tools have been developed to assess the probability of detecting a mutation according to a patient’s characteristics and family history of cancer. 45-48 However, even though the development of guidelines and advances in DNA sequencing technology have allowed substantial progress on the identification of mutation carriers, there are still several challenges to overcome. Mutation testing, although prices dropped dramatically lately, is still costly and not widely available in low-income countries. When genetic testing is available, other concerns may arise such as the possible psychological distress associated with genetic testing, 49 concerns about cancer risks, prophylactic surgeries, family planning, passing the gene to their children, future life insurance or health care insurance discrimination. 50 Nevertheless, these possible sources of anxiety are generally discussed during the counseling process preceding genetic testing and, over time, the emotional consequences of BRCA testing appear to be minimal. 51

OVARIAN CANCER RISKS IN BRCA1/2 MUTATION CARRIERS

Since BRCA1/2 have been identified, 52-54 several studies have aimed at estimating the average cumulative ovarian cancer risk associated with mutations in these genes. Available risk estimates vary substantially between studies, depending on the population included and on the methodological approach applied. 17,18,55,56 The first estimates (30% [95%CI: 8%-47%] by age 60 for BRCA1 and 27% [95%CI: 0%-47%] by age 70 for BRCA2 mutation carriers) were based on the multiple-case families that had been included in the linkage studies for the identification of the disease loci in the nineties. 57,58 Subsequently, studies based penetrance estimates on the incidence of cancer in relatives of mutation-carrying index cases (case-based studies). 59-62 Aiming at improving the precision of penetrance estimates, a meta-analysis including 10 studies was conducted, revealing that by age 70 ovarian cancer risk was 40% (95%CI: 35-46%) for BRCA1 and 18% (95%CI: 13-23%) for BRCA2 mutation carriers. 17

The wide variation in reported age specific penetrance estimates has been mainly attributed to two factors: population differences (e.g. genetic factors, environmental factors) and methodological differences (e.g. ascertainment of study participants, methods of risk estimation, bias correction methods). A study investigating the effects of methods of risk estimation and bias correction on the cumulative life time risks of breast cancer in retrospective cohort studies concluded that much of the variation on estimates can be attributed to the method of bias correction used. 56 It has also been reported that penetrance estimates may vary according to type of cancer (i.e. breast or ovarian) of index case 15,63-65 and according to birth cohort. 15,55,65,66 Furthermore, most previous studies had a retrospective design and may be biased by inaccurate or incomplete reports of family history. A prospective study was conducted to partly overcome the limitations of retrospective analyses, however it presented
other limitations, such as a relatively small sample size (N=988) and short follow-up time (median 2.6 years). 18

The variation in cancer risks observed in previous studies suggests that cancer risks in BRCA1/2 mutation carriers may be modified by both genetic and non-genetic factors. 67 The most widely investigated cancer risk modifiers are described below.

- Mutation position on the gene

Shortly after BRCA1/2 were first described, it was observed that families with mutations on the central regions of BRCA2 presented higher proportions of ovarian cancer, in relation to breast cancer. This difference was attributed both to lower risks of breast cancer and to higher risks of ovarian cancer associated with mutations in this region when compared to mutations in other regions of the gene. 68 Similar observations were made for mutations in the central region of BRCA1, in which a lower risk of breast cancer was observed when compared to mutations in other parts of the gene, while a lower risk of ovarian cancer was observed in mutations 3’ to nucleotide 4191 when compared to mutations in the 5’ part of the gene. 69 These central regions of BRCA1/2 were named Ovarian Cancer Cluster Regions (OCCRs).

A recent study using the largest dataset evaluated to date supports the earlier observations regarding the OCCR in both BRCA1 and BRCA2, reporting an increase in ovarian cancer risks relatively to breast cancer risks among women with mutations in the central part of the genes. 70 Additionally, this study reported multiple Breast Cancer Cluster Regions, in which mutations were associated with a relatively higher breast cancer risk and a relatively lower ovarian cancer risk. 70

- Other genetic factors

Although about 90% of hereditary ovarian cancer cases are attributed to BRCA1/2 mutations, germ-line mutations in other genes may also lead to increased ovarian cancer risks. Mismatch repair genes such as MLH1, MSH2, MSH6, and PMS2 (Lynch syndrome), STK11 (Peutz-Jeghers Syndrome), as well as more recently identified DNA repair genes (RAD51C, RAD51D and BRIP1) have been associated with increased risks of developing ovarian cancer. 12, 13, 71 Furthermore, genome wide association studies (GWAS) have identified common susceptibility variants which modify the risk of ovarian cancer, both in the general population and in BRCA1/2 mutation carriers. 72 Other loci that appear to modify ovarian cancer risks specifically in BRCA1/2 mutation carriers and present no association with risk in the general population have also been identified. 73

It is also important to remark that BRCA1/2 and the genes described above, can play a role in cancer development through germ-line mutations as well as through somatic mutations. Several studies reported the presence of somatic BRCA1/2 mutations in ovarian cancer tissue. 74 Moreover, it is important to consider that in some cases gene function is lost not due to a mutation, but due to epigenetic silencing (for example because of promoter hypermethylation), which could also impact ovarian cancer risks. 74, 75 Molecular similarities
between ovarian cancers associated to \textit{BRCA1} mutations and to epigenetic-induced silencing of \textit{BRCA1} have been observed.\textsuperscript{74}

- Family history

Approximately 15\% of all patients with ovarian cancer have a family history of this disease.\textsuperscript{76} Regardless of the \textit{BRCA1/2} mutation status, a family history of ovarian cancer is considered to be an important risk factor for developing the disease. Women with a first-degree relative with ovarian cancer present a relative risk of developing the disease of 2.0 - 6.8 when compared to the general population.\textsuperscript{53,64,77,78} Having more than one affected relative increases this risk even further, with relative risks of up to 24.\textsuperscript{64,78,79} Among \textit{BRCA1} mutation carriers it has been observed that the risk of ovarian cancer is further increased 1.6 fold for each first- or second-degree relative affected with the disease.\textsuperscript{80}

\textbf{MANAGING BRCA1/2 MUTATION CARRIERS}

Identifying \textit{BRCA1/2} mutation carriers is very important for the prevention of ovarian cancer. Traditional screening methods for ovarian cancer, such as transvaginal ultrasound and serum CA-125 measurements have shown poor ability to detect early-stage disease, even in high-risk populations\textsuperscript{81,82} and are therefore not recommended for women with \textit{BRCA1/2} mutations. In the absence of effective screening methods, women at high risk are advised to have a timely risk reducing salpingo oophorectomy (RRSO) after childbearing is complete, preferably between ages 35-40 (for \textit{BRCA1} carriers) and 40-45 (for \textit{BRCA2} carriers). When performed at the appropriate age window (before ovarian cancer incidence rises), RRSO can reduce ovarian cancer risks by 96\%.\textsuperscript{83}

Among women who already developed ovarian cancer, identifying a \textit{BRCA1/2} mutation can be of relevance for guiding treatment choices. Recent clinical studies observed that \textit{BRCA1/2} deficient tumors are sensitive to Poly ADP-ribose polymerase (PARP) inhibitors,\textsuperscript{84-86} which have demonstrated durable antitumor activity in advanced \textit{BRCA}-mutated ovarian cancer.\textsuperscript{87} PARP is a protein that plays a role in DNA repair by homologous recombination (HR), together with \textit{BRCA1/2}.\textsuperscript{88} Cells with deficient HR due to a \textit{BRCA1/2} mutation become hypersensitive to inhibition of PARP activity. Several PARP inhibitors are currently under evaluation in clinical trials for their potential use in \textit{BRCA1/2}-deficient cancers and some have already been approved for clinical use in ovarian cancer treatment in the European Union and United States.\textsuperscript{87}

\textbf{LONG-TERM HEALTH CARE FOR BRCA1/2 MUTATION CARRIERS}

With increasing uptake of risk reducing salpingo-oophorectomy (RRSO),\textsuperscript{89} life expectancy of these women will increase. With increasing life expectancy and decreasing breast cancer related morbidity, it becomes essential to raise awareness over non-cancer endpoints in this population.

RRSO at premenopausal age induces acute and early onset of menopause, which
may have several undesirable consequences for women’s health and subjective wellbeing.\textsuperscript{90,91} Several studies have evaluated the possible consequences of oophorectomy, observing short- and long-term adverse effects of this procedure. Particularly when performed before menopausal age, bilateral oophorectomy was associated with hot flushes, decreased quality of life and sexual functioning, adverse bone and cardio-metabolic health and loss of cognitive function.\textsuperscript{92,93} Although there is substantial evidence of the adverse effects of early acute menopause through oophorectomy with or without hysterectomy, most available studies included women with several different indications and operative procedures. So far there is little knowledge of the short- and long-term impact of RRSO in the particular population of \textit{BRCA1/2} mutation carriers. Initial studies observed that although 5 years after RRSO women presented elevated bone turnover markers when compared to age-matched women, bone mineral density (BMD) and fracture risk were not significantly different from the general population.\textsuperscript{94,95} Moreover, another study observed that after a comparable follow-up time, women who had RRSO presented an even more favorable cardiovascular risk profile than age-matched controls.\textsuperscript{96} Due to the small number of studies on the topic and the lack of sufficient evidence, no specific guideline for these women is available so far.

**THE GLOBAL CANCER DIVIDE**

There are large inequities on access to health care around the world. These inequities are especially notable when it comes to prevention, diagnosis and treatment of cancer,\textsuperscript{97} which are often associated with high-costs. This disparity between high- and middle-/low-income countries has been coined as the global cancer divide.\textsuperscript{98} Currently, only 5\% of global investment on cancer is directed towards low- and middle-income countries, where the highest burden of the disease is concentrated.\textsuperscript{99} Cancer is associated with disability, premature death and disruption of family life.\textsuperscript{100} The health care costs and disability associated with cancer have long lasting social and economic consequences not only for the patient but also for their families. Female cancers are particularly devastating, especially when they occur at young age, at which women usually have a pivotal role in caregiving for their families. A mother’s disability and ultimately death may have substantial negative consequences on her children, increasing their vulnerability to health- and social complications,\textsuperscript{101} which can exacerbate the cycle of poverty in low-income countries. These marked inequities raise the attention for the urgent need of more investment on cancer control in low- and middle-income countries.

In this thesis we will take Brazil as an example of middle-income country. In low- and middle-income countries, access to health care depends largely on geographic factors and on socio-economic conditions. In this sense, Brazil is a typical example of a middle-income country. Brazil is a large country with a projected population of over 207 million people in February 2017.\textsuperscript{102} The Brazilian population presents unique characteristics, marked by considerable miscegenation mainly of Amerindian, European and African people, which took place since the XVI\textsuperscript{th} century.\textsuperscript{103} According to the last Brazilian population census, held in 2010, 7.6\% of Brazilians defined themselves as black, 43.1\% said they were mixed race, and 47.7\% labelled themselves white.\textsuperscript{104}
An unified health care system (Sistema Único de Saúde, SUS) was instituted in Brazil after the promulgation of the seventh Brazilian Federal Constitution, in 1988, which states that “health is a right of each citizen and duty of the State”, guaranteeing free universal access to health promotion, protection and care for every Brazilian citizen. The public Brazilian Health System is one of the largest in the world, responsible for health care of more than 70% of the country’s population. The Brazilian Constitution establishes that the public health system should be maintained through budget provided by the State, Provinces and Municipalities and that no less than 15% of the net current revenue of the respective financial year should be invested in the health system. However, data from the Organization for Economic Co-operation and Development indicates that in 2013 the national health expenditure per capita in Brazil was USD 904, representing only 3.4% of the Gross Domestic Product (GDP).

Although Brazil is the world’s ninth largest economy at market exchange rates according to the International Monetary Fund (IMF), the GDP per capita in 2016 was USD 15,048, putting Brazil in the 77th position according to IMF data. This discrepancy reflects the marked socioeconomic inequality in Brazil, where great inequalities in GDP are observed among different regions of the country. São Paulo state, in the Southern region of Brazil, is the most densely populated in the country, with almost 45 million people. It accounts for 32.1% of the Brazilian GDP, four other states (Rio de Janeiro, Minas Gerais, Paraná and Rio Grande do Sul) contributing with another 33.5% of it, while the other 22 Brazilian states account for only 34.4% of the GDP.

The regional disparities are also evident concerning access to healthcare across different regions of Brazil, with lower availability and more difficult access to health care resources in areas that are distant from the state capitals and from the more developed regions. Furthermore, the rate of health services utilization is higher among people from upper socio-economic classes, with higher education levels and with private insurance than among those from lower socio-economic classes, with lower education and who rely solely on the public health care system. Although rates of services utilization do not necessarily reflect the quality of the available services, these differences are an important reflection of the social inequality in Brazil. Disparities in access to genetic counseling, hereditary cancer screening and prevention are even more evident. Although the coverage of genetic testing by Brazilian private health insurances is mandatory since 2012, these services are not available for those who depend solely on the public health care system. Women without private health insurance would have to pay for genetic testing on a private laboratory, among which the prices for BRCA1/2 testing with multiplex ligation-dependent probe amplification vary widely, ranging from approximately USD 450 to more than USD 3000, a considerably high price when taking into account the average income in Brazil. With the given limitations for identifying BRCA1/2 mutation carriers, preventive strategies for ovarian cancer are still unavailable for the great majority of Brazilian women.

It is widely known that cancer represents an important economic burden and that investments in women’s health are associated with substantial economic returns. However, more research is needed to support the development of a cost-effective strategy for the im-
plementation of genetic counseling and testing in the public health care system in low- and middle-income countries. It is important to remark that the threshold for cost-effectiveness might be different in these settings than in high-income countries, since the criteria for evaluation of cost-effectivity is not only dependent on the costs associated with an intervention in relation to the health benefits resulting from it, but also on the countries context (e.g. disease burden and available budget).\textsuperscript{114}
THESIS OUTLINE

- Aim

Although substantial progress has been achieved on prevention of \( BRCA1/2 \)-associated ovarian cancer, there are still several challenges to overcome in order to optimize the management of women with a \( BRCA1/2 \) mutation. The general aim of this thesis is improving care of women with hereditary increased risk of ovarian cancer, from the moment of genetic testing to the awareness of long-term consequences of RRSO. In the first part of this thesis, the selection of patients for genetic testing and possible ways of improving this selection in limited resources settings are evaluated. In the second part, potential sources of variation in hereditary ovarian cancer risk are investigated, aiming at providing more accurate risk estimates for \( BRCA1/2 \) mutation carriers. Finally, the consequences of RRSO are explored, in order to understand what should be the concerns of medical professionals involved in the long-term care of this specific patient group.

PART I – GENETIC TESTING IN LIMITED RESOURCES SETTINGS

In countries where the availability of genetic testing is restricted to wealthy people, there is overall limited information on epidemiologic data and on characteristics of patients suspected to have hereditary ovarian cancer. In chapter 2 the characteristics of a cohort of women with ovarian cancer in Brazil are described. Additionally, the association of risk factors for ovarian cancer with the probability of having a \( BRCA1/2 \) mutation according to a widely available tool for assessing the mutation detection probability is tested.

In countries with limited access to genetic testing it is essential to acquire information on the genetic profile of the population, investigating the prevalence of mutations and the presence of possible founder mutations. Furthermore, it is important to have accessible and reliable tools for estimating the risk of having a \( BRCA1/2 \) mutation, in order to select the patients with the highest probability of having a mutation for genetic testing. Chapter 3 describes the genetic profile of 100 Brazilian women diagnosed with ovarian cancer unselected for family history of the disease. Subsequently, chapter 4 evaluates the accuracy of algorithms widely used for predicting the probability of identifying a \( BRCA1/2 \) mutation on that population, investigating whether other factors could be added to these algorithms for improving their performances.
Life-time ovarian cancer risk estimates for \textit{BRCA1/2} mutation carriers vary considerably among previous studies. A family history of ovarian cancer is considered a strong predictor for developing the disease. It has also been suggested that ovarian cancer risk vary according to the position of the mutation on the gene. It is plausible that the risk variation associated with the type of family history of cancer is related to the position of the familial mutation on the gene. In \textit{chapter 5} the combined impact of mutation position on the gene and type of cancer family history on age-related ovarian cancer risks is assessed in a cohort of women from \textit{BRCA1/2} mutation families from the northern Netherlands, a genetically homogenous population, with a relatively low frequency of mutations in certain regions of the genes. \textit{Chapter 6} investigates on a larger and more heterogeneous cohort of patients from all regions of The Netherlands whether the impact of a family history of cancer on ovarian cancer risks in \textit{BRCA1/2} mutation carriers can be explained by the mutation position on the gene.

\textbf{PART III – THE CONSEQUENCES OF RRSO}

Currently, RRSO is the only effective preventive strategy for ovarian cancer and therefore it is recommended for all women with a highly increased risk of developing the disease. Thus far there is insufficient evidence on what are the long-term consequences of the early hormone depletion induced by this procedure in this very specific group of women. In \textit{chapter 7} the impact of RRSO on the cholesterol levels of women from families with hereditary ovarian cancer is assessed. \textit{Chapter 8} systematically reviews and meta-analyses results of available studies on the effects of surgical menopause on bone health.

Finally, \textit{chapter 9} summarizes and discusses the most relevant findings of this thesis, highlighting the remaining gaps in existing knowledge and suggesting potential areas for future research.


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Part I

GENETIC TESTING IN LIMITED RESOURCES SETTINGS