Chapter 9
Summarizing discussion
SUMMARY OF MAIN FINDINGS

Women with BRCA1/2 mutations present a markedly increased risk of ovarian cancer, the most lethal of gynecologic malignancies, with life-time risk estimates reaching up to 60% for BRCA1 and 20% for BRCA2 mutation carriers.\(^1,2\) Although BRCA1 and BRCA2 have been first identified more than 20 years ago and since then a substantial body of knowledge on BRCA1/2-associated ovarian cancer has been accumulated, there are still several aspects that demand further investigation. The general aim of this thesis was to improve the overall care of women with a hereditary increased risk of ovarian cancer due to BRCA1/2 mutations. In order to achieve this goal, three aspects of BRCA1/2-associated ovarian cancer were addressed: first, optimizing the identification of a BRCA1/2 mutation in women with ovarian cancer; secondly, the variation on ovarian cancer risk in BRCA1/2 mutation carriers according to mutation position on the gene and the family history of cancer; and finally, the non-oncologic long-term adverse consequences of risk-reducing salpingo-oophorectomy (RRSO).

In recent years, international guidelines have recommended that all patients diagnosed with invasive ovarian cancer should be offered further genetic evaluation.\(^3,4\) However, in low- and middle-income countries, where resources for genetic counseling and testing are often limited, it is not possible to follow these recommendations. Part I of this thesis is focused on the identification of ovarian cancer patients with a BRCA1/2 mutation in a limited resources setting. The three chapters that compose this first part describe sub-studies of a larger project based on a cohort of women undergoing treatment or follow up for ovarian cancer at a reference medical center in Brazil. This project aimed not only to assess the genetic profile of this population and to optimize the selection of ovarian cancer patients for genetic testing, but also to evaluate the cost-effectiveness of DNA testing in this setting. Thus far, no studies had evaluated the entire coding sequence of BRCA1/2 for mutations on a cohort of patients selected solely on basis of an ovarian cancer diagnosis in Brazil, and epidemiological information on the demographic, clinical and genetic characteristics of ovarian cancer patients in the Brazilian population was lacking.

In chapter 2, the risk profile and family histories of cancer of 51 ovarian cancer patients from Brazil were described, and the association between their clinical/demographic characteristics and a family history suggestive of hereditary disease was assessed. For this initial study, the risk of having BRCA1/2 associated disease was assessed with the Manchester Scoring System, a simple method that allows the calculation of estimated risks by hand, without the need of any additional resources. In this small series, it was observed that 12 out of the 51 patients presented an estimated risk higher than 10% of having a BRCA1/2 mutation, and that having comorbidities (such as hypertension, diabetes, dyslipidemia, hormonal disorders) was associated with a lower estimated risk of having a mutation. None of the other clinical/demographic characteristics were associated with the risk of having a BRCA1/2 mutation.

Because information on the prevalence of BRCA1/2 mutations on the Brazilian population was very limited and based on few studies, none of which focusing specifically on ovarian cancer patients, we aimed to acquire more information on the genetic profile of ovar-
ian cancer patients in Brazil. Therefore, the entire coding sequence of *BRCA1* and *BRCA2* genes were screened for mutations in a series of 100 Brazilian ovarian cancer patients, unselected for family history of cancer or age at diagnosis of the disease (chapter 3). Nineteen pathogenic mutations were identified (17 in *BRCA1* and 2 in *BRCA2*), two of which had not been previously reported. Three of the *BRCA1* mutations were identified in more than one patient, two of which had been previously identified in Brazil and reported to be common in other Latin populations.

The relatively high prevalence of *BRCA1/2* mutations observed in chapter 3 emphasized the relevance of optimizing the selection of ovarian cancer patients for genetic testing, by identifying which of them are at the highest risk of having a mutation when available resources do not allow testing of all women with the disease. Hence, we evaluated the accuracy of four widely used algorithms for predicting *BRCA1/2* mutation status probability in a cohort of Brazilian ovarian cancer patients described in chapter 3 and investigated whether other factors could be added to these algorithms to improve their accuracy (chapter 4). Mutation carrier probability was calculated according to BOADICEA, BRCAPRO, Myriad and Manchester Scoring System. BOADICEA outperformed the other algorithms with a sensitivity of 73% and a specificity of 88%. Furthermore, age at menarche was significantly associated with the *BRCA1/2* mutation status (OR: 1.44, 95%CI: 1.06-1.94) and adding information on age at menarche tended to improve performance of all algorithms. These results indicate that available algorithms for estimating mutation-carrier probability can be a valuable tool for the selection of ovarian cancer patients for genetic testing and could be an important first step for the cost-conscious implementation of genetic testing on the public health care system of low- and middle-income countries.

Identifying which ovarian cancer patients have a *BRCA1/2* mutation is important not only for the patients themselves, but also for their unaffected relatives. Because *BRCA1/2* mutations are associated with increased life-time risks of breast and ovarian cancer, individuals in whom a *BRCA1/2* mutation has been identified should be offered counselling regarding strategies for prevention of breast cancer (intensive screening or risk reducing mastectomy) and ovarian cancer (RRSO). Counseling is based on age related risk estimates, however there is substantial evidence that cancer risks vary considerably among *BRCA1/2* mutation carriers. Although the basis of this inter-individual risk variation is still not fully understood, earlier studies observed that the type of family history of cancer and the mutation position on the gene were associated with ovarian cancer risks in *BRCA1/2* mutation carriers. Part II of this thesis focuses on the evaluation of these two possible sources of variation on ovarian cancer risk among *BRCA1/2* mutation carriers.

Chapter 5 focuses on the combined impact of mutation position on the gene and type of cancer family history on the ovarian cancer risk of women from families with *BRCA1/2* mutations from the northern part of the Netherlands. We observed that among women from *BRCA1* families, mutations within the central regions of *BRCA1/2* (Ovarian Cancer Cluster Regions, OCCR) were statistically significant associated with higher risks of ovarian cancer, even when adjusting for the family history of cancer. For women from *BRCA2* families the type of cancer family history had a stronger impact on ovarian cancer risks, and this impact
remained stable after taking the mutation position on the gene into account, suggesting that among \textit{BRCA2} mutation carriers the family history effect is not completely explained by the mutation position on the gene. However, the population included in this study was ascertained from a limited geographical area, which was reflected by a limited spectrum of observed mutations across the \textit{BRCA1/2} genes. Therefore, in a subsequent study we assessed whether the family history effect could be explained by the mutation position on the gene on a larger, nationwide cohort study (Hereditary Breast and Ovarian Cancer study Netherlands – HEBON), including \textit{BRCA1/2} mutation carriers from all regions of the Netherlands (chapter 6). A significant association between type of family history of cancer and ovarian cancer risk was observed both among \textit{BRCA1} and \textit{BRCA2} mutation carriers. After adjusting for mutation position on the gene, the association between family history of cancer and ovarian cancer risk remained comparably strong for \textit{BRCA1} and \textit{BRCA2} mutation carriers, although no longer significant. These results confirmed the findings from the previous study, suggesting that the effect of family history of cancer is not completely explained by mutation position on the gene, and that other genetic and environmental factors may be involved.

Regardless of the variation on cancer risks estimates and the possible factors associated with it, ovarian cancer risks are incontestably increased in \textit{BRCA1/2} mutation carriers. Because there are currently no effective screening options available for the early detection of this malignancy, women with \textit{BRCA1/2} mutations are advised to have timely RRSO, as it is the only effective way of reducing the ovarian cancer risk in this population. Part III of this thesis focuses on the long term consequences of the early acute menopause induced by RRSO, especially on the non-oncological health risks of these women. Understanding the possible consequences of RRSO becomes increasingly important, since, with the increasing uptake of RRSO, \textit{BRCA1/2} mutation carriers tend to live longer and with less cancer-related morbidity.

It has been long known that the changes in hormonal levels observed during menopausal transition are associated with metabolic changes. Furthermore, it had been previously observed that premenopausal bilateral oophorectomy was associated with a more atherogenic lipid profile (based on increased total cholesterol and decreased HDL-cholesterol) and with higher risks of cardiovascular disease when compared to natural menopause. On chapter 7, the impact of premenopausal RRSO on the cholesterol profile of women with hereditary increased risk of ovarian cancer was evaluated. We observed that, even though women who had RRSO before the mean age of natural menopause (52 years of age) presented an overall healthier life style when compared to age-matched women from the general population, RRSO was associated with a more atherogenic cholesterol profile, with lower HDL-cholesterol levels and higher non-HDL-cholesterol levels.

Besides its impact on lipid metabolism, natural menopause has also been associated with decreased bone mineral density (BMD) and increased fracture rates. Thus far it was unclear whether surgical menopause enhanced this effect, since previous studies on the nature and magnitude of the impact of surgical menopause on bone health reached inconsistent results. Therefore we performed a systematic review of the literature and meta-analyses of the available estimates of the impact of surgical menopause on BMD and on fracture
rates (chapter 8). In this review we observed that BMD was significantly lower in women who had surgical menopause than in premenopausal age-matched women. However, when comparing women with surgical and with natural menopause, no differences were observed. Furthermore, no impact of surgical menopause on fracture rates could be observed. Nevertheless, the included studies were prone to bias and had a relatively short follow-up, hence their observations need to be interpreted carefully.

GENERAL DISCUSSION

When a woman is suspected to have a hereditary increased risk of ovarian cancer she must face difficult decisions. First, there is a decision regarding the (timing of) genetic testing and the possible psychological distress associated with it. Furthermore, patients may worry about genetic discrimination. Since individuals applying for a private health/life insurance are generally obliged to disclose all relevant information, it is a reasonable concern that insurers could discriminate based on genetic testing results, imposing higher premiums or deny coverage for those considered to be at a higher health risk. Aiming to manage concerns about genetic discrimination and to avoid that patients deny genetic testing because of such concerns, policymakers from several countries (including European countries and Brazil) formulated laws restricting the use of genetic information by insurance companies. In patients who decide to undergo testing and in whom a BRCA1/2 mutation is identified other concerns arise, such as disclosure of the information to other members of the family, family planning, options to prevent offspring with the same mutation, cancer risks, preventive strategies etc. Broadening our understanding of aspects associated with hereditary ovarian cancer, such as who are the patients at the highest risk of carrying a mutation, what are their age-related cancer risks and what are the best strategies for cancer prevention, as well as the potential side effects of these preventive strategies, is essential to optimize counseling of these women, by providing them with accurate information to support the decisions they have to make during phases in their life.

The first step on improving care of women at a hereditary increased risk of ovarian cancer is identifying which ovarian cancer patients would benefit the most from genetic counseling and testing. Most recent international guidelines recommend that all women with invasive ovarian cancer are offered further genetic evaluation, however, in low- and middle-income countries it is not always possible to follow this recommendation. The prevalence of BRCA1/2 mutations in an unselected cohort of Brazilian ovarian cancer patients reported in this thesis (19%) is amongst the highest described in the literature. Another recent study investigating the germ line BRCA1/2 mutation status of 349 unrelated Brazilian individuals at risk for hereditary breast and ovarian cancer (with personal and family history of breast and/ or ovarian cancer) observed an even higher proportion of BRCA1/2 mutations (21.5%). The high prevalence of BRCA1/2 mutations observed in these studies emphasizes the relevance of this topic for Brazilian public health and the importance of discussing the implementation of genetic counseling in the Brazilian public health care system. It is important to remark that the health expenditure per capita in Brazil is lower than in developed countries and below
what is determined by the Brazilian constitution (which states that no less than 15% of the net current revenue of the respective financial year should be invested in the health system). If the determination made by the Brazilian constitution was respected, more funds would be available for the public health care system and could be invested on the implementation of a genetic counseling network.

After identification of patients with a BRCA1/2 related ovarian cancer, it is important to identify family members at risk and offer them counseling regarding their possibly increased cancer risks, testing for the familial mutation and, for those who test positive, timely preventive measures to reduce their highly increased cancer risks. This counseling is currently based on age-related risk estimates, which show varying results on the literature. From all factors possibly underlying the observed variation on ovarian cancer risks among BRCA1/2 mutation carriers, this thesis explores the impact of the family history of cancer and the mutation position on the gene on the estimated age-related ovarian cancer risks of these women.

Regarding the impact of a family history of ovarian cancer on ovarian cancer risks, the magnitude of estimated effects varied considerably across previous studies, with estimates ranging from approximately 2.0 to 7.0 for women with one affected relative and from 10.0 to 24.0 for women with multiple affected relatives. These estimates were mostly derived from cohorts of women with unknown BRCA1/2 mutation status. Among BRCA1 mutation carriers, a hazard ratio of 1.6 per relative affected with ovarian cancer had been previously reported. The results observed on this thesis agree with this previous observation, suggesting that the impact of family history of breast or ovarian cancer on ovarian cancer risks in women with a known hereditary predisposition for the disease is not as large as the impact estimated for women with unknown mutation status.

Concerning the mutation position on the gene, the first indications of a possible genotype-phenotype correlation among BRCA1/2 mutation carriers derived from studies that observed significantly different proportions of breast:ovarian cancer in families with mutations in different portions of the genes. Subsequently, studies further investigated the differences in the proportion of breast:ovarian cancer, evaluating possible differences in cancer risks according to the mutation position on the gene. These studies confirmed the previous observations, reporting a higher proportion of ovarian over breast cancers in families with mutation in central portions of the genes and observing a lower risk of ovarian cancer (RR 0.81) for mutations in the 3’ third of BRCA1 and a higher risk of the disease (RR 1.88) for mutations on the central portion of BRCA2. The central portions of BRCA1/2 were thus named Ovarian Cancer Cluster Regions (OCCR). Recently, the largest cohort study published to date confirmed the earlier genotype-phenotype correlation, observing higher risks of ovarian cancer relatively to breast cancer on the central regions of both BRCA1 and BRCA2. That study additionally observed higher breast cancer risks relatively to ovarian cancer risks for mutations on the 5’ and 3’ ends of BRCA1/2, suggesting the existence of Breast Cancer Cluster Regions (BCCR). The estimates of the impact of mutation position on ovarian cancer risks reported in this thesis are comparable in magnitude to the other reports available in the literature.
It is important to remark that regardless of the possible sources of ovarian cancer risk variation in \textit{BRCA1/2} mutation carriers and despite the relevance of understanding this variation to provide the patients with the most precise risk estimates possible, it is not clear how far variation on risk estimates will impact patients’ decision making. It has been suggested that an individual’s perceived risk of cancer, rather than objective numeric risk estimates, plays a major role on decision making regarding genetic testing and risk reducing options.\textsuperscript{21} Furthermore, a number of other factors associated with the uptake of risk reducing surgery has been identified, such as age, education level, family history of breast and ovarian cancer, as well as psychosocial factors and personal values and beliefs.\textsuperscript{22,23}

Irrespective of the several aspects that may impact women’s decision-making regarding cancer risk-reducing surgery, recent reports indicate that the uptake of RRSO among \textit{BRCA1/2} mutation carriers is high.\textsuperscript{24,25} The long-term consequences of the early menopause induced by RRSO in the general health of this very specific group of women remain unclear. Current guidelines for the management of women with hereditary increased risk of ovarian cancer state that the possible consequences of early menopause should be discussed with women considering RRSO, and that, in the absence of contra-indications, hormonal replacement therapy (HRT) should be offered to women who had premenopausal RRSO up until the average age of natural menopause.\textsuperscript{26,27} However, none of these guidelines offer specific guidance regarding the need of a long term follow up or additional screening for non-malignant disease for these women. In the absence of clear guidelines for the follow up of \textit{BRCA1/2} mutation carriers after RRSO, it is possible that additional interventions might be offered for this patient group, which could induce unnecessary costs to the health care system and additional burden to the patients in case the impact of RRSO on their health risks does not justify such measures. Previous literature on the topic provided conflicting results, which were most often based on cohorts of patients with oophorectomy for several different indications, not only due to an increased risk of ovarian cancer. Therefore the third part of this thesis investigated the impact of RRSO on the non-oncological health risks of women at hereditary increased risk of ovarian cancer. The results observed indicate that the impact of RRSO on lipid and bone metabolism is not a reason for major concern. However, although these results are an important contribution to the existing knowledge on the topic and are relevant for professionals involved in counseling and long term health care of these women, it is important to remark the possible limitations of the studies presented on this thesis. The impact of surgical menopause by RRSO on lipid profile was investigated after a median follow-up time of only 5 years. Concerning the impact of surgical menopause on bone health, important methodological limitations were identified on the studies included in our systematic review, and high heterogeneity was observed between the studies included in the meta-analyses.

It should be noted that this thesis focused only on \textit{germ line mutations} on \textit{BRCA1} or \textit{BRCA2}, which explain the majority of hereditary ovarian cancer cases and are the most widely studied causes of this condition. The remaining cases of hereditary ovarian cancer are mostly explained by mutations in one of the mismatch repair (MMR) genes in Lynch syndrome (which is associated with cases diagnosed at early stages and with better prognosis\textsuperscript{28,29}), or several other genes of lower penetrance that have been more recently iden-
tified,\textsuperscript{30,31} while a proportion of cases remain unexplained. The frequency of mutations in newly identified genes, as well as the association between mutation frequency and clinical characteristics that could be used as predictors of mutation status (e.g. histology) is currently under investigation.\textsuperscript{32} With recent advances on molecular biology techniques and the advent of next-generation sequencing, genetic testing has become more affordable and wide-spread. Consequently, the investigation of hereditary ovarian cancer is rapidly shifting towards a multi-gene panel testing, rather than sequencing of \textit{BRCA1/2} exclusively. Although this approach represents an important advancement on the field, since it will increase the diagnostic capability of genetic testing allowing the identification of more individuals at increased risk of ovarian cancer, it also raises important concerns. The information obtained with multi-gene testing can be difficult to interpret, since the cancer risks associated with mutations in many of the tested genes have not been precisely estimated yet, and it is unclear how patients with mutations in these genes should be counseled regarding preventive strategies.

Furthermore, germ line mutations are not the only possible cause of impaired expression of \textit{BRCA1/2}. Somatic mutations of \textit{BRCA1/2} have also been identified in ovarian cancer tissue.\textsuperscript{33} Moreover, expression of \textit{BRCA1/2} genes may also be impaired due to epigenetic alterations, most importantly hypermethylation of promoter regions, a phenomenon that has been associated with inactivation of genes.\textsuperscript{34} Hypermethylation of the promoter region of \textit{BRCA1} has been detected in 5\%-15\% of sporadic ovarian cancer cases.\textsuperscript{35} Tumors associated with impaired expression of \textit{BRCA1/2} due to other causes might have a similar phenotypic behavior as those associated with germ line \textit{BRCA1/2} mutations, which might have clinical implications. Because in recent years a promising novel therapy for ovarian cancer has been proposed (based on the inhibition of poly [ADP-ribose] polymerase), and the highest response rates to this new therapy have been observed among \textit{BRCA1/2} mutation carriers,\textsuperscript{36} it is important to identify not only tumors associated to \textit{BRCA1/2} germ line mutations but also those that might behave similarly. In line with this demand, a study aiming to implement a diagnostic workflow in which genetic testing for newly diagnosed ovarian cancer patients is initiated by the pathologist, using tumor tissue, is currently ongoing.\textsuperscript{37} This approach could be useful not only as a pre-screening test for germ line mutations but also for the identification of sporadic cases associated with somatic \textit{BRCA1/2} mutations. The advantage of this approach over germ line mutation testing is that patients whose tumors tested negative for a mutation would not need genetic counseling, which is costly. This strategy might be of special interest for low- and middle-income countries, since it could reduce the number of genetic counseling procedures, potentially reducing the costs associated with \textit{BRCA1/2} mutation testing in ovarian cancer patients.\textsuperscript{37}
IMPLICATIONS FOR CLINICAL PRACTICE

The first part of this thesis concentrates on the implementation of genetic testing for \( BRCA1/2 \) mutations in ovarian cancer patients from low- and middle-income countries, where resources for health care are limited and genetic diagnosis is often not available. In such countries there will always be a dilemma between favoring equality (e.g. offering the same options to all patients) or equity (e.g. offering each patient what they need).\(^{38}\) If genetic diagnosis is not available for all patients, should it be offered to some? The frequency of \( BRCA1/2 \) mutations observed in the studies from Part I of this thesis, which is among the highest reported in the literature, stresses the urgency of raising awareness over hereditary ovarian cancer in low- and middle-income countries, where genetic evaluation is not yet offered to all women diagnosed with invasive ovarian cancer, as currently recommended by international guidelines. In the Brazilian setting in particular, approximately 6000 cases of ovarian cancer are diagnosed yearly.\(^{39}\) Considering that 70% of the Brazilian population relies on the public health care system, on average 4200 ovarian cancer patients would need to be offered genetic testing with public resources every year. Taking into account the high frequency of \( BRCA1/2 \) mutations observed, we believe that offering genetic testing to these patients would be affordable and very beneficial in the Brazilian setting. The next step would be to implement a network of services for counseling of family members and cancer prevention strategies for unaffected mutation carriers.

Furthermore, our observations suggest that available algorithms for estimating the mutation carrier probability, although still limited in their accuracy, could be an important tool for selecting patients (and relatives) that would profit the most from genetic testing when resources are not enough to test all ovarian cancer patients. In the cohort of ovarian cancer patients evaluated in this thesis, BOADICEA presented the highest sensitivity (74%) from all evaluated algorithms. BOADICEA is a user-friendly algorithm which is freely available online. Spreading its use could be a first step for the implementation of genetic testing on the public health care system of low- and middle-income countries.

The next concern regarding genetic testing of ovarian cancer patients in a setting of limited resources are the additional preventive actions that will be necessary when a patient tests positive for a mutation. Family members will also need to be tested. Genetic counseling and risk-reducing interventions should be offered to the women in whom a pathogenic mutation is identified. If these services are not available, genetic testing could create major anxiety without bringing any real benefit for the patients and their families. Thus, genetic testing should only be implemented when resources for the appropriate management of mutation carriers and their families are also available.

The second part of this thesis investigated possible sources of variation on ovarian cancer risk in \( BRCA1/2 \) mutation carriers, indicating that the impact of the two most widely studied potential sources of risk variation (mutation position on the gene and family history of cancer) is likely to be small. Although the level of risk change that would justify altering patient counseling is still to be determined, we do not believe that the observed impact of family history and mutation position on the gene on ovarian cancer risks is large enough
to adjust the counseling of BRCA1/2 mutation carriers according to these factors. Because ovarian cancer is a disease with very dismal prognosis and there are no effective screening strategies available for the early detection of this malignancy, the potential consequences of these observations for clinical practice are null and should be considered with caution.

Finally, the third part of this thesis investigated the potential long term sequelae of RRSO. Our results demonstrated that RRSO is associated with a more atherogenic cholesterol profile after 5 years of follow up. This observation emphasizes the importance of advocating a healthy life-style and raising awareness over cardio-metabolic health during the perioperative counseling of these women. Additionally, this information should be taken into account by health care professionals evaluating the cardiovascular risk of women with a hereditary increased risk of ovarian cancer who had early surgical menopause. Lastly, the impact of RRSO on bone health was investigated by meta-analyzing the available evidence on the topic. This systematic review demonstrated that there is not enough evidence in the literature that RRSO impacts fracture prevalence to a greater extent than natural menopause. However the available studies on the topic present heterogenous results and important methodological limitations. Although better, long term studies are still needed, currently available literature does not justify systematic BMD screening or any additional measures for the prevention of osteoporosis or fractures after RRSO.

FUTURE PERSPECTIVES

Although we observed a relatively high frequency of BRCA1/2 mutations, and investigated possible strategies for refining the selection of patients for genetic testing, more research is still needed before genetic testing can be implemented in the public health care system of countries with limited resources for health care. Future research should focus especially on the cost-effectiveness of genetic testing and counseling in these settings. With the aim of meeting this demand, an ongoing study, which is part of the same project as the studies presented on Part I of this thesis, is focused on assessing the cost-effectiveness of genetic testing of ovarian cancer patients in the setting of public health care system in Brazil.

The second part of this thesis indicates that the type of cancer family history is associated with the ovarian cancer risks in BRCA1/2 mutation carriers, and that part, but not all of this effect may be explained by a genotype-phenotype correlation. Efforts have been made on identifying other genetic and non-genetic risk modifiers among BRCA1/2 mutation carriers, however, so far the sources of variation on cancer risks in this patient group have not been fully elucidated. Furthermore, the reported variation on ovarian cancer risks according to the mutation position on the gene are based on epidemiological studies and the biological mechanisms underlying this genotype-phenotype correlation remain poorly understood. Therefore, more research aimed at elucidating the molecular mechanisms affecting tumorigenesis in different BRCA1/2 mutations is necessary. Reverse genetics techniques, such as targeted gene editing (CRISPR/Cas, TALEN, ZINC finger), could be applied to mimic various types of mutations (not only in BRCA1/2 but also in other genes known to interact with BRCA1/2 on DNA repair pathways). Such studies could be performed in in-vivo models, to investigate the
phenotypes associated with different types of mutations, on different parts of the gene, elucidating the role of different mutations in tumorigenesis. Furthermore, gene editing could also be used to investigate the phenotype associated with different BRCA1/2 mutations in specific cell lines, such as breast cancer cell lines or ovarian cancer cell lines to study the underlying molecular mechanism in cell proliferation.

Furthermore, other genes associated with hereditary ovarian cancer besides BRCA1/2 have been identified. Mutations in such genes (BARD1, BRIPI, CHEK2, MRE11A, MSH6, NBN, PALB2, RAD50, RAD51C, RAD51, TP53) may justify genetic counseling and preventive measures. More studies are needed to clarify the incidence of mutations on genes other than BRCA1/2 in cohorts of patients with ovarian cancer and to estimate the age related ovarian cancer risks associated to these mutations in prospective observational studies. More precise information on prevalence and penetrance of such mutations is essential for defining the implications they should have on counseling regarding preventive strategies and on the clinical management of mutation carriers. Furthermore, this information would be important when evaluating the cost-effectives of multi-gene testing and comparing it with the testing of highly penetrant mutations only. With the advent of next generation sequencing, costs of multi-gene testing are rapidly decreasing. Even on the setting of a middle-income country like Brazil, we estimate that costs of multi-gene testing would be similar to BRCA1/2 testing through next generation sequencing in a centralized public testing facility. Therefore, it is likely that multi-gene testing would be cost-effective for ovarian cancer prevention when compared to testing of BRCA1/2 alone.

Lastly, this thesis explores the consequences of RRSO on the non-oncological health risks of BRCA1/2 mutation carriers. It is important to further investigate the effects of RRSO on quality of life and non-oncological disease in prospective studies with appropriate control groups and longer periods of follow-up. It is also important to explore alternative strategies for the prevention or early detection of ovarian cancer, which, when proven effective, could enable screening for the disease and represent an alternative for RRSO. It has been observed that tumor cells release circulating tumor DNA (ctDNA) into the blood and that the use of ctDNA as a liquid biopsy could potentially improve cancer diagnosis, monitoring of treatment and early identification of disease recurrence. Furthermore it was reported that micro RNAs (small non-coding RNA molecules involved in the post-transcription regulation of gene expression) are abnormally expressed in ovarian cancers and might have a potential role as biomarkers for early diagnosis of the disease. More efforts on the identification of novel biomarkers, on the development of multi-marker panels for the effective screening of ovarian cancer and on the elucidation of the role of ctDNA and microRNAs as potential tools for the early diagnosis of the disease, are therefore urged.

Furthermore, because it was postulated that the majority of high-grade serous ovarian cancers actually originate from the fallopian tube, research should focus on proving this hypothesis. Together with the concerns regarding potential adverse consequences of RRSO, this theory encouraged the proposal of an alternative strategy for ovarian cancer prevention on high-risk women: early salpingectomy with delayed oophorectomy. This approach would delay the onset of menopause and consequently the short and long-term mor-
bidity associated with it. However, it remains uncertain whether this intervention will indeed reduce ovarian cancer risk and to which extent. Because this strategy is still experimental and its safety has not been determined yet, it should only be performed in the context of clinical studies. Further research is needed to investigate whether it is safe to perform salpingectomy alone, delaying oophorectomy to later ages in women at high risk for ovarian cancer. For the time being timely RRSO is a safe strategy that has been proven to reduce ovarian cancer risks by up to 96% without severe consequences for women’s health. RRSO should be strongly advised for all BRCA1/2 mutation carriers at the appropriate age window, before the incidence of ovarian cancer starts to rise, followed by appropriate counseling on how to minimalize potential consequences of RRSO on their cholesterol profile (e.g. with a healthier diet and frequent physical activity) and no additional interventions concerning BMD assessment or osteoporosis prevention besides what would normally be offered to women from their age group.
Chapter 9

Summarizing discussion

References


44. Seidman JD, Zhao P, Yemelyanova A. „Primary peritoneal“ high-grade serous carcinoma is very likely metastatic from serous tubal intraepithelial carcinoma: assessing the new paradigm of ovarian and pelvic serous carcinogenesis and its implications for screening for ovarian cancer. Gynecol Oncol 2011 Mar;120(3):470-473.

