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GENETIC PREDISPOSITION FOR CANCER; GENES AND GENETIC COUNSELING

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To my family

ABSTRACT

Breast cancer accounts for one third of all female cancer cases worldwide. A hereditary component accounts for 10-15% of all breast and ovarian cancer cases. The overall aim of this thesis is to evaluate and improve genetic diagnostic and genetic counseling in hereditary cancer patients.

A total of 215 counselees were enrolled to a questionnaire study which aimed to conceptualize risk perception and worry for cancer before and one week after initial oncogenetic counseling and one year after completed genetic investigations. The most incorrect risk perceptions were identified among unaffected counselees with low or the same risk than the general population. The unaffected counselees showed more accurate risk perceptions and decreasing worry for cancer after oncogenetic counseling. The affected counselees overestimated the risk of cancer for children and did not show any change in cancer worry. The relevance of preventive programs was well understood among counselees. (Paper I)

Germ-line mutations in *BRCA1* and *BRCA2* genes predispose to high risk for breast- and ovarian cancer. Penetrance of cancer among *BRCA1/2* mutation carriers is incomplete suggesting that genetic- and environmental factors play a role as risk modifier. A large-scale genome-wide association study was performed to identify genetic modifiers of risk for developing breast and ovarian cancer in *BRCA1* mutation carriers. The results revealed five SNPs on 19p13 associated with breast cancer risk. Two of these SNPs showed independent associations (rs8170, HR 1.26, 95% CI 1.17-1.35 and rs2363956 HR 0.84, 95% CI 0.80-0.89). The two SNPs showed similar association with estrogen receptor-negative tumors and with triple-negative tumors (Paper II)

A randomized questionnaire study was conducted as described above (Paper I). The aim was to evaluate the oncogenetic counseling process and to compare the impact of the initial part of the oncogenetic counseling, when conducted via telephone versus in-person. The results indicate that telephone pre-counseling works as well as in-person pre-counseling. The counselees showed high satisfaction rates with the oncogenetic counseling process. A considerable number of counselees experienced difficulties with the process of creating a pedigree and dissatisfaction with information on surveillance and prevention. The counselees were unsatisfied with the received emotional support during genetic counseling and information on recommended cancer prevention and surveillance. (Paper III)

To identify additional breast cancer predisposing genes, a genome-wide linkage study on fourteen large non-BRCA1/2 hereditary breast cancer families was performed. The linkage analyses identified five candidate loci with a HLOD above one. Regions indicating evidence of linkage are located on 6p21, 8q13, 11p12, 18q21 and 22q11. (Paper IV)

LIST OF PUBLICATIONS

This thesis is based on the following four publications.

I. **Rantala J**, Platten U, Lindgren G, Nilsson B, Arver B, Lindblom A, Brandberg Y
Risk perception after genetic counseling in patients with increased risk of cancer
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CONTENTS

LIST OF ABBREVIATIONS

BRCA1	Breast cancer susceptibility gene 1
BRCA2	Breast cancer susceptibility gene 2
DNA	Deoxyribonucleic acid
DS	Double strand break
ER	Estrogen receptor
GWAS	Genome wide association study
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
LD	Linkage disequilibrium
LOD	Logarithm of the odds
NHEJ	Non-homologous end-joining
NPL	Non-parametric linkage
OR	Odds ratio
PL	Parametric linkage
PR	Progesterone receptor
SNP	Single nucleotide polymorphism
UTR	Untranslated region

1 INTRODUCTION

Cancer befalls individuals not only at a physiological condition and not only in individual manner. Individuals afflicted with cancer and their family members search for explanatory factors for disease and feel often psychosocially deprived. Many family members with hereditary cancer in the family have experienced the consequences of the disease by seeing suffering and through the loss of close relatives from cancer. Many of these individuals seek support from oncogenetic clinics to investigate the legitimacy of their worry and to receive information about preventive interventions. Genetic counseling in familial cancer presents therefore unique challenges. Counselees may wish to discover their own risk of developing cancer and behaviors to reduce the risk, or to find out whether they are carriers of deleterious mutation presented in the family. If they have cancer themselves, they may wish to know whether they have a detectable cancer predisposing mutation. An accurate understanding the risk of developing cancer reduces psychological distress among low-risk individuals. Reduction of economic costs due to less unnecessary examinations benefits health care system. Counseling about cancer prevention is crucial in order to reduce cancer incidence and mortality in high-risk individuals.

Unraveling mechanisms behind cancer has produced evidence that cancer is a common disease and that it is multi-factorial in nature involving the interaction of genetic and environmental factors clustering in the families. Most cancers develop due to somatically acquired mutations, but mutations can also be present in the germ-line, predisposing the individual to increased risk of developing cancer. Inherited mutations in *BRCA1* and *BRCA2* genes cause early onset breast- and ovarian cancer [1, 2].

The *BRCA1* and *BRCA2* and other high-risk predisposing genes account for approximately 15-20% of all familial breast cancer cases [3]. Due to incomplete penetrance, not all mutation carriers will develop cancer, suggesting other modifying genetic and environmental factors clustering in families. In the past few years, candidate gene approaches to find associations between common polymorphisms and breast/ovarian cancer risk have been replaced by studies of bigger consortiums, such as CIMBA, with large sample sizes. CIMBA collaboration studies (Consortium of

Investigators of Modifiers of *BRCA1/2*) and availability of high throughput techniques have made it possible to reliably investigate modifying factors. CIMBA consortium has studied 1) polymorphisms in candidate genes indicated by smaller studies in mutation carriers or in the general population, 2) polymorphisms from genome-wide association studies of breast/ovarian cancer in the general population, and 3) associations in *BRCA1/2* carriers with a genome-wide association approach [4]. Implications for risk prediction consider the genetic variations either in isolation or jointly with other risk modifiers. In the future mutation carriers could benefit from clinical applications and receive individualized risk management.

Intensive research has been trying to reveal novel high-penetrance breast cancer genes, but genetic determinants of many of the common familial cancers have remained unknown. Today the effort is to identify moderate and low penetrance genes, which in combination with other genetic and environmental variants can contribute to increased risk in some families. Family based linkage study-strategy aims to identify moderate penetrance genes, and candidate gene approach tries to reveal low penetrance genes. The missing heritability for familial cancers includes additional SNPs, causal SNPs/variants and genetic heterogeneity (gene-gene interaction and gene-environmental). Genetic heterogeneity presents a major obstacle. While rare high- and moderate risk variants explain less than 20% of familial risk of breast cancer, the other identified variants contributes less than 10% [5].

1.1 BREAST CANCER

The progress from normal cell to cancerous cell is described as a multistep model and involves the acquisition of a number of genetic modifications. Carcinogenesis is characterized by ultimately re-programming the cell to undergo uncontrolled cell division and resulting malignant transformation by causing an abnormal balance between normal proliferation and cell death and leading to the somatic evolution of cancer cells by natural selection. A massive proliferation and genomic instability gives a foundation for effective evolutionary process. A new generation of mutations will arise giving the tumor better survival characteristics under poor conditions or the ability to persist the immune response of the host or a treatment. Combinations of alterations, that can be tolerated and co-works optimally, will survive. This rapid proliferation of

cells can lead to benign first stage tumors such as atypical hyperplasia. Malign tumor development requires several (5-10) mutations in critical genes. Usually, malign tumors have approximately 80 different mutations in the genome. Not all of these mutations are probably crucial for tumor development and only a minority of these mutations is part of the important stages such as angiogenesis and resistance for apoptosis. Other mutations in tumor genesis have more basal function such as ion-transport and RNA metabolism [6, 7].

1.2 EPIDEMIOLOGY OF BREAST CANCER

Breast cancer remains one of the most immense health related problems for women with an annual global incidence rate of 1.4 million breast cancer cases (23% of all female cancer cases). Global mortality is around 460 000, representing 14% of all female cancer deaths [8]. In Sweden the incidence is close to 8000 accounting for 30% of all female cancer cases [9]. Sweden is one of the countries showing reducing mortality. Today there is a five-year survival rate of 85% compared to only 65% in the 1960´s.

Over the last decades breast cancer incidence has increased globally and incidence is higher in more recent birth cohorts due the consequences of the changing patterns in environmental, lifestyle, reproductive and hormonal factors. The same phenomenon has not been shown for ovarian cancer [4, 10]. Incidence among immigrant women from developing countries is often lower compared to local populations in developed countries demonstrating the influence of life style factors to cancer risk. Mortality does not differ between immigrants and local populations in developed countries indicating equal access prevention programs. Some sub-populations of immigrants have poorer survival rates, which emphasize the need for targeted interventions for women who are not attending screening or not following prescribed cancer treatment. Irrespective of country of birth, women with the highest socioeconomic status often have higher incidence but better survival compared to women with the lowest socioeconomic status [11, 12].

1.3 GENETIC RISK FACTORS

1.3.1 *BRCA1* and *BRCA2* genes

1.3.1.1 Inheritance of BRCA1 and BRCA2

A mutation of *BRCA1* and *BRCA2* genes are inherited in an autosomal dominant mode with incomplete penetrance. Thus, only one defect allele from one ancestor causes lifetime predisposition for developing cancer. Statistically 45-65% of mutation carriers will develop cancer by age 70 and 11-40% will develop ovarian cancer due to this predisposition indicating that other factors modify the risk [13].

1.3.1.2 The function of BRCA1 and BRCA2 genes

The functions of tumor suppressor genes *BRCA1* and *BRCA2* are to block cell division and to promote DNA reparation. *BRCA1* mediated reparation of double strand brakes (DBs) occurs via two major pathways that are homologous recombination and non-homologous end-joining (NHEJ). Homologous recombination is a vital process using undamaged sister chromatid as a template to carry out repairs of breaks, while in NHEJ overhang micro homologies is used to guide repair. In addition, *BRCA1* plays a role as repairer of inter-strand crosslink. *BRCA1* has even other functions such as recruitment to DNA damage sites, DNA end resection and checkpoint during different cell division phases. In contrast to multifunctional *BRCA1*, the prime function of *BRCA2* is to work as a mediator of the core mechanism of homologous recombination. *BRCA2* works in conjunction with *BRCA1* to guard the genome from double-strand DNA damages during the replication process [14]. *BRCA2* is a crucial component which brings the RAD51 module onto single-stranded DNA [15].

The loss of wild-type alleles of *BRCA1* gene in the majority of breast cancer tumors among women carrying an inherited heterozygous mutation in the breast cancer susceptibility gene underlines the crucial function as a tumor suppressor gene. The tumor suppressor gene can be inactivated by a mutation in gene sequence or by deletion of chromosome regions including the gene. In order to inactivate the entire gene and to induce the carcinogenesis, both of the gene copies need to be inactivated. Transcription of tumor suppressor genes can be silenced by tumor cells by way of methylation of

promoter sequence [16]. *BRCA1* gene has a vital role in genomic integrity since a bi-allelic deficiency in *BRCA1* gene leads to early embryonic lethality and lack of functional *BRCA1* gene causes a proliferation defect or cell death.

The inactivation of tumor suppressor genes is described as a two hit model, where tumor development triggers when both alleles become inactivated after independent mutations [17]. Cells from a wild type individual has to lose one allele first to receive the same probability to develop a tumor as cells from an individual with inherited heterozygous mutation. Consequently, sporadic tumors occur less frequently than tumors in mutation carriers. However, it has been suggested that in a minority of tumor suppressor genes a single hit is sufficient to contribute to tumorigenesis. Reduction in gene dosage prevents the wild type allele to sustain its normal function. This condition is entitled as haploinsuffiency. An inactivation of an allele leads to genetic instability that promotes additional genetic alterations in heterozygous BRCA1/2 cells [18, 19, 20] and makes breast epithelial cell vulnerable to mitotic recombination [21]. Haploinsuffiency also delays DNA damage recognition, disturbs cell cycle checkpoint and inhibits DNA repair [19, 22].

1.3.1.3 Swedish BRCA1 and BRCA2 founder mutations

The most common deleterious mutation in Sweden, c.3171insTGAGA in *BRCA1* gene also known as “the west coast mutation”, originated 50 generations ago. This mutation accounts for up to 77% of identified mutations in a limited part of western Sweden. Other recurrent *BRCA1* mutations are c.2594delC, c.1806C>T, c.1201del11 occurring primarily in southern Sweden and duplication of exon 13 also known as the “Vallonish” founder mutation. A mutation, c.4486delG is the most common of the *BRCA2* mutations in Sweden [23].

1.3.1.4 Prevalence of BRCA1 and BRCA2 mutations

Mutation prevalence varies depending on ethnicity and is influenced by founder mutations. Penetrance may be predisposed by mutation specific phenotypes and by genetic and environmental modifying factors [24].

The prevalence of *BRCA1/2* mutation carriers in the European population is approximately 0.2% for *BRCA1* and 0.1% for *BRCA2* mutations. In other populations, such as the Canadian population, the frequencies are higher (0.32% and 0.69%) [25]. In Stockholm region frequency of *BRCA1* mutation in unselected breast cancer case cohort was found to be <1% [26]. In some specific case cohorts in Sweden such as in young females with breast cancer, 6.8% of the cases carried deleterious *BRCA1* mutation and 2.1% *BRCA2* mutation [27]. In unselected ovarian cancer cohort in Sweden the *BRCA1* and *BRCA2* mutation frequencies were 7.4% respective 0.6% [28].

1.3.1.5 The risk of developing cancer

Carriers of germ-line mutation in *BRCA1* have an average cumulative risk by age 70 of 65% for breast cancer and 39% for ovarian cancer. The equivalent estimates for *BRCA2* carriers are 45% and 11% [13]. The risk of developing cancer in *BRCA1* and *BRCA2* mutation carriers varies depending on age of diagnosis and the type of cancer (i.e. unilateral and contralateral breast cancer or ovarian cancer) among family members. The differences in risks of developing cancer among families suggest that there are additional genetic and environmental modifiers.

Mutation in the central region of *BRCA1* (nucleotides 2401-4190, exon 11) confers a lower risk for breast cancer (RR 0.71) [29, 30]. *BRCA2* mutation families with ovarian cancer are more likely to harbor mutations in the central region of *BRCA2* gene (nucleotides 3035-6629, exon 11), also referred as an ovarian cancer cluster region (OCCR), than elsewhere in the gene. The OCC-region is associated with a higher ratio of ovarian than breast cancer [31, 32].

Deleterious germ-line *BRCA1* and particularly *BRCA2* mutations contribute to predisposition for cancer in other organs. A germ-line *BRCA2* mutation confers 8.6 fold risk, implicating 15% cumulative risk of developing prostate cancer by the age of 65 [33, 34]. Prostate cancer patients harboring a germ-line *BRCA2* mutation show more aggressive outcome of cancer with poorer survival, independent of other predictors [35, 36]. It has been found that risk of dying in prostate cancer in *BRCA2* families was 70% higher than in *BRCA1* families [37]. Increased risk of pancreas cancers (RR 4.1) and uveal

melanoma (RR 99.4) has also been confirmed among *BRCA2* carriers as well as risk for esophagus- (RR 4.1) and stomach cancer (RR 2.7) [34].

For *BRCA1* mutation carriers the conferred relative risk of prostate cancer is 3.7 fold translating to approximately 9% cumulative risk by the age of 65 [38] as well as risk of esophagus (RR of 2.9) and stomach cancer (RR 2.4) [34].

1.3.2 Other high- and moderate penetrance genes

Beyond *BRCA1/2* genes, there are two other rare high-risk genes associated with a relative risk of >10 of developing breast cancer as a part of distinct genetic syndromes with high risk for other cancers. Germ-line mutations in *TP53* gene, causing Li-Fraumeni syndrome, are characterized by an increased risk of soft tissue carcinoma and osteosarcoma, leukemia, brain tumor, adrenocortical carcinoma and breast cancer [39]. Mutations in *PTEN* gene, underlying Cowden syndrome, affects multiple organs. The primary concern is high risk of cancer of the breast, endometrium and thyroid [40]. The frequencies of mutations in *TP53* and *PTEN* gene are <0.1% in the general population and ~1% among breast cancer patients from non-*BRCA1/2* high-risk families [39, 41, 42]. Somatic mutations in *TP53* and *PTEN* genes are frequently present in breast tumors and are the most common first events in breast cancer tumorigenesis [43].

Mutations in rare high-risk penetrance genes *STK11* (Peutz-Jegers syndrome) [44], *CDH1* (diffuse gastric and lobular breast carcinoma) [45] and *CDKN2A* (melanoma/pancreas/breast cancer) [46] are associated with 4-10 fold increased risk of breast cancer. Moderate-penetrance genes *CHEK2*, *ATM*, *PALB2*, *BRIP1*, *NBS1*, *RAD51C*, *RAD50*, *BARD1*, *MRE11A*, *RAD50* and *NBN* are associated with 2-4 fold increased risk of breast cancer [47]. An ongoing Swedish project aims to evaluate the prevalence of these mutations in Swedish non-selected breast cancer cohort and build risk prediction programs that can help in making surveillance and prophylactic management decisions.

1.3.3 Low penetrance variants

1.3.3.1 BRCA1 mediated breast cancer

A number of studies to evaluate associations between genetic variants and risk of developing breast and/or ovarian cancer have been performed. In *BRCA1* mutation carriers, six loci (8 SNPs) associated with breast cancer risk have thus far been discovered by CIMBA consortium. For *BRCA2* mutation carriers, fourteen loci (14 SNPs) associated with breast cancer risk have been discovered to modify breast cancer risk.

A candidate gene approach has revealed an association of minor allele of SNP D302H in *CASP8* gene giving approximately 15% reduced risk of breast cancer in *BRCA1* carriers [48, 49]. By investigating SNPs identified through *population based genome wide association studies*, four SNPs at three separate loci have been identified. At 6q25.1, two SNPs (rs2046210 and rs9397435) are independently associated with elevated breast cancer risk in *BRCA1* carriers [50]. The other two associated variants are located in *TOX3/TNRC9* gene (rs3803662) and intergenic at 2q35 (rs13387042) respectively giving higher risk of breast cancer [51].

Of the SNPs identified to have association with breast cancer risk in the general population, five have been validated in large CIMBA cohorts in *BRCA1* mutation carriers (Table 1). The SNPs rs3803662 in the *TOX3/TNRC9* [52] and rs13387042 at 2q35 [53] are associated with slightly increased risk for breast cancer while D302H in *CASP8* is associated with decreased risk for breast cancer risk in *BRCA1* mutation carriers. The two SNPs (rs2046210 and rs9397435) at 6q25.1, close to *ESR1* gene, also gives increased risk for breast cancer in *BRCA1* mutation carriers [50]. The SNP rs10771399 in *PTHLH* gene was associated with reduced breast cancer risk in *BRCA1* mutation carriers overall (HR 0.87 CI 0.81-0.94 p=3.2x10^{-4}) and further classification by different mutation classes showed association with class 1 mutation (a truncated protein as predicted functional consequence) (HR 0.82 CI 0.74-0.90 p=3.1x10x^{-5}). No association was shown in class 2 mutation carriers (predicted to generate stable mutant protein). The *PTHLH* SNP was associated with ER-negative tumors for both *BRCA1*

and *BRCA2* carriers producing reduced risk of developing breast cancer (HR 0.81 respective 0.78) [54].

Table 1. SNPs associated with breast cancer risk for *BRCA1* mutation carriers

BRCA1					
Gene/loci	SNP	# of carriers	HR (95% CI)	*p*	Ref.
CASP8/10p14[a]	D302H	4844	0.85 (0.76-0.97)	0.01	[49]
TOX3/*TNRC9*/16q12[b]	rs3803662	8403	1.09 (1.03-1.16)	0.0049	[51]
Intergenic 2q35[b]	rs13387042	9937	1.11 (1.01-1.21)	0.026	[53]
C19orf62/*ANKLE*/19p13[c]	rs8170	8363	1.26 (1.17-1.35)	$2x10^{-9}$	[55]
C19orf62/*ANKLE*/19p13[c]	rs2363956	8359	0.84 (0.80-0.89)	$6x10^{-9}$	[55]
ESR1/6q25.1	rs2046210	10817	1.17 (1.11-1.23)	$4.5x10^{-9}$	[50]
ESR1/6q25.1	rs9397435	12575	1.28 (1.18-1.40)	$1.3x10^{-8}$	[50]
PTHLH	rs10771399	12558	0.87 (0.81-0.94)	3.2x10-4	[54]

[a] SNP identified through candidate gene studies
[b] SNP identified through GWAS in the general population
[c] SNP identified through GWAS of BRCA1 mutation carriers

A *genome wide association study in BRCA1 carriers*, have revealed two SNPs at 19p13 (rs8170 and rs2363956) that are associated with breast cancer risk. The results are described in detail later on (Paper II).

1.3.3.2 BRCA2 mediated breast cancer

The first gene reliably identified as a strong genetic modifier was *RAD51* modifying the cancer risk in *BRCA2* mutation carriers. The *RAD51* gene is part of the prior candidate pathway for breast cancer susceptibility genes, functioning in the homologous recombination DNA repair mechanism. Evidence of association was first discovered by smaller *candidate gene approach* [56, 57, 58] and in one larger multistage GWAS study [59]. In time, the association of *RAD51* was confirmed by the CIMBA study. A SNP in the 5´ UTR of RAD51, 135GrC, gives hazard ratio of 3.18 (95% CI 1.39-7.27) among rare

CC homozygotes. The 135GrC variant affects *RAD51* splicing within the 5´UTR and thus alters the expression of *RAD51* [60].

Additional 13 loci have been discovered by *a population based genome-wide screening approach* and validated by the CIMBA consortium showing evidence of association with breast cancer risk for *BRCA2* mutation carriers (Table 2). The strongest of these is the SNP rs2981582 in *FGFR2* gene conferring 30% increased risk. The three SNPs in gene *TOX3*/*TNRC9*, at 2q35 and at 6q25.1 (HR 1.15, 1.18 and 1.14) were also associated with *BRCA2* breast cancer risk as indicated for *BRCA1* breast cancer risk as well. The rest of the SNPs (the minor allele of *LSP1*/*LOC643714*, *MAP3K1*, *SLC4A7*/*NEK10*, and *MRPS30* at 5p12 and intergenic SNP at 1p11.2) indicated hazard ratios between 1.09 and 1.14. These SNPs were not associated with *BRCA1* breast cancer risk [51].

GWAS investigation in BRCA2 carriers identified the previously known variant associated with increased risk for breast cancer in gene *FGFR2*, rs2982582 (HR 1.28 95% CI 1.18-1.39, p=1.2x10^{-8}) and the variant rs3803662 near to *TOX3*-gene (HR 1.20 95%CI 1.10-1.31, p=4.9x10^{-5}). Two novel loci, rs16917302 (HR 0.75, 95% CI 0.66-0.86, p=3.8x10^{-5}) on gene *ZNF365* and rs311499 (HR 0.72, 95% CI 0.61-0.85, p=6.6x10^{-5}) in the region including *GMEB2* among others were associated with decreased risk of developing breast cancer among *BRCA2* mutation carriers [61].

Table 2. SNPs associated with breast cancer risk for *BRCA2* mutation carriers.

BRCA2					
Gene/loci	SNP	# of carriers	HR (95% CI)	*p*	Ref.
RAD51/14q24[a]	rs1801320	2748	3.18 (1.39-7.27)	0.0004	[60]
FGFR2/10q26[b]	rs2981582	4876	1.30 (1.20-1.40)	$6.8x10^{-11}$	[51,61]
TOX3/*TNRC9*/16q12[b]	rs3803662	4814	1.17 (1.07-1.27)	0.00029	[51,61]
MAP3K1/5q11[b]	rs889312	5122	1.10 (1.01-1.19)	0.0022	[51]
LSP1/11p15[b]	rs3817198	5902	1.14 (1.06-1.23)	0.00079	[51]
Intergenic 2q35[b]	rs13387042	5449	1.18 (1.04-1.33)	0.008	[53]
SLC4A7/*NEK10*	rs4973768	6153	1.10 (1.03-1.18)	0.006	[51]
MRPS30/5p12	rs10941679	5854	1.09 (1.01-1.19)	0.03	[51]
ESR1/6q25.1	rs9397435	7117	1.14 (1.01-1.28)	0.031	[50]
Intergenic 1p11.2	rs11249433	6250	1.09 (1.02-1.7)	0.015	[50]
ZNF365	rs10995190	7119	0.90 (0.82-0.98)	0.015	[54]
CDK2NA/B	rs1011970	7123	1.09(1.00-1.18)	0.048	[54]
9q31.2	rs865686	7111	0.86 (0.78-0.95)	0.007	[54]
12q24	rs1292011	4872	0.84 (0.72-0.99)	0.03	[54]

[a] SNP identified through candidate gene studies
[b] SNP identified through GWAS in the general population

In the near future large-scale replication studies will evaluate previously identified discoveries. Current variations found to be associated with *BRCA1* breast cancer risk accounts for 3% of the genetic variability and corresponding estimation for *BRCA2* breast cancer risk is 6% [4].

1.3.3.3 Association with ER-, PR- and HER2 status

The SNPs identified to have association with estrogen receptor status in the general population show a similar association pattern with SNPs associated in mutation carriers defined by estrogen-receptor status. This suggests that morphological ER-defined tumor subtypes could explain differences in the associations of SNPs with breast cancer risk in *BRCA1* and *BRCA2* tumors. The majority of *BRCA1* tumors are ER-negative while most of the *BRCA2* tumors are ER-positive [62].

1.3.3.4 BRCA1 and BRCA2 mediated ovarian cancer

Through a candidate gene approaches and GWAS in the general population, some SNPs have been revealed to have an association with ovarian cancer. The minor allele of SNP rs3814113 at 9p22.2 was found to protect against ovarian cancer (HR=0.82) for both *BRCA1* and *BRCA2* mutation carriers [63, 64]. This association with ovarian cancer susceptibility was confirmed by genotyping the SNP in 10 029 *BRCA1/2* mutation carriers, revealing the risk similar to primary analysis (0.79, 95% CI 0.73-0.84, p=2.0x10^{-11}) [65]. When stratifying by tumor characteristics, the association was stronger (OR 0.77) for serous ovarian cancer. Serous tumors are the common histological subtype of ovarian cancers, which is also shown in tumors for *BRCA1/2* carriers (67% serous, 1% mucinous, 12% endometrioid and 2% clear-cell cancer) [66]. Some suggestion for association between ovarian cancer risk and SNP rs10771399 in *PTHLH* gene has been shown, especially in rare homozygotes GG (HR 1.67 CI 10.5-2.64 p=0.03). This SNP has been shown to be associated with reduced breast cancer risk. Even SNP rs614367 at11q13 has been shown to have a weak association with ovarian cancer risk (HR 0.83 CI0.72-0.96 p=0.03) [54].

The minor allele of SNP D302H in *CASP8* gene even modifies ovarian cancer risk in *BRCA1* mutation carriers. The ovarian cancer risk is reduced by 30%. The same SNP has been mentioned above as a modifier of BRCA1 breast cancer risk [49].

The other ovarian cancer susceptibility loci have not yet been confirmed with large-scale studies, but there are four good candidate SNPs, which can modify the risk of ovarian cancer. These SNPs are located at 8q24, 2q31, 3q25 and 17q21 and have a strong association with tumors aroused from epithelial cells in Fallopian tubes (i.e. serous ovarian cancer) [64].

1.3.3.5 Implication for risk prediction

The susceptibility alleles in complex disorders such as breast cancer may have a role in stratifying individuals into different risk groups. Classification is important in the context of prevention and treatment programs in order to facilitate individualized prevention and manage public health policy. The common genetic variants *per se*

modify the risk for cancer at a modest level meaning that the risk alteration by one single allele is small since risk alleles seem to act multiplicatively [4, 62]. Depending on how many of the risk alleles the individual has inherited, the combined risk varies considerable. The calculated combined risk, based on the 18 identified risk SNPs in the general population, show that individuals at lowest risk (5% of women in the general population) have a lifetime risk ≤5.7%. Individuals (5% of women in the general population) at highest risk have ≥19% lifetime risk.

The combined risk profile for *BRCA1/2* mutation carriers has greater consequences due to the underlying high risk. The combined hazard ratio across the seven SNPs (rs2981582 in *FGFR2*, rs3803662 in *TOX3/TNRC9*, rs889312 in *MAP3K1*, rs3817198 in *LSP1*, rs13387042 in 2q35 region, rs4973768 in *SLC4A7/NEK10*, and rs10941679 in the 5p12 region) associated with breast cancer risk in *BRCA2* mutation carriers illustrates that the individual who is homozygote for the protective allele in all the seven SNPs has a hazard ratio of 1. An individual who is homozygote for all risk alleles reaches a HR of 5.75. The individuals at the lowest risk (5^{th} percentile) have a HR ≤1.3 while the carriers at highest risk (95^{th} percentile) have a HR ≥3.0 (Figure 1A). Depending on how many risk alleles the individual has inherited, the absolute risk of developing breast cancer in *BRCA2* mutation carriers varies from 42% to 96%. The individuals at lowest risk (5^{th} percentile) have ≤ 50% risk and the individuals at highest risk (i.e. homozygote for all risk alleles) have ≥80% (Figure1B) [51, 62].

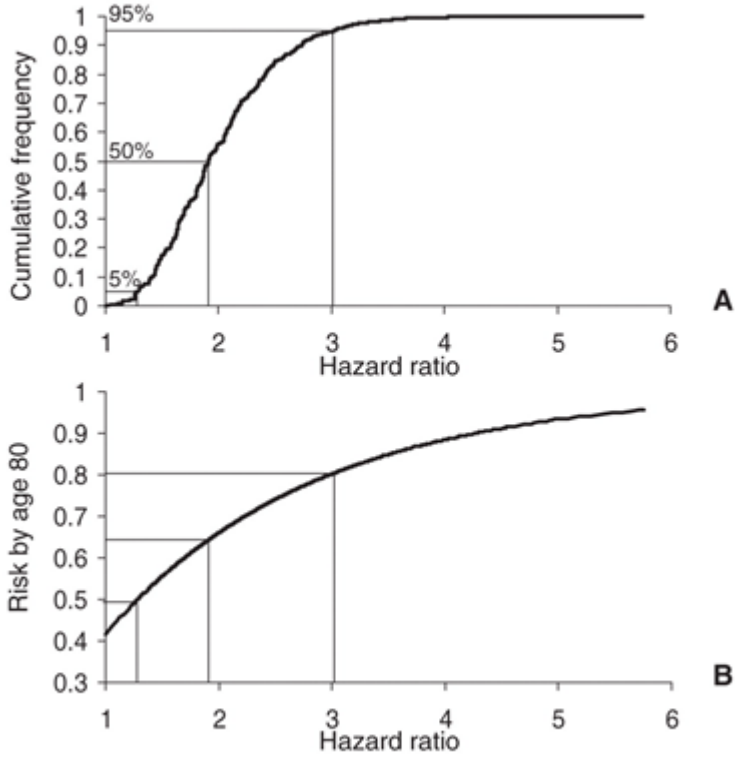


Figure 1. **A:** cumulative combined hazard ratio for breast cancer risk for BRCA2 mutation carriers at SNPs in *FGFR2, TOX3/TNRC9, MAP3K1, LSP1*, 2q35 region, *SLC4A7/NEK10* and in the 5p12. **B:** predicted cumulative risk of developing breast cancer by age 80 by the combined HR at the same SNPs [51].

Despite the set of identified modifying loci, the majority of the genetic variability for risk of developing cancer in mutation carriers remains unsolved. Thus, the SNP profiling is still underpowered and the weight on clinical risk prediction is limited. Currently these SNPs are not tested clinically due to cost-infectivity (prevalence of mutations in each individual is very low) and because it is still difficult to evaluate attribute to *BRCA1* and *BRCA2* mutations. [67, 68].

1.4 CHARACTERISTICS OF *BRCA1* AND *BRCA2* TUMORS

BRCA1 and *BRCA2* associated tumors display different pathologic characteristics. Morphologic, the most of the breast tumors in *BRCA1* mutation carriers are ductal carcinoma. However, the tumors are more often of medullary or atypical medullary subtype, which generally accounts for less than 5% of all breast cancer subtypes [69]. *BRCA1* tumors express basal cytokeratin and tend to have lymphocytic infiltration. Negative prognostic factors such as high grade, high mitotic amount, pleomorphic

pattern, poor differentiation and high proliferation rate makes *BRCA1* tumors aggressive [70, 71]. Predominantly *BRCA1* tumors lack estrogen-, progesterone- (approximately 80% of tumors) and HER2 receptors (approximately 90%) and approximately 70% are triple-negative (estrogen, progesterone and HER2 negative) tumors [72, 71, 66]. It has been proposed that women below the age of 50 with triple-negative tumors should be offered *BRCA1* mutation screening due to the fact that these individuals have >10% likelihood to carry a mutation [71].

BRCA2 tumors are more heterogeneous than *BRCA1* tumors. The most of the breast tumors arising in *BRCA2* mutation carriers are ductal carcinomas, but lobular subtype is more often exhibited compared to *BRCA1* and sporadic tumors [73, 74, 66]. The tumors also exhibit higher grade, have a luminal molecular subtype, express seldom basal cytokeratin and are associated with a positive expression of estrogen (80% of tumors) and progesterone (65% of tumors). *BRCA2* tumors are less likely to be HER2 overexpressed/amplified (90% HER2 negative) [74, 66].

Stratification of tumors by grade at different age of onset shows that the grade of tumor decreased with increasing age in *BRCA1* mutation carriers. Similar trends were shown in *BRCA2* carriers, although this was not a statistically significant result. This implies that older breast cancer patients were diagnosed with higher differentiated tumors. In *BRCA1* mutation carriers, the frequency of ER- and PR-negative tumors decreased with increasing age, but HER2 frequency was stable with increasing age. The tendency for *BRCA2* tumors was the opposite; the frequency of ER- and PR-negative tumors increased with increasing age, whereas HER2 frequency was also stable. ER-negative tumors were of higher histologic grade (i.e. less differentiated) tumors than ER-positive tumors in both *BRCA1* and *BRCA2* mutation carriers [66].

The same pathology study shows that in *BRCA1* mutation carriers, two third of mutations were class 1 mutation and on third class 2 mutations. In *BRCA2* carriers, the frequency of class 2 mutations was low. No significant differences were found between tumor pathology and class of *BRCA1* mutations. No analysis in *BRCA2* mutation carriers was carried out. Tumor characteristics did not differ depending on whether the mutation was located in the ovarian cancer cluster region (OCCR region) or outside the region in *BRCA2* gene [66].

ER- and PR-status of the first breast cancer was predictive of ER-status of the asynchronous contralateral breast cancer in both *BRCA1* and *BRCA2* mutation carriers [66].

The majority of ovarian cancer cases in *BRCA1/2* carriers are serous and classified as grade 3 at the time of diagnosis. Grade and age did not show any association. Further, morphology or grade of ovarian cancer was not influenced by history of breast cancer. No significant differences were shown between *BRCA1* and *BRCA2* mutation carriers in regards to morphology or grade of ovarian cancer [66].

1.5 NON-GENETIC RISK FACTORS

Environmental/lifestyle, hormonal and reproductive factors as breast/ovarian cancer risk modifiers in *BRCA1/2* carriers has been widely studied, though often in small cohorts and some of the results are contradictory.

The post-menopausal women carrying a *BRCA1/2* mutation have been recommended to avoid hormone replacement therapy (HRT) as a treatment for menopause because of increased breast cancer risk. Many pre-menopausal women after prophylactic oophorectomy elect to use short-term HRT to relieve symptoms of abrupt menopause. Postsurgical breast cancer risk has not been shown to alter due to short-term HRT [75].

Breast-feeding has shown to protect against *BRCA1/2* breast cancer [76]. Women who breast-fed for at least one year had 30%-50% lower risk compared to women who never breast-fed. Breast-feeding for two years or longer confers as risk reduction of 50%. The similar reduction could not be shown in *BRCA2* carriers [77, 78]. Breast-feeding did not seem to have a protective impact on the ovarian cancer risk in *BRCA1/2* carriers [79].

An increased number of full-term pregnancies among mutation carriers, as shown in the general population as well, is associated with a slight decrease in the risk of breast cancer [76]. The risk of developing ovarian cancer in mutation carriers does not differ between null parity compared to at least one full-time pregnancy. However, *BRCA1*

mutation carriers with more than two children were at a lower ovarian cancer risk compared to carriers with only one child [79].

Use of oral contraceptives confers increased risk of breast cancer for *BRCA1/2* mutation carriers. Particularly long duration and usage before first full-time pregnancy were associated with increased risk of breast cancer. Today´s use of oral contraceptives confers equal risk compared to past use [80]. However, use of oral contraceptives and tubal ligation are associated with reduced risk of ovarian cancer [79].

Women in the general population or *BRCA1/2* carriers who had got infertility treatment were not at increased risk of breast or ovarian cancer [81, 82]. Radiation exposure from chest X-rays is associated with breast cancer risk [30], particularly in younger generations and exposure before age the of 20. The location of the mutation in the *BRCA1* and *BRCA2* genes does not influence the risk caused by X-ray [83]. Other risk environmental risk factors are heavy smoking (more than 21 packs annually) (HR 2.09) [31], and lack of physical activity and obesity [84].

1.6 RISK REDUCING INTERVENTIONS

Management options of the breasts for high-risk women include enhanced surveillance programs, chemoprevention and risk reducing surgery.

Bilateral prophylactic mastectomy reduces future primary breast cancer in asymptomatic high-risk women efficiently. Follow-up studies indicate that the risk remains low [85, 86]. Prophylactic mastectomy is offered at early age, because of the risk of early development of breast cancer [87].

In addition to prevent ovarian cancer, salpingo-oophorectomy has been shown to be an effective protective method against breast cancer since most of the breast tumors are ER-positive. Therefore, oophorectomy as a hormonal barricade can inhibit tumor development. Removing both tubes and ovaries is recommended because *BRCA1/2* mutation carriers often develop fallopian tube carcinoma and peritoneal papillary serous carcinoma. Preventive salpingo-oophorectomy before the age of 40 has been shown to reduce ovarian/fallopian tube cancer risk by 80% and breast cancer risk by

50% in mutation carriers. The high-risk women are offered to undergo oophorectomy after childbearing [87]. Risk reduction is similar in *BRCA1* and *BRCA2* mutation carriers [88].

Estrogen, through its metabolites, plays an important role in development of breast cancer. Estrogen promotes the growth of estrogen receptor positive breast cancer. Chemoprevention aims to target estrogen receptor signaling pathways or synthesis and therefore prevent the incidence of ER-positive breast cancer in mutation carriers. Tamoxifen is an antagonist of the estrogen receptor. Metabolite of tamoxifen binds to the estrogen receptors and thus prevents estrogen binding. Tamoxifen is a traditional endocrine therapy in pre-menopausal breast cancer women while aromatase inhibitors are frequently used in post-menopausal women. Aromatase inhibitors inhibit the action of the enzyme aromatase, which converts androgens into estrogens [89].

1.7 SURVIVAL

Clinically, the median age of diagnosis of breast cancer in *BRCA1* and *BRCA2* mutation carriers is approximately 40 [66], thus occurring at an earlier age than sporadic cases. A meta-analysis of survival reveals that *BRCA1* mutation carriers have lower short- and long-term overall survival rates compared to sporadic cases. The rate of local recurrence, rate of contralateral breast cancer and rate of metastasis was higher in *BRCA1* mutation carriers compared to non-carriers. The survival rate or risk of local recurrence in *BRCA2* mutation carriers does not differ from non-carriers [90].

2 STRATEGIES FOR CANCER GENE DISCOVERY

2.1 LINKAGE ANALYSIS

Traditionally, the search for a phenotypic similarity i.e. to find a particular gene responsible for monogenic Mendelian inherited human disorders begins with linkage analysis. Linkage analysis is based on the co-segregation of predisposing genetic loci in pedigrees and is therefore a family-specific phenomenon where affected individuals in a family share the same ancestral predisposing DNA segment at a given trait locus. Ability to identify the alleles and parental origin of markers shows if recombination has taken place. In this approach, the aim is to find out the rough position of the gene relative to DNA sequence called a genetic marker, which has its known position in the genome. Recombination event i.e. crossing-over occurs during meiosis more frequent between two distant loci and the closer two loci are the more likely they will be transmitted together. A chromosomal region harboring responsible disease gene can be localized by identifying markers that co-segregate with the trait more often than would be expected by the rules of random assortment. Genomic distance is expressed in terms of centimorgan (cM) and is defined as the distance between genes for which one product of meiosis in 100 is recombinant. A recombinant frequency of 1% is equivalent to 1 cM.

The statistical method to calculate and show evidence of linkage between loci is LOD (logarithm (base 10) of odds) scores [91]. LOD is a likelihood-based parametric linkage approach and relies on the pattern of certain parameters, relating to a known mode of inheritance. The LOD score demonstrates the likelihood of true linkage compared to the likelihood of observing the same data purely by chance. A positive LOD score favors the presence of linkage whereas a negative LOD score indicates the opposite. The recombination fraction θ is the probability of recombination between two loci. If the recombination fraction is 0, the two loci are in perfect linkage and no recombination has occurred between the loci. Recombination fraction of 0.5 refers to no linkage between the loci. Recombination fraction between 0 and 0.5 indicates some degree of linkage.

LOD scores above three is an indicator of linkage and strong evidence that the disease and genetic markers are located close to each other and thus rarely separated by meiotic recombination. LOD Score ≤ -2 indicates no linkage, conferring that disease is not linked to the marker. Values between ≥-2 and ≤ 3 suggest linkage and require further investigations. Once a region of linkage is identified, a high-resolution mapping with additional markers to narrows down the region that may harbor the gene.

The non-parametric linkage approach (NPL) is a robust alternative to infer the location of a region linked with a complex disease [92]. The NPL approach allows contribution of several genes and environmental factors to risk of trait and do not rely on a known mode of inheritance. The objective of the NPL approach is an allele sharing analysis and aims to calculate the probability that family members have the same alleles at a locus (identical by state) regardless of whether the allele is actually inherited from a common ancestor (identical by descent).

Traditionally for linkage analysis are used microsatellite markers, which are highly polymorphic in the population. The repeated sequence is often simple, consisting of two, three or four nucleotides. The simple CA nucleotide repeats are very frequent in human genome and are present every 1000 bp. Markers for linkage analysis are evenly spaced through the genome, composing several hundreds of markers. Genotypes are often fully informative and ancestors in pedigrees can often be identified making the microsatellite markers ideal for recombination analysis.

Past successes in finding high predisposing breast cancer genes using linkage analysis are identification of *BRCA1* and *BRCA2* genes in the mid-1990s [1, 2]. The two breast cancer susceptibility genes were discovered by the positional cloning approach analyzing a large cohort of families with young affected individuals in several generations. The pure linkage approach has led to identification of some syndromic breast cancer traits such as Cowden syndrome, where inactivating mutations in the *PTEN* gene causes the trait that is associated with not only breast cancer but also includes even predisposition to thyroid cancer, mucocutaneous lesions and macrocephaly [93]. Other loci associated with syndromic breast cancer are *STK11* causing Peutz-Jegher syndrome which is characterized by gastrointestinal hamartomatous polyposis and increased risk of benign and malignant tumors in many organs [94], *CDH1*

gene associated with diffuse gastric cancer [95] and *TP53* causing Li-Fraumeni syndrome which refers to high risk of breast- and other cancers [96].

Since the initial success, nearly two decades ago, many linkage studies have been performed in non-*BRCA1/2* families without leading to identification of additional high-risk breast cancer susceptibility genes. The known germ-line mutations in high- and moderate penetrance genes contribute to no more than 15-20% of the total risk of heritable breast cancer [97], which indicates that underlying etiology in the majority of cases and families is still unsolved. One reason for lack of success could be locus heterogeneity meaning that only a small proportion of families in the studies are linked to particular loci. Remaining familial risk is explained by multiple low or moderate risk alleles or rare high-risk cancer loci that occur at a low prevalence within the population.

To focus on subsets of families from more phenotypically and geographically homogenous populations such as the Finnish or Ashkenazi Jewish populations, is an alternative method to find loci, which occur at a low prevalence within a population. The gene *TMPRSS6* is associated with breast cancer in the eastern Finnish population [98] and *RAD50* and *NSB1* genes in the northern Finnish population [99].

Focusing on candidate genes within the pathway of double strand DNA breaks through homologous recombination has led to identification of moderate risk genes such as *PALB2* [100], *ATM* [101], *CHEK2* [102], *RAD51C* [103], *BRIP1* [104]. Mutations in these moderate susceptibility genes confer a 2-3 fold higher risk of breast cancer. However, these variants account for only a few families and frequency is less than 1% in most populations. Some variants confer to higher risks in specific populations. A good exception is a founder mutation in *PALB2* gene that is associated with of HR 6.1 in the Finnish population. The risk is comparable to risk for carriers of mutations in *BRCA2* [105].

2.2 ASSOCIATION ANALYSIS

In the beginning of the 20th century linkage analyses were still used to find additional susceptibility genes and association studies were initiated. Association studies intend to identify common variants that are significantly more common in a case cohort than in

the general population. The initial focus was on single nucleotide polymorphisms (SNPs) in the biologically plausible candidate genes functioning in DNA repair, cell cycle control, apoptosis or hormone signaling pathway. The case cohort was usually females affected with breast cancer and the aim was to identify a locus that regulates a heritable trait for oligo-or polygenic (non-Mendelian) disorders.

Soon genome-wide association (GWAS) approach was the tool to be used. GWAS is based on the population-specific phenomenon where affected individuals in a population share the same ancestral predisposing DNA segment at a given trait locus. GWAS studies are possible due to the development of high-throughput techniques and biostatistics. GWAS studies of today include large sample and SNP sets. SNPs are distributed through the whole genome based on known linkage disequilibrium (LD) of SNPs and by designating tagging SNPs in LDs the whole genome can be captured. SNPs may have a direct functional effect or are associated with other SNPs in LD. Currently, the size of SNP set in GWAS study is more than 610K and the preferred number of cases is more than 10K in order to provide strong statistical power.

The most common approach of association studies is the case-control approach, whereby frequencies of SNPs are compared between unrelated affected cases and healthy controls. A GWAS study is usually conducted as a two-stage study. In stage 1, a smaller number of cases selected for example by age of onset (young affected) and controls (old healthy) are genotyped for large numbers of SNPs. In stage 2, the best hits of SNPs are genotyped for additional cases independent of current age or age of onset. Another study design for association is family-based design where association is assessed within family, which is a good way to eliminate population heterogeneity. Controls for this approach are often matched from population. Therefore family-based association design compliments traditional linkage study and case-control association study. Design for family-based association studies can be conducted as a transmission disequilibrium test (TDT) or case-parent (trio) test.

Controls should reflect the ethnic and genetic composition of the case samples, to avoid false associations due to population stratification (multiple subgroups with different allele frequencies within a population). Population stratification and admixture may lead to spurious association biases the gene-disease association [106].

The effect size of associations is inconsistently given as odds ratio (OR) and hazard ratio (HR). OR is the ratio between the odds of an event occurring in one group and the odds of same the event occurring in another group. Odds ratio is used in retrospective studies to show if being exposed to a factor increases the risk of cancer. In case-control studies OR describes the strength of association or non-independence between two binary variables. Hazard ratio measures the ratio of the risk rates corresponding to a given disease. Hazard ratio represents instantaneous risk over the study period. Relative risk (RR) is cumulative risk of event and should not be computed for case-control study design because the prevalence of the given disease is artificially constrained.

3 ONCOGENETIC COUNSELING

Until the beginning to the 1990s oncogenetic counseling comprised only rare cancer syndromes such as familial adenomatous polyposis and retinoblastoma. The need of oncogenetic counseling was restricted and families were mainly counseled by specialist doctors. While accumulation of other more common cancers in families has been observed and knowledge of molecular biology has increased, the oncogenetic counseling field has become broader. Families with a suspected or identified hereditary predisposition of cancer need specific genetic services. Greater request for genetic information, support, screening possibilities and genetic testing by the public has led to increased numbers of referrals to oncogenetic counseling. There has been wide variation in the quality of genetic service and organization within and between clinics until recent years and more focus has been invested in equalizing counseling service.

3.1 ONCOGENETIC COUNSELING PROCESS

The process of oncogenetic counseling aims to identify and to stratify counselees with risk of developing cancer into the following subtypes; high risk (highly penetrant cancer syndromes), moderate risk (multifactorial etiology or low penetrant alleles) and population risk groups. The goal is to provide the individuals at increased risk of developing cancer adequate counseling [107]. As a counselee´s understanding of their genetic risk may influence risk management decision and communication with family members, it is crucial for counselor to observe how counselees construct and interpret risks.

Today´s oncogenetic counseling focuses on quantifying and communicating the risk of cancer, informing options for managing the risk, understanding individual concerns and giving recommendations for long-term risk management strategies. Adequate risk counseling also includes information about modifying risk factors. All this is discussed together with the patient [108, 109]. Considerable effort is invested to exam how genetic services can meet patient's needs. To evaluate genetic counseling in its entity is difficult and therefore one of the focuses of studies has been on how patients

understand their risk of developing cancer. This issue is further discussed in the next chapter.

3.2 RISK PERCEPTION AND PSYCHOLOGICAL DISTRESS

Risk communication about inheritance raises many issues. It includes assessments of probabilities of a genetic susceptibility in the family and an individual's risk of developing hereditary cancer, the probabilities of increased risk for children, siblings and other relatives, consequences of cancer and the outcome of undergoing genetic testing. Both benefits and limitations related to genetic testing are addressed as well as the consequences of a positive or negative test result. Information about available support groups has been shown to benefit the counselees [108].

Since the demand for genetic counseling has increased, the need for evidence that it improves counselee's knowledge of genetics and risk perception has been manifested. Accuracy of individuals perceived likelihood of developing cancer is essential for risk management orientation. Several different educational tools as an intervention in genetic counseling have been practiced in order to improve the outcomes of genetic counseling. An earlier meta-analysis [110] evaluated 12 prospective designs and randomized controlled trials studying the impact of genetic counseling on generalized anxiety, depression, breast cancer anxiety, risk perception, knowledge of genetics and breast cancer screening uptake. Quantitative synthesis of studies revealed that general anxiety decreased and accuracy of risk perception was improved owing to genetic counseling [110]. Another meta-analysis [111] evaluated five controlled trials and 16 prospective studies on short-term and long- term differences in risk perception, knowledge, anxiety, cancer-specific worry, depression and cancer surveillance between intervention and control groups. The interventions for controlled trials were for example trial of problem-solving training vs general health counseling, trial of multidisciplinary genetic assessment vs surgical assessment and trial of breast cancer risk vs general health counseling. The meta-analysis of controlled trials revealed that knowledge of genetics was improved but the levels of risk perception did not change after genetic counseling; neither decreased general anxiety or cancer-specific worry. On the contrary, prospective studies showed more accurate risk perceptions and decreased short-term general anxiety and cancer-specific worry. The potential effect of

interventions is contradictory between heterogenic study designs and there is need for more research [111].

Zimmerman reviewed additional 56 randomized studies investigating the effectiveness of psychological interventions (e.g. education, support) in breast cancer patients. The results of the meta-analysis indicated similar effect of interventions as previous meta-analysis. Psycho-education as an intervention had the strongest effect on outcomes [112].

Bjorvatn *et al*. [113] suggested that other psychosocial variables predicting distress such as intrusion and avoidance should also be observed. The total of one fourth of the participants in her study reported severe levels of intrusion before genetic counseling. A low level of self-efficacy before genetic counseling and a high level of worry after genetic counseling were predictors for intrusions and avoidance. This means that some subgroups should be identified and offered additional support.

3.3 WORRY FOR CANCER

Rather than being a stand-alone concept, worry for cancer, as a psychological well-being outcome is something lived and experienced and often combined with risk perception during the genetic counseling process.

A study based on 4911 women from three Scandinavian countries evaluated if genetic counseling process is considered as a stressful event and associated with anxiety and/or depression. Results reveal that risk counseling does not have major effects on psychological well-being [114]. Another most recent study based on unaffected first-degree relatives to breast cancer patients, revealed that baseline cancer worry did not differ between genetic risk groups (low, moderate or high risk). The mean worry for cancer was 7.4 (on a scale from 4 to 16) [115].

3.4 SATISFACTION WITH THE ONCOGENETIC COUNSELING PROCESS

The need for more information about recommendations usually arise when asking about satisfaction of oncogenetic counseling. Reasons for this can be that the counselee lacks basic scientific knowledge and does not understand medical terms or information

about disease, prognosis, treatment and risk probabilities. Counselees have different references and make own interpretations based on experiences and life situations. The given information is usually emotionally and intellectually challenging, and counselees are supposed to make informed and essential decisions based on that information. Obliviousness is also a problem with genetic counseling; the counselee can recall only one fourth of given information and barely half of the key-points. Emotional barriers can block the counselee from making important enquiries and essential concerns often arise later at home instead. The counselee usually has some expectations, which give a direction on the counseling situation. Interaction between the counselor and the counselee should be free of counselor bias and it is important to create an open and safe atmosphere [116, 117].

3.5 ALTERNATIVE METHODS FOR ONCOGENETIC COUNSELING

The use of alternative methods to deliver oncogenetic counseling is needed to improve access and to be able to meet the increasing demand for oncogenetic counseling services. The possibility to choose between different service delivery methods such as telephone counseling is emphasized and appreciated by counselees, especially by those hindered by being able to travel. The initiative is to offer alternative user-friendly methods, which are easy to implement, better attend to the counselee´s needs and improve productivity and reduce costs. A concern with telephone counseling may include a lack of face-to-face human contact, but can be out weighted by perceptions of greater integrity. Certain counseling aspects are more suitable using alternative methods, while other situations require in-person meetings [118]. In addition to in-person and telephone counseling service delivery models, group counseling and telegenetics models have been described as alternative ways. In a group counseling, several counselees (unrelated individuals or members from the same families with own purpose) with a common indication are counseled together. Telegenetics refers to video conference or web-based counseling. In practice, these model are currently used at low extend in Sweden [119].

4 AIMS OF THE THESIS

The overall aim of this thesis is to evaluate and improve genetic diagnostic and genetic counseling in hereditary cancer patients. It is important to identify individuals with increased cancer risk and offer them adequate risk assessment, possibility of genetic testing, information about prevention in order to reduce morbidity and mortatility in cancer. The identifying of genes gives a better understanding about underlying mechanisms and can even mean new therapeutic tools to cure breast cancer.

The specific aim of each paper was:

Paper I

To conceptualize risk perception and anxiety about cancer in individuals attending oncogenetic counseling.

Paper II

To identify genetic modifiers of the risk for developing breast and ovarian cancer in BRCA1 mutation carriers by performing a large-scale genome-wide association study.

Paper III

To evaluate the oncogenetic counseling process and to compare the impact of the initial part of the oncogenetic counseling, when conducted via telephone versus in-person.

Paper IV

To identify genes associated with high or moderate risk of developing hereditary breast cancer by performing linkage study in large cancer families.

5 METHODS

5.1 ONCOGENETIC COUNSELING

Papers I and III are based on the data collected from the same questionnaires. The questionnaires were completed at three points in time: before and after oncogenetic nurse counseling and one year after the entire counseling process. The number of included participants was 215 individuals. Regarding question about risk perception and worry for cancer, the participants responded different questions depending on affected status. Risk perception was evaluated and displayed separately for each risk assessment group (population, low, moderate and high risk).

Test statistics were performed with SPSS (Statistical Package for the Social Sciences) or Statistica 8 software. Beyond the traditional descriptive test statistics of participants, dissimilarities from non-participants were analyzed. For this purpose Mann-Whitney test or Pearson´s exact χ^2 test was used. Differences in risk perception (over time and between groups) were calculated by paired and unpaired students´ t-test and ANOVA. Correlations between risk perceptions were evaluated with Spearman´s rho test. Differences in cancer worry over time were evaluated with Wilcoxon matched pair test. Pearson´s exact χ^2 test was performed to evaluate differences in satisfaction and experiences. The effect of cofounders was calculated with binary logistic regression analysis.

5.2 LINKAGE ANALYSIS

A genome-wide linkage analysis was performed on the 102 family members from 14 non-*BRCA1/2* breast cancer families. A total of 540 fluorescently labeled microsatellite markers covering the whole human genome with an average spacing of 7.25 cM were used. Genotypes were performed by DeCode with a success rate of 94.3% of the genotypes.

Statistical linkage analyses were calculated with Simwalk v2.91 software package for autosomal chromosomes and with Merlin v1.1.2 software for chromosome X. Possible mistyped genotypes inconsistent with Mendelian inheritance were calculated for each

marker by mistyping analysis and observed mistyped genotypes were removed. Multipoint parametric LOD scores, heterogeneity LOD scores (HLOD) and non-parametric LOD score (NPL) were used to measure the significance and to determinate linkage. For parametric linkage analysis, the following parameters of inheritance mode were assumed: dominant mode of inheritance (50%), disease allele frequency of 0.0001, penetrance of 80% and phenocopy rate of 5% of the observed affected individuals.

Two different affected status criteria, strict and loose, were used in linkage analysis. In the strict criteria analysis exclusively females affected by breast cancer were coded as affected while spouses (non-related family members) were coded as unaffected and all other family members as unknown. In the loose criteria analysis females with breast cancer as well as other family members with any type of cancer were coded as affected.

5.3 ASSOCIATION ANALYSIS

The whole genome genotyping was performed with a human 610K array on the Illumina Infinium array platform. The other sample cohorts were genotyped for the most significant SNPs with following platforms; general population sample cohort inclusive the controls with TaqMan assay and detection with ABI Prism 7900HT sequence detection system; *BRCA2* mutation carriers with Sequenom iPlex; triple-negative breast cancer cases with Illumina 660K array or Sequenom iPlex and controls for triple-negative breast cancer study were genotyped either with Illumina Infinium 550K array or custom Illumina Infinium 1.2M array.

The samples with subsequent exclusion criteria were excluded: call rate <99%, sex errors, sample duplications and ethnic outliers (>15% non-European ancestry). Criteria to exclude SNPs were: call rate below 95%, minor allele frequency <1%, minor allele frequency between 1-5% and call rate <98% or HWE $p<10^{-7}$.

Analysis of association was carried out by using the model of the retrospective likelihood of the observed genotypes dependent on the disease phenotypes. Genotype frequencies were compared between cases and controls using a 1-degree-of-freedom score trend test. A kinship-adjusted version of the score test was used to allow for non-

independence among individuals from the same families. To account and correct for population-specific variations in alleles distribution on the SNPs due to hidden population stratification (non-European ancestry) and due to hidden relatedness (genomic kinship) between all pairs of individuals, a multi-dimensional scaling (MDS) calculation was performed. MDS approach aims to mitigate false associations and to maximize power to detect true associations. The first two principal components of the genomic kinship matrix were calculated in a selection of 37 804 uncorrelated SNPs (pairwise $r^2 < 0.10$) between all pairs of *BRCA1* mutation carriers plus with 210 HapMap samples with the origin of Chinese, African and European populations. MDS displays the structure of distance-like data as a geometrical picture of populations. The test statistic inflation factor (λ) for kinship was calculated from the lower 90% of the χ^2 statistics and displayed as a quantile-quantile plot. As the computational tools to analyze and display results R-coding in GenABEL and SNPMatrix libraries were used.

The effect of each SNP was displayed on $\log_{10}$P-scale as a per-allele hazard ratio (HR) (multiplicative model) or as HRs for heterozygotes and homozygotes. A survival analysis framework, a Cox-proportional-hazards model, was used to analyze hazard ratios.

A competing risk framework was performed to investigate whether the SNPs were associated with ovarian cancer. HRs were simultaneously estimated for breast and ovarian cancer following up the individuals to the age of developing either breast or ovarian cancer and therefore classified as affected or up to the age of bilateral mastectomy or bilateral oophorectomy and therefore classified as unaffected. The individuals without cancer or any intervention were classified as unaffected.

SNP associations with estrogen and progesterone status were evaluated as a case-only analysis using logistic regression whereas differences in associations were compared between groups defined by ER and PR status. Both OR for each genotype and per-allele OR were displayed.

For genotype imputation analysis the selected 1055 SNPs based on phased haplotypes from the 1000 Genome project together with 59 SNPs available from the stage 1 and 5

SNPs available from stage 2 were analyzed with MACH software. The number of available genotyped individuals was 2383 from stage 1 and 5986 from stage 2.

6 STUDY SUBJECTS

6.1 ONCOGENETIC COUNSELING COHORT (PAPERS I AND III)

During the year 2000 all new patients, admitted to clinical genetics at Karolinska University Hospital for oncogenetic counseling in Solna, were asked to participate in a research study. A total of 309 patients were referred to oncogenetic counseling and invited to participate in the questionnaire study. After referral, 253 of the patients showed interest in genetic investigations. A total of 215 returned at least one of the questionnaires and were included in the study.

The first questionnaire was sent to all patients immediately after the clinic received a referral for oncogenetic counseling. The second questionnaire was distributed after completion of oncogenetic nurse and physician counseling. The third questionnaire was requested to be returned after completion of the entire counseling process. The questionnaire data was collected during the study period of three years.

6.2 *BRCA1*- AND *BRCA2*- MUTATION CARRIER COHORT (PAPER II)

Upon joining the international CIMBA (Consortium of Investigators of Modifiers of *BRCA1/2*) collaboration group in 2007, oncology clinics and clinical genetics in Stockholm have contributed with DNA samples and phenotype data on 361 *BRCA1* or *BRCA2* mutation carriers. The patient cohort was collected from the patient register at the Department of Clinical Genetics, Karolinska University Hospital and all females over 18 years of age with *BRCA1* and *BRCA2* mutations were invited to participate. The patients had received genetic counseling at the Department of Oncology and Pathology at Radiumhemmet or Södersjukhuset or at the Department of Clinical Genetics. A researcher in Lund collected the mutation carriers from other districts (the departments of clinical genetics in Linköping, Lund, Gothenburg, Uppsala and Umeå) in Sweden. All patients included in the study either have been screened positive for a *BRCA1* or *BRCA2* mutation or tested positive for the existing mutation in the family at the Department of Clinical Genetics. After genetic testing and receiving the test results,

the mutation carriers were invited to participate in a research study on genetic and environmental modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers.

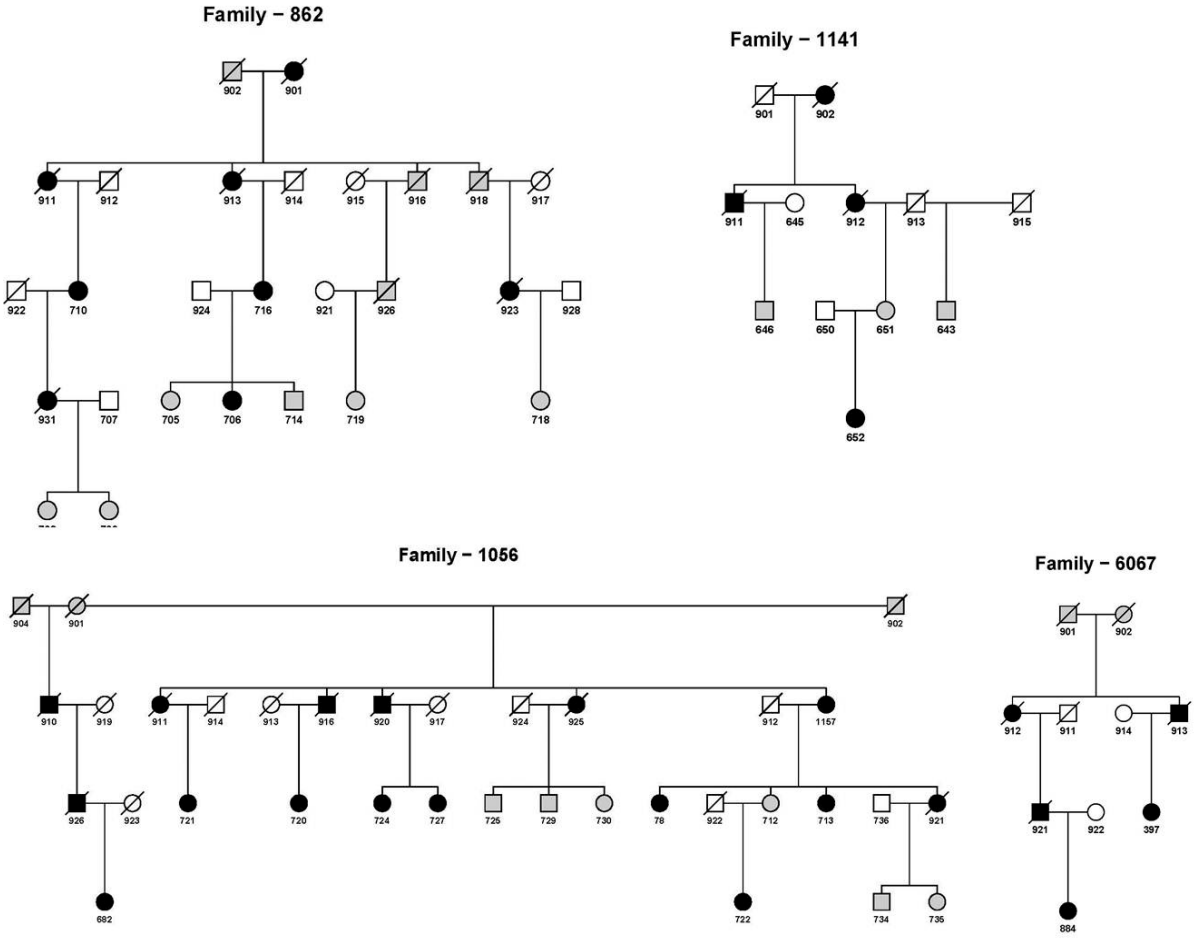
At the first inclusion in the year 2007, a total of 201 carriers were included in the study of which 43 carriers were deceased. The deceased persons had been included in another research project before death and found to be carriers of *BRCA1* or *BRCA2* mutations. For these deceased individuals, the phenotypic data is insufficient. In the following years identified mutation carriers were invited after completed carrier testing or screening of *BRCA1* or *BRCA2*. As of today, the total number of carriers included from Stockholm is 363 whereof 282 (78%) are *BRCA1* mutation carriers.

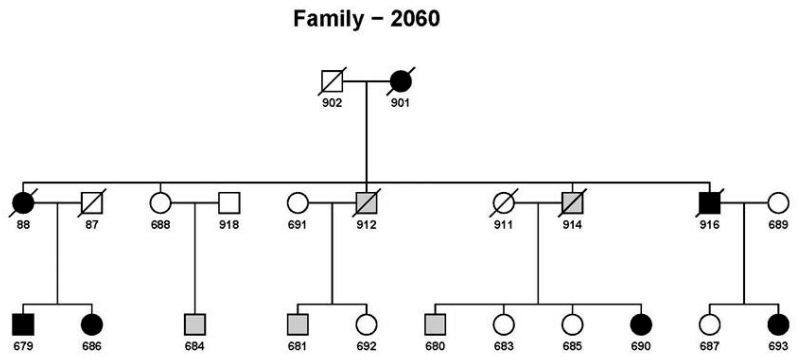
At stage one a total of 1250 female *BRCA1* mutation carriers with invasive breast cancer diagnosis under 40 years of age and 1250 female *BRCA1* mutation carriers without cancer diagnosis at 35 years of age or above were selected from 11 countries (20 centers) for the genome-wide screening. At stage two, additionally 6332 carriers from 17 countries were included for analysis. To evaluate the contribution of the two most significant SNPs to breast cancer risk in the general population, 6800 affected and 6613 controls from the general population based study cohort were used. The cases in the general population were females diagnosed with breast cancer before the age of 55 between the years 1991 and 1996 and females diagnosed with breast cancer before the age of 70 between the years 1996 and 2006. The controls in this cohort were from an epidemiological study cohort randomly collected from the same geographic region. To evaluate the contribution of the two SNPs in triple-negative breast cancer cases, a total of 2301 females were analyzed together with 3949 controls from the same geographic region.

From Stockholm, Sweden the number of samples fulfilling the criteria for stage 1 was 105 females and 279 for stage 2.

6.3 LINKAGE ANALYSIS COHORT (PAPER IV)

This study is based on the cohort of 14 familial non-*BRCA1/2* breast cancer families (Figure 2). Breast cancer families were counseled at the Department of Clinical Genetics, Karolinska University Hospital, Stockholm. Fourteen large pedigrees with 102 members were included in linkage analysis. A total of 39 of the family members were considered as breast cancer affected. The number of genotyped family members varied from two to sixteen individuals between families and the number of breast cancer affected females varied from two to seven for every family.

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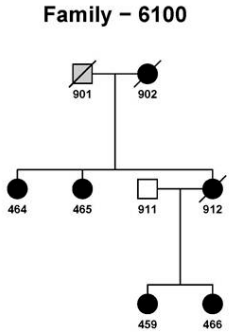
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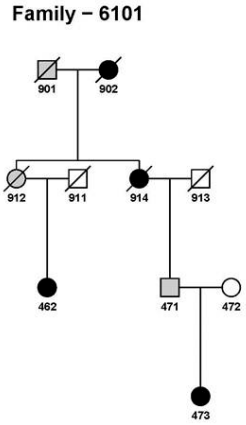
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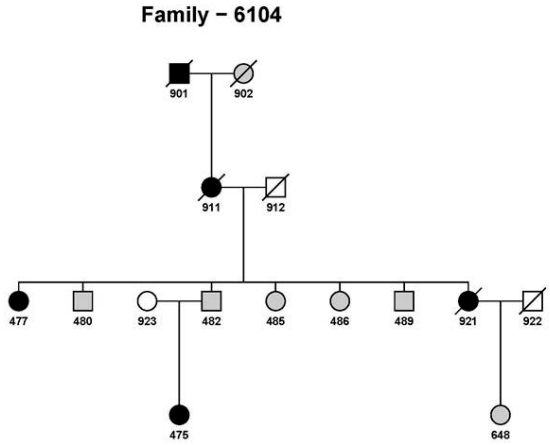
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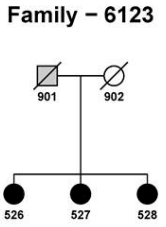
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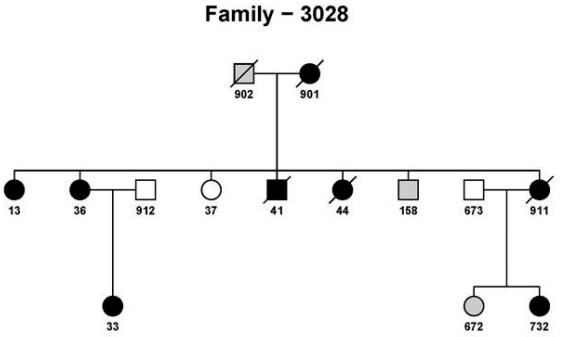
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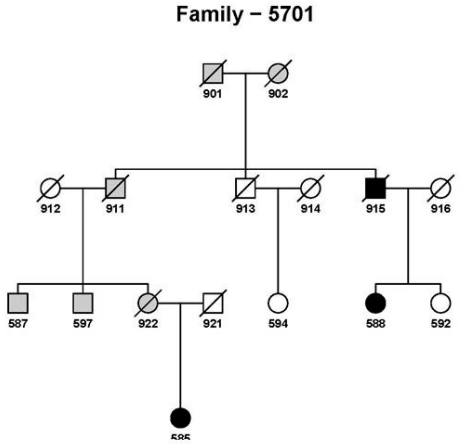
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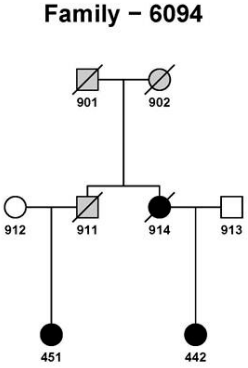
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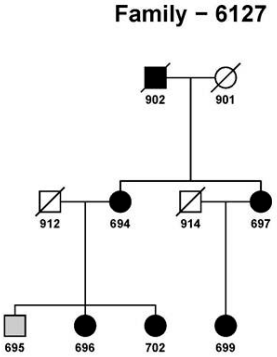
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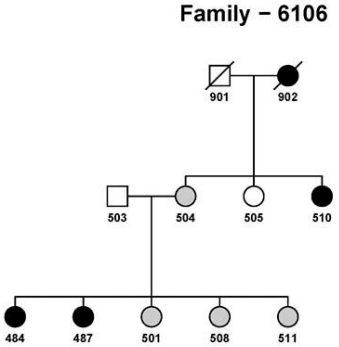
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Figure 2. Pedigrees of the 14 Swedish families included in the genome-wide linkage analysis. Affected individuals are marked in black, individuals with unknown cancer status marked in grey and unaffected marked in white. Deceased individuals have a line through them. Affected status is displayed according the loose criteria.

7 RESULTS AND DISCUSSION

7.1 PAPER I

This study aimed to conceptualize risk perception and worry for cancer in individuals seeking hereditary cancer services for oncogenetic counseling. The main findings of this questionnaire survey were 1) the unaffected counselee overestimated their own risk of developing cancer as well as the risk for children/siblings; 2) both the unaffected and affected counselees overestimated risk for the general population; 3) the affected counselees overestimated the risk for children/siblings; 4) the overall risk estimations were more accurate after genetic counseling and 5) the counselees except the high-risk and affected counselees expressed lower levels of worry for cancer after genetic counseling.

The unaffected counselees were stratified into four risk groups: the same risk as the general population, low-, moderate- and high risk regarding the objective risk of developing the type of cancer running in their family. The counselees with different cancer types were analyzed composed because the outcomes did not vary between the two most prevalent cancer types in this cohort i.e. breast and colon cancer.

Before genetic counseling, the counselees in all risk-groups overestimated their personal risk of developing cancer. The counselees with the same risk than the general population or with a slightly increased risk displayed most overestimated risks prior counseling. Between pre- and post-counseling the reductions of risk perception were most prominent in these particular risk groups. Moderate risk counselees did not show any difference in risk perception over time. One year after genetic investigations, the counselees were asked if prevention and surveillance program would have or not have an effect on personal risk of developing cancer. All counselees reported lower risks if included in prevention program, especially the moderate and high-risk counselees.

When the counselees were asked to estimate the risk for children/siblings, they reported lower risks compared to personal risks and even lower risks for the general population even if the estimate risks were too high. The risk estimations for children/siblings and for the general population were significantly more correct after genetic counseling. The estimated risks were lower if children/siblings would hypothetically attend prevention program, indicating that the effect of program was well understood.

The affected counselees were asked to evaluate the risk for their children/siblings and for the general population. The results indicated overestimated risks before oncogenetic counseling with a decrease over time.

Post-counseling worry for developing cancer was lower than pre-counseling worry in all other risk groups than in the high-risk counselees. Worry for cancer was strongly in correlation with personal risk perception at all measurement time points. The affected counselees did not report any changes in worry of relapse.

The results of our study are in concordance with the results from the latest review of genetic counseling outcomes [120] evaluating 10 trials including risk perception and psychological distress. Overall, that review demonstrated improved psychological well-being and suggested that genetic counseling helps to reduce distress and leads to more accurate risk perception and increases knowledge of genetics. The results suggest that genetic counseling do not cause any harm and can have a positive effect on health related distress. However, the authors do not make any firm conclusions due to limited number of trials. Studies were heterogeneous in terms of populations, settings, interventions and outcomes and therefore the data was presented as a narrative synthesis of the studies rather than as a meta-analysis.

One smaller study with 150 participants had very similar results compared to our study of risk perception and cancer worry [115]. Mean perceived risk was 64% for low risk individuals, 65% for moderate and 69% for high-risk individuals, indicating heavy overestimated risk. Most of the individuals (65%) in this study group were confident that they will very likely develop cancer and 70% reported higher risk perception compared to risk for women in general population.

7.2 PAPER II

After stage 1 genotyping, 96 SNPs showed significant association at the $p<10^{-4}$ level. A total of 86 SNPs, seven surrogate SNPs and three additional SNPs were selected for stage 2 genotyping in additional 6332 mutation carriers. After stage 2 genotyping, the five top SNPs locating on 19p13 remained significant at $p<10^{-3}$ level with hazard ratios showing the same direction as in stage 1 analysis. When combining genotyping results from both stages, the five SNPs showed significant associations at $p=2.3x10^{-9}$ to $p=3.9x10^{-7}$ level. The two most significant SNPs were associated with increased breast cancer risk (HR 1.26) while the other three SNPs were associated with decreased risk for breast cancer (HR 0.84 and 0.86).

Table 3. Association with breast cancer risk in *BRCA1* mutation carriers for the most significant SNPs on 19p13. Affected status conferring breast cancer.

SNP Position Allele1/2	Stage	Number of unaffected/ affected	Allele 2 freq. unaffected/ affected	HR (95%CI) Per allele[1]	Heterozygote	Homozygote[2]	P_{trend}[3]
rs8170	Stage 1	1 193/1 190	0.16/0.20	1.25(1.12-1.39)	1.23 (1.08-1.41)	1.61 (1.13-2.30)	1.1x10-4
17,250,704	Stage 2	3 010/2 970	0.17/0.20	1.26 (1.15-1.38)	1.28 (1.14-1.43)	1.54 (1.17-2.03)	4.1x10-6
G/A	Combined	4 203/4 160	0.17/0.20	1.26 (1.17-1.35)	1.26 (1.16-1.37)	1.57 (1.26-1.95)	2.3x10-9
rs4808611	Stage 1	1 191/1 190	0.16/0.19	1.26 (1.13-1.41)	1.23 (1.08-1.41)	1.72 (1.21-2.45)	7.9 x10-5
17,215,825	Stage 2	3 000/2 964	0.16/0.19	1.26 (1.15-1.39)	1.30 (1.16-1.46)	1.43 (1.06-1.92)	6.4 x10-6
G/A	Combined	4 191/4 154	0.16/0.19	1.26 (1.17-1.35)	1.27 (1.17-1.39)	1.53 (1.22-1.93)	2.7 x10-9
rs8100241	Stage 1	1 191/1 189	0.53/0.47	0.81 (0.74-0.88)	0.82 (0.71-0.95)	0.65 (0.55-0.77)	1.8 x10-6
17,253,894	Stage 2	3 008/2 972	0.51/0.49	0.86 (0.80-0.92)	0.93 (0.82-1.05)	0.74 (0.63-0.79)	1.1 x10-4
G/A	Combined	4 199/4 161	0.52/0.48	0.84 (0.80-0.89)	0.88 (0.81-0.97)	0.71 (0.63-0.79)	3.9 x10-9
rs2363956	Stage 1	1 193/1 190	0.53/0.47	0.81 (0.74-0.88)	0.82 (0.71-0.95)	0.65 (0.55-0.77)	1.5 x10-6
17,255,124	Stage 2	3 006/2 970	0.51/0.49	0.87 (0.81-0.93)	0.92 (0.82-1.04)	0.75 (0.65-0.86)	1.7 x10-4
A/C	Combined	4 199/4 160	0.52/0.48	0.84 (0.80-0.89)	0.88 (0.80-0.97)	0.71 (0.64-0.79)	5.5 x10-9
rs3745185	Stage 1	1 193/1 190	0.46/0.40	0.83 (0.76-0.90)	0.81 (0.71-0.93)	0.69 (0.57-0.82)	2.3 x10-5
17,245,267	Stage 2	3 009/2 972	0.44/0.41	0.88 (0.82-0.95)	0.89 (0.80-1.00)	0.77 (0.67-0.89)	1.2 x10-3
G/A	Combined	4 202/4 162	0.44/0.41	0.86 (0.81-0.91)	0.86 (0.81-0.91)	0.74 (0.66-0.83)	3.9 x10-7

[1] Per copy of allele 2
[2] Two copies of allele 2
[3] Kinship-adjusted score test

No heterogeneity in associations between nations was shown. When excluding females with breast cancer more than five years before inclusion to the study, i.e. assuring that association are not survival related, the HRs were similar indicating no effect of

prevalent cases. The SNPs were not concomitant with risk for ovarian cancer and the associations with breast cancer were not confounded by the competing risk of ovarian cancer. When further evaluating whether SNP associations are related to the functional consequence of mutation type (class1 and 2), the predicted HRs were stronger in class2 mutation carriers indicating an eventual stronger modifying effect on breast cancer risk in class 2 mutation carriers.

The five most significant SNPs were associated with estrogen receptor negative breast cancer tumors, especially the SNPs associated with decreased risk for cancer (rs8100241, rs2363956 and rs3745185) (Table 4).

Two of the most significant SNPs (rs8170 and rs2363956) were genotyped in the set of samples and controls from the general population. No contribution to breast cancer risk was shown in the general population. Although, when stratifying the tumors by hormone receptor status, the two SNPs were associated with estrogen receptor negative breast cancer tumors in the general population as well. To further evaluate the association related to hormone receptor status, an analysis in triple negative breast cancer tumors in the general population was performed. The five SNPs were all associated with triple negative breast cancer tumors and ORs were in the same magnitude as HRs in *BRCA1* mutation carriers. This is consistent with the observation that *BRCA1* mutation breast cancers have predominantly ER-, PR- and HER2 receptor negative phenotype [55, 121]. Two SNPs (rs8170 and rs2366956) were genotyped in *BRCA2* mutation carriers in simultaneously ongoing GWAS in *BRCA2* mutation carriers. The SNPs were not associated with breast cancer in *BRCA2* mutation carriers.

Table 4. ORs of associations between SNPs on 19p13 and different tumor characteristics in *BRCA1* mutation carriers and in triple negative breast cancer tumors.

***BRCA1* mutation carriers**

Estrogen receptor

SNP	# of ERpos / ERneg breast cancer cases	OR[1] (95%CI)	P[1]
rs8170	295 / 889	1.21 (0.93-1.58)	0.15
rs4808611	293 / 886	1.21 (0.93-1.59)	0.16
rs8100241	294 / 888	0.78 (0.64-0.96)	0.018
rs2363956	295 / 886	0.77 (0.63-0.95)	0.013
rs3745185	295 / 887	0.76 (0.62-0.93)	0.009

Progesterone receptor

SNP	# of ERpos / ERneg breast cancer cases		
rs8170	243 / 778	1.24 (0.92-1.68)	0.16
rs4808611	243 / 774	1.33 (0.97-1.82)	0.07
rs8100241	243 / 778	0.79 (0.63-0.99)	0.04
rs2363956	244 / 775	0.78 (0.62-0.98)	0.03
rs3745185	244 / 776	0.79 (0.63-0.99)	0.044

Estrogen and progesterone receptor

SNP	# of ERposPRpos / ERnegPRneg breast cancer cases		
rs8170	356 / 718	1.24 (0.96-1.60)	0.10
rs4808611	335 / 715	1.28 (0.98-1.66)	0.066
rs8100241	356 / 718	0.76 (0.62-0.93)	0.007
rs2363956	357 / 715	0.75 (0.62-0.92)	0.006
rs3745185	357 / 716	0.74 (0.60-0.90)	0.003

Triple negative breast cancer cases

SNP	Allele1/Allele2	Cases/Controls	OR[2] (95%CI)	p_{trend}	pHet
rs8170	G/A	2285/3941	1.28 (1.16-1.41)	$1.2x10^{-6}$	0.993
rs4808611	G/A	2265/3277	1.25 (1.13-1-38)	$2.4 x10^{-5}$	0.984
rs8100241	G/A	1383/2774	0.80 (0.73-0.89)	$1.6 x10^{-5}$	0.968
rs2363956	A/C	2279/3931	0.80 (0.74-0.87)	$1.1 x10^{-7}$	0.996
rs3745185	G/A	1384/3419	0.82 (0.74-0.91)	$8.1 x10^{-5}$	0.992

[1] Odds ratio estimate and p-value adjusted by nation and age at diagnosis

[2] OR per copy of allele, with fixed effect of nation

[3] significance of heterogeneity between nations

When analyzing the joint effect of single SNPs it was not possible to distinguish which SNPs together are causative for increased risk of breast cancer. Therefore, a set of SNPs was selected from 1000Genome project for imputation analysis. The imputed genotypes together with the real genotypes revealed that eight of the imputed SNPs were correlated with four of the top SNPs suggesting that one or more of the imputed SNPs can be causally associated with breast cancer risk (Figure 3).

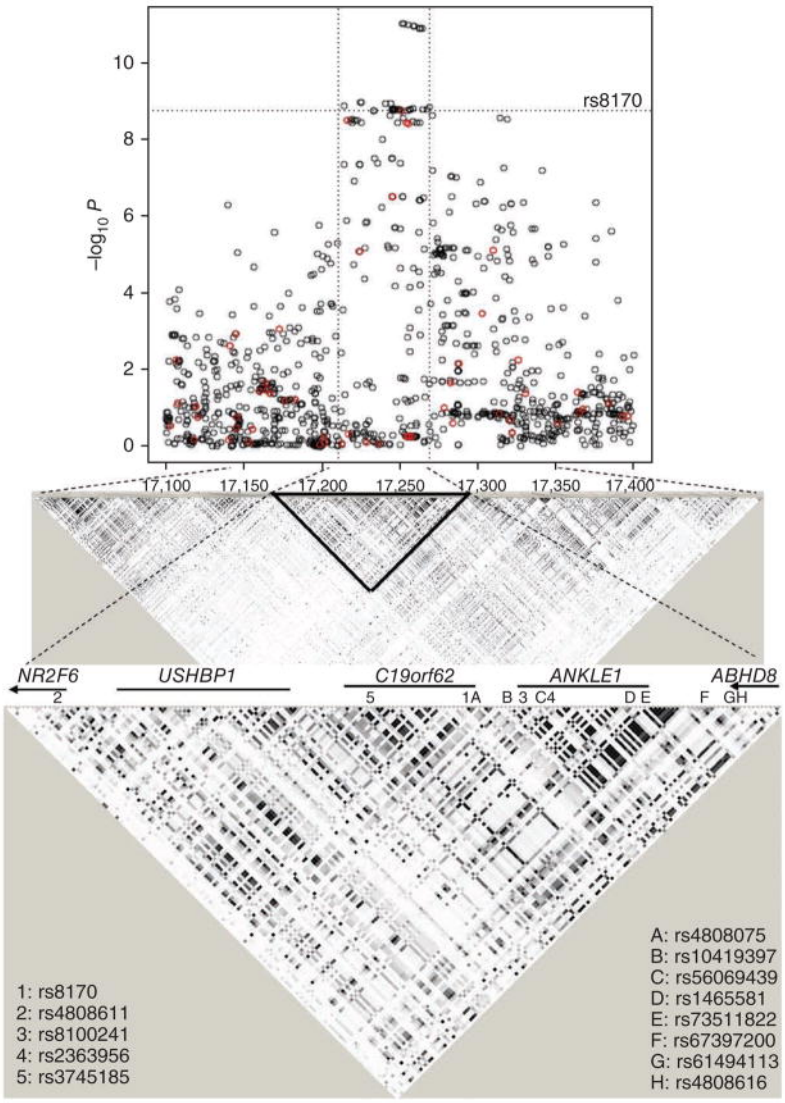

Figure 3. The genotyped SNPs are shown in red and imputed SNPs in black. The horizontal dotted line indicates the p-value for the strongest association among genotyped SNPs (rs8170). The middle figure shows LD blocks around the best five SNPs. Below, details of the region containing the genotyped SNPs (shown by numbers 1-5) and imputed SNPs (shown by letter A-H)

The region 19p13 with the five SNPs and eight imputed SNPs encloses three genes, whereof the gene C19orf62 is the most interesting. The gene *C19orf62* transcripts the protein MERIT40 (Mediator of Rap80 Interactions and Targeting 40 kd), which assists *BRCA1* localization and enforces DNA damage repair response [122]. The SNPs modifying the function or expression of MERIT40 may have an impact on increased breast cancer risk.

Most recently, a replication study was performed with 5408 additional *BRCA1* mutation carriers to confirm the previously identified association with the most significant SNP on 19p13, rs8170, and one of the imputed SNPs, rs67397200. The

combined analysis with the previous and new mutation carriers was consistent with the original analysis (rs8170 combined HR 1.20, 95% CI 1.17-1.35 p=2.3x10^{-9}). The SNP rs67397200, which was imputed in original analysis, was genotyped in the whole sample set showing strongly significant association with an increased breast cancer risk (HR 1.17, 95% CI 1.11-1.23, p=2.4x10^{-8}). In the analysis of a possible joint effect of the two SNPs, the rs67397200 remained significant. Further analysis of association with ER status in *BRCA1* tumors revealed that this SNP was slightly more associated with ER negative tumors (HR1.22) compared to ER positive tumors (HR 1.14), but the difference was significant (p=0.41). However, in *BRCA2* mutation carriers this SNP rs67397200 was associated with ER negative breast tumors (HR1.29, 95% CI 1.1-1.49, p=8.7x10^{-4}), but no association was shown with ER positive tumors. Competing risk analysis with the combined set of samples revealed that the SNPs rs8170 and rs67397200 were both association with ovarian cancer risk in both *BRCA1* (rs8170 HR1.15, rs67397200 HR 1.16) and *BRCA2* (rs8170 HR 1.34, rs67397200 HR 1.30) mutation carriers [121].

7.3 PAPER III

In this study we set up to investigate the oncogenetic counseling process. The main findings were 1) telephone pre-counseling worked as well as traditional in-person counseling; 2) the counselees were satisfied at high level with oncogenetic counseling; 3) the counselees experienced difficulties with the process of creating a pedigree and 4) the counselees showed dissatisfaction with information on surveillance and prevention.

Given the results of this study, the telephone pre-counseling as an option of pre-counseling process is an equally satisfying delivery model from counselee´s perspective. This is in concordance with the only past randomized study of telephone versus in-person counseling in the area of cancer [123]. Results from a small randomized trial, which focused to compare the disclosure of genetic testing result on telephone and in-person, suggests that genetic test disclosure by phone is a reasonable alternative to traditional model showing equal outcomes of anxiety, well-being, knowledge and satisfaction and does not show any negative psychological outcomes [124]. An ongoing large randomized study on counselees´ outcomes aims to determine the impact of telephone counseling model versus in-person counseling model prior *BRCA1/2*

mutation testing [125]. The collection of study material started 2008 and continues 5 years aiming to collect 600 participants. The results from a study based on individuals with increased breast cancer risk which were identified from the general population based cohort after an email invitation, support the effort to build up oncogenetic counseling process to respond needs from counselees as well as economic and organizational requests. Indeed two thirds of the participants indicated that they attended genetic counseling only because it was available by telephone [126].

The counselees were very well satisfied regarding the contact with oncogenetic nurse in both telephone- and in-person counseling groups. The results are in concordance with several other studies showing that overall counselees at hereditary cancer clinics are satisfied at large extent with the process [116, 117, 127-129].

One of the most profound findings among counselees in our survey was that they experienced difficulty with the process of creating a pedigree. A fifth of the participants were not interested to gain information from relatives while almost half of the participants did not feel comfortable with contacting relatives. Almost 40% had practical difficulties to gain information about relatives. This can be a problem because missing essential information can ground obstacles in implications of the risk of developing cancer in the family as the counselees have an active role in collecting information from relatives.

A desire of more emotional support from caregivers during oncogenetic counseling process is a frequent issue [117, 127]. This was the case in our survey as well revealing that one in ten found it emotionally challenging to expose themselves and the family. This indicates the need to identify the counselees, which would benefit from additional support.

A considerable number among both affected and unaffected participants expressed dissatisfaction with information on cancer prevention and surveillance. Recommendations on risk reducing lifestyle factors can are difficult give because of the lack of trustworthy scientific evidence. Individualized risk management and prevention have presumably a key role in health care and it is a challenge for caregivers.

7.4 PAPER IV

The success rate of genotyping was 94.3%. None of the markers failed genotyping. Sporadic single marker genotypes were excluded after analysis of mistyping errors. The final number of individuals for linkage analysis was 102 individuals.

The linkage analysis revealed five candidate loci with a HLOD above one (Table 5.) whereof two loci (chr18q and chr22q) had HLOD above one in both models of affected status criteria (loose and strict). Regions in these two chromosomes showing evidence of linkage are very broad. Region on chromosome 18q covers 35cM (69-104cM) (loose criteria model). Within the same region, strict criteria model showed only a single marker, D18S450, with significant linkage with α-value 0.60. Region at 22q11.1-q22.3 covers 732Kb. These regions showed some evidence of association (HLOD above 1) for both of the top markers with both affected criteria models. The region in chromosome 6p (two markers) revealed positive linkage only with loose criteria model whereas the region in chromosome 8q (one marker) showed positive linkage with strict criteria model. The region on chromosome 11p12-q13.2 shows evidence of linkage with strict criteria model for three markers spanning across centromere.

Table 5. LOD scores, HLOD, α-values, NPL and p-values with both loose and strict criteria models in the candidate regions.

Locus	cM	Model	Marker	LOD (α=1)	HLOD[a]	α value[1]	NPL	p value
6p21.1-p12.3	70-72	Loose	D6S459	-2.544	1.333	0.40	0.732	0.185
			D6S452	-3.283	1.338	0.40	0.673	0.212 ←
		Strict	D6S459	-1.367	0.335	0.30	0.476	0.334
			D6S452	-2.155	0.349	0.30	0.448	0.357
8q13.3	84	Loose	D8S279	-4.592	0.000	0.00	0.475	0.335
		Strict	D8S279	1.193	1.193	1.00	0.549	0.282 ←
11p12-q13.2	57-72	Loose	D11S1360	-3.567	0.098	0.15	0.626	0.237
			D11S4191	-3.465	0.104	0.15	0.733	0.185
			D11S4087	-2.307	0.242	0.30	0.821	0.151
		Strict	D11S1360	1.053	1.053	1.00	0.534	0.293
			D11S4191	1.711	1.711	1.00	0.779	0.166
			D11S4087	1.942	1.942	1.00	0.778	0.167 ←
18q21.1-q22.3	69-104	Loose	D18S450	-1.812	1.301	0.40	0.865	0.136
			D18S474	-4.371	0.773	0.25	0.745	0.180
			D18S64	-5.717	1.359	0.20	0.709	0.196
			D18S1134	-5.830	1.348	0.20	0.690	0.204
			D18S1147	-6.516	1.354	0.20	0.673	0.212
			D18S465	-5.101	1.403	0.25	0.722	0.190 ←
			D18S469	-6.104	1.088	0.20	0.565	0.272
		Strict	D18S450	0.342	1.164	0.60	0.946	0.113 ←
			D18S474	-1.052	0.549	0.35	0.763	0.173
			D18S64	-1.263	0.548	0.35	0.660	0.219
			D18S1134	-1.571	0.526	0.35	0.640	0.229
			D18S1147	-2.806	0.513	0.30	0.590	0.257
			D18S465	-2.551	0.528	0.35	0.638	0.230
			D18S469	-2.317	0.454	0.30	0.470	0.339
22q11.1-q11.21	3-6	Loose	D22S420	1.006	1.159	0.70	1.006	0.099 ←
			D22S427	0.062	1.162	0.60	1.058	0.088
		Strict	D22S420	1.560	1.560	1.00	0.758	0.175 ←
			D22S427	1.215	1.437	0.80	0.849	0.142

[1] the proportion of families linked to that marker.

← the maximum LOD score in the candidate regions

8 CONCLUSIONS AND CLINICAL IMPLICATIONS

Paper I

Even though the today´s counselees have more knowledge about hereditary cancer and the oncogenetic counseling process has been improved, the counselees still estimate the risk of developing cancer too high, which leads to increased worry for cancer and health care burden in form of unnecessary controls and need of psychological interventions. Oncogenetic counseling could benefit from changing from the traditional risk information to more prevention-focused counseling. Numerical risks are hard to interpret and remember, therefore risk counseling could rely more on descriptive risk counseling.

Paper II

Identification of genetic modifiers for risk of developing hereditary breast and ovarian cancer is essential because age of onset and cancer incidence varies in *BRCA1* and *BRCA2* mutation carriers. The identified genetic variants in combination with other risk modifiers will improve individual risk assessment in *BRCA1* and *BRCA2* mutation carriers. The knowledge of modifying factors may also be adapted to risk assessment in the general population.

Paper III

The results from our study indicate that the pre-counseling telephone model is an equally good approach to provide the first stage of oncogenetic counseling than the in-person pre-counseling model. Economic and administrative advantages make it a profitable alternative to traditional in-person counseling.

Oncogenetic counseling providers should pay more attention to that the counselees experienced difficulties with the process of creating a pedigree and were dissatisfied with information on recommended surveillance and prevention. These aspects should be improved in order to face counselee´s needs and expectations.

Paper IV

The knowledge we obtain from linkage studies can point us in the right direction to identify additional genes, which predispose to hereditary breast cancer. We are currently performing fine-mapping in some of the candidate regions and are sequencing some candidate genes, which are located in the regions revealed by fine-mapping. Association studies of the candidate SNPs will also be performed.

9 POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

I vissa familjer förekommer en ansamling av personer som drabbat av tumörsjukdom. Flera som insjuknat i familjen, låg ålder vid insjuknande, multipla tumörer och associerade tumörer talar starkt för en ärftlig komponent som orsak till sjukdomen. Cirka 10-15% alla bröstcancer fall beräknas ha en ärftlig bakgrund. Familjer med en ökad risk för bröst- och/eller äggstockscancer erbjuds idag genetisk vägledning för att få råd och rekommendationer om förebyggande av cancer.

Hos en liten andel av kvinnor med bröstcancer kan en medfödd mutation i en av två kända gener, *BRCA1* och *BRCA2,* identifieras. Mutationer i dessa gener är förenat med en ökad risk att utveckla bröstcancer och äggstockscancer. Individer som bär på en mutation bör inkluderas i kontrollprogram för förebyggandet av cancer. Ålder vid insjuknande varierar mycket mellan mutationbärare och ungefär en femtedel förblir friska, vilket indikerar att det finns modifierande genetiska- och miljöfaktorer som påverkar risken för sjukdomen.

Trots att många familjer med mutationer i nämnda gener har identifierats och erbjudits vägledning och prevention, finns många familjer med en sannolik ärftlig bröstcancer där man ännu inte har identifierat den bakomliggande genetiska orsaken. Familjemedlemmar med en ökad risk kan således inte erbjudas genetisk testning utan rekommenderas delta i kontrollprogram.

Förståelse för patientens riskuppfattning före och efter genetisk vägledning ger verktyg för att förbättra kommunikation och därmed bidra till en mer korrekt riskuppfattning hos patienten och att patienten uppfattar betydelsen av kontrollprogram. Individer med låg eller lätt förhöjd risk för cancer skulle gynnas av minskad oro och ångest medan individer med hög risk skulle vidta adekvata pre-symptomatiska åtgärder om de kunde uppfatta sina risker korrekt och då skulle möjligheten att upptäcka cancer i tidigt stadium öka.

Exempel på metoder för att försöka hitta den bakomliggande ärftliga faktorn i familjära fall är kopplings- och associationsstudier. Med kopplingsanalys menas lokalisering av olika positioner i arvsmassan som är kopplade till sjukdom. Ett samband mellan fenotyp och genetisk region kan upptäckas genom att individer med en specifik fenotyp ovanligt ofta delar anlag som har ärvts från samma person långt tillbaka i familjen. Med hjälp av statistiska verktyg beräknas ett värde på hur pass gemensam nedärvingen är. I en associationstudie jämförs kvinnor med bröstcancer och konmed friska kvinnor för att hitta samband mellan risken att drabbas av sjukdomen och genetiska markörer. För att studera nedärvingen använder man sig av markörer spridda i hela genomet. Dessa markörer varierar mellan individer.

Huvudsyftet med avhandlingsarbetet var att utveckla genetisk diagnostik samt öka kunskap vid genetisk vägledning i familjer med ärftlig cancer. Denna avhandling har fyra delarbeten som undersöker ovanstående.

I **arbete I** använde vi oss av enkäter från 215 patienter vid tre uppföljningstillfällen; före och precis efter genetisk vägledning samt ett år efter avslutat genetisk utredning. Syftet var att utvärdera patientens uppfattning av den information som ges samt hur denna uppfattning påverkar individens upplevda oro efter given information. Detta anses vara viktigt eftersom information om risk är en mycket viktig del av den genetiska vägledningen. Informationen avser att hjälpa patienten att förstå den egna och familjemedlemmarnas risk eftersom den har medicinsk och psykologisk innebörd.

Resultaten visar att alla förutom hög-risk patienter överskattade sina risker att drabbas av ärftlig cancer. Individer med samma risk som populationen och de med låg risk hade mest inkorrekta uppfattningar om sina risker. Patienternas riskuppfattning för barnen och för populationen var lägre än den personliga uppfattade risken även om den också var överskattad. Efter genetisk vägledning var riskuppfattningen mer korrekt, speciellt i låg-risk gruppen. Oron för cancer minskade hos alla utom hög-risk individer och hos individer som hade haft cancer. Studien visade ett starkt samband mellan upplevd oro och riskuppskattning. Syftet att delta i kontrollprogram för att minska cancer incidens och mortalitet hade förståtts väl av patienterna.

I **arbete II** utvärderades hur genvarianter modifierar risken för bröst- och äggstockscancer hos *BRCA1* mutationbärare. En associationstudie med över 600 000 markörer utfördes för att jämföra genotyper och kliniska uppgifter mellan fall och kontroller.

Studien identifierade fem ovanliga varianter som modifierar risken för cancer hos *BRCA1* bärare, varav två medför 20% förhöjd risk och tre medför 20% skyddande effekt mot bröstcancer jämfört med bärare som har den vanliga varianten. På sikt kan enskilda individens risk beräknas som en kombination av genetiska varianter där effekten sammanvägs vilket skulle innebära att mutationsbärare skulle få mer individuell riskbedömning vid genetisk vägledning.

I arbete III utvärderades genetisk vägledning ur patientens synvinkel. Studiematerialet var samma som i delarbete I. Arbetet jämförde vägledning per telefon med personligt besök för den första delen av vägledningen som syftar till att kartlägga familjeträdet och bekräfta cancerdiagnoser inför läkarbesöket. Följande aspekter evaluerades också: patientens förväntningar, tillfredställelse, erfarenhet av genetisk vägledning, oro för att drabbas av familjär cancer och hälsorelated livskvalité.

Resultaten visar att deltagarna var generellt väldigt nöjda med vägledningsprocessen oavsett om den första kontakten skedde via telefon eller genom personligt besök. Telefonvägledning förefaller fungera lika bra som traditionell ansikte-mot-ansikte vägledning. Resultaten visar även att det svåraste i processen var att generera ett släktträd och att kontakta anhöriga. Känslomässigt stöd under vägledningsprocessen bör därför förbättras. Deltagarna rapporterade missnöje med information av preventiva åtgärder och kontrollprogram och detta bör också förbättras.

Arbete IV syftade till att hitta regioner som är kopplade till förhöjd risk för cancer hos familjer som har en anhopning av både bröst- och ovarialcancer. Vid en genom-vid kopplingstudie genotypades 540 mikrosatellitmarkörer i 14 stora non-*BRCA1/2* familjer med 102 familjemedlemmar. För statistiska analyser beräknades parametrisk LOD score, icke-parametrisk LOD score och HLOD analys. Analyser genomfördes med två olika insjuknande status. I den ena analysen kodades endast kvinnor med bröstcancer

som drabbade, medan vid den andra analysen kodades de med bröstcancer och de med andra cancer typer som drabbade.

Fem kandidatregioner med en sannolik koppling till bröstcancer kunde identifieras. I framtida analyser fortsätter man identifiera dessa kandidatregioner.

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