70-gene signature for clinical decision making in breast cancer

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CHAPTER 1

INTRODUCTION AND OUTLINE

BREAST CANCER

Breast cancer is the most common cancer among women worldwide with 2.3 million new cases and 685.000 deaths in 2020.[1] Incidence varies between countries, the highest breast cancer incidences are reported in Europe, with Belgium, Luxembourg and the Netherlands representing the top three countries in 2018.[2] Breast cancer mortality has improved drastically since the 1980s with the introduction of breast cancer screening programs, increased patient awareness, adjuvant systemic therapies and improved locoregional treatments.[3]

SCREENING

In the Netherlands, the national breast cancer screening program started in 1990 and achieved full coverage in 1998. Screening consists of biennial mammography for women aged 50 to 75 years. The Netherlands has a very high attendance rate, ranging from 75-80% over the past 10 years.[4–7] The goal of screening is to detect breast cancers in an earlier stage, thus being able to initiate treatment early, ultimately resulting in a decrease in breast-cancer related mortality. Since the late 1980's there has been a steady increase in breast cancer incidence in the Netherlands, partly due to the introduction of the national breast cancer screening [4,6] There has mainly been a large increase in the incidence of small, early-stage tumors and a small decrease in the incidence of large tumors, resulting in a significant stage shift.[4,8] In the Netherlands 85% of the incident breast cancers in 2019 were stage I or II tumors (47% stage I, 38% stage II) with respective 10-year survival rates of 95% and 85%, versus a 15% incidence of stage III and IV tumors (9% stage III, 6% stage IV) with 10-years survival rates of 60% and 12%.[9] Breast cancers detected by screening have more favorable tumor characteristics than interval cancers found in between two screening rounds. However, screen-detection itself is also associated with a better prognosis of breast cancer, independent of clinical-pathological characteristics.[5]

BREAST CANCER SUBTYPES

The identification of different subtypes of breast cancer has resulted in better and more targeted treatment of breast cancer.[10] In addition to the four main molecular subtypes of breast cancer - Luminal A, Luminal B, Human Epidermal growth factor Receptor 2 (HER2) enriched and Triple negative subtypes - continuing research is uncovering many differences in the underlying biology of breast cancer within these subtypes, providing new knowledge for the development of new targeted

treatments.[10] Luminal type cancers, expressing the estrogen receptor (ER) and progesterone receptor (PR), are the most common subtype, representing between 70-80% of breast cancers. The majority of these are Luminal A-like (60-70%, of all breast cancers), which have a high expression of ER and PR and are HER2-negative (HER2-), with typically low histological grade and a low proliferation index. Luminal B-like cancers are ER+, but with lower expression of ER and PR than Luminal A-like cancers, they can be either HER2- (10-20%) or HER2+ (10%), and have a higher histological grade and a higher proliferation index. HER2-enriched, non-luminal cancers are ER and PR negative, HER2+, high grade and with a high proliferation index (3-5%). Lastly, Triple negative cancers are ER, PR and HER2 negative, high grade and with a high proliferation index, and represent 10-15% of breast cancers.[10] The prognosis of breast cancer is strongly related to these molecular subtypes, in combination with tumor size and axillary lymph node status.

ADJUVANT SYSTEMIC TREATMENT

The current adjuvant treatment guidelines for early breast cancer use prognostic features of breast cancer (tumor size, grade, lymph node status) and the main molecular subtypes for the recommendation of different treatments. There are many international and national adjuvant treatment guidelines, and although they mostly overlap, slight differences between guidelines exist. All guidelines agree on the treatment of patients with Triple negative tumors; chemotherapy is always recommended, except in patients with very small tumors (≤0.5 cm or ≤1 cm) that are lymph node negative (N0).[11–17] Similarly, all guidelines agree that patients with HER2+ tumors should be treated with anti-HER2 targeted therapy, combined with chemotherapy, except in patients with small tumors (≤0.5 cm, N0 or N1mi) where chemotherapy can be omitted but anti-HER2 therapy seems to give some benefit.[11–17]

For Luminal-like cancers, most guidelines state that all patients with ER+ invasive cancers should receive endocrine therapy. The Dutch guideline has an exception here: endocrine therapy can be omitted in patients with negative lymph nodes and grade 1 tumors ≤2 cm or grade 2 or 3 tumors ≤1 cm.[16] The recommendations for chemotherapy in Luminal-like patients are less clear between different guidelines. There is agreement that patients with high tumor burden, meaning large tumors (>5 cm) or ≥4 positive lymph nodes, should receive chemotherapy. Similarly, patients with small tumors (≤0.5 cm or ≤1 cm, N0) are not considered for chemotherapy in all guidelines.[11–17] For patients with tumors ranging from 1 to 5 cm and 0 to 3

positive lymph nodes, the majority of breast cancer patients, guidelines are less clear in their recommendation for chemotherapy. Most guidelines recommend using (online) decision-making tools, such as PREDICT, Nottingham Prognostic Index (NPI) and Adjuvant! Online, to help predict the individual patients' risk of recurrence and the potential benefit from systemic treatments.[18–20] The Dutch guideline further specifies groups that are not considered for chemotherapy, namely patients with negative lymph nodes and grade 1 tumors ≤3 cm, grade 2 tumors ≤2 cm and grade 3 tumors ≤1 cm, and patients with positive lymph nodes and grade 1 tumors ≤2 cm.[16] All guidelines currently recommend using gene expression assays in patients where there is an uncertainty regarding the indication for chemotherapy.[11–17]

GENE EXPRESSION ASSAYS: A BETTER INSIGHT INTO TUMOR BIOLOGY

In the past 20 years several gene expression assays have been developed and validated that give an insight into the biology of breast tumors and improve the prediction of the risk of distant metastases based on the expression of genes associated with, amongst others, tumor proliferation and metastasis.[21] These assays were developed to aid in the selection of patients eligible for chemotherapy. Although chemotherapy is effective in eradicating micrometastases and has significantly contributed to the reduction of breast cancer mortality over the years, it is associated with many short and long-term serious side-effects and should therefore be avoided in patients already cured by locoregional therapy.[3,22–24]

One of these assays is the 70-gene signature, commercially known as MammaPrint. The 70-gene signature was developed to identify patients with a high or low risk of developing distant metastases within 5 years of breast cancer diagnosis.[25] The 70-gene signature produces an index score ranging from –1 to 1; an index score equal to or < 0 is classified as high risk and an index score > 0 is classified as low risk. The 70-gene signature was developed in a historic cohort of patients that received no adjuvant systemic treatment and has been validated extensively, both retrospectively and prospectively, including in subgroups of patients with HER2+ tumors and with 1-3 positive lymph nodes.[25–35]

An overview of the gene expression assays that are currently available and endorsed by guidelines is presented in Table 1. The analyses presented in this thesis focus on the 70-gene signature.

Table 1 Gene expression assays currently available

	MammaPrint	Oncotype DX	Prosigna/PAM50	EndoPredict	Breast Cancer Index
Number of genes in assay	70 genes	21 genes (16 genes + 5 reference genes)	50 genes (+ 5 reference genes)	12 genes (8 genes + 4 reference genes)	7 genes (5 genes + 2 gene ratio)
Technique	Micro-Array	qRT-PCR	qRT-PCR	qRT-PCR	qRT-PCR
Provided by	Agendia (Amsterdam, NL)	Exact Sciences (Madison, WI, USA)	Nanostring Technologies (Seattle, WA, USA)	Myriad Genetics (Salt Lake City, UT, USA)	Biotheranostics, Inc. (San Diego, CA, USA)
Tissue Sample	Frozen or FFPE	Frozen or FFPE	Frozen or FFPE	FFPE	FFPE
Output	High or Low risk	Continuous variable (Recurrence Score), categorized into 3 groups; High, Intermediate and Low	Continuous variable (Risk of Recurrence score), categorized into 3 groups; High, Intermediate and Low	Continuous variable (EP score), categorized in 2 groups; High and Low	High or Low risk
Molecular subtyping	Yes (Blueprint)	No	Yes	No	No
Retrospective clinical trials	TRANSBIG[28]	NSABP B14[39] NASBP B20[40] SWOG 8814[41] TransATAC[42]	ABCG8[43,44] ATAC[42,44]	GEICAM 9906 [45] ABCG6[46] ABCG8[47]	TransATAC[48] Stockholm Trial[49]
Prospective clinical trials	RASTER[35] MINDACT[27,50]	TAILORx[36] RxPONDER[37]	OPTIMA (ongoing)[51]	-	-
ASCO/NCCN recommendation	Yes (strong)	Yes (strong)	Yes (moderate)	Yes (moderate)	Yes (moderate)
Guideline recommendations for candidate patients	ER/PR+, HER2-, LN0 or LN+ (1-3 positive nodes)	ER/PR+, HER2-, LN0 or LN+ (1-3 positive nodes)	ER/PR+, HER2-, LN0	ER/PR+, HER2-, LN0	ER/PR+, HER2-, LN0
Adjuvant therapy recommendation	Chemotherapy	Chemotherapy	Chemotherapy	Chemotherapy Extended Endocrine therapy	Extended Endocrine therapy
Combination with clinical-pathological characteristics in score assessment	Yes (Adjuvant! Online)	No	Yes (proliferation score, tumor size)	Yes (tumor size and nodal status (EPclin))	No
Assumptions	Assumes no therapy	Assumes 5 years of endocrine therapy	Assumes 5 years of endocrine therapy	Assumes 5 years of endocrine therapy	Assumes 5 years of endocrine therapy

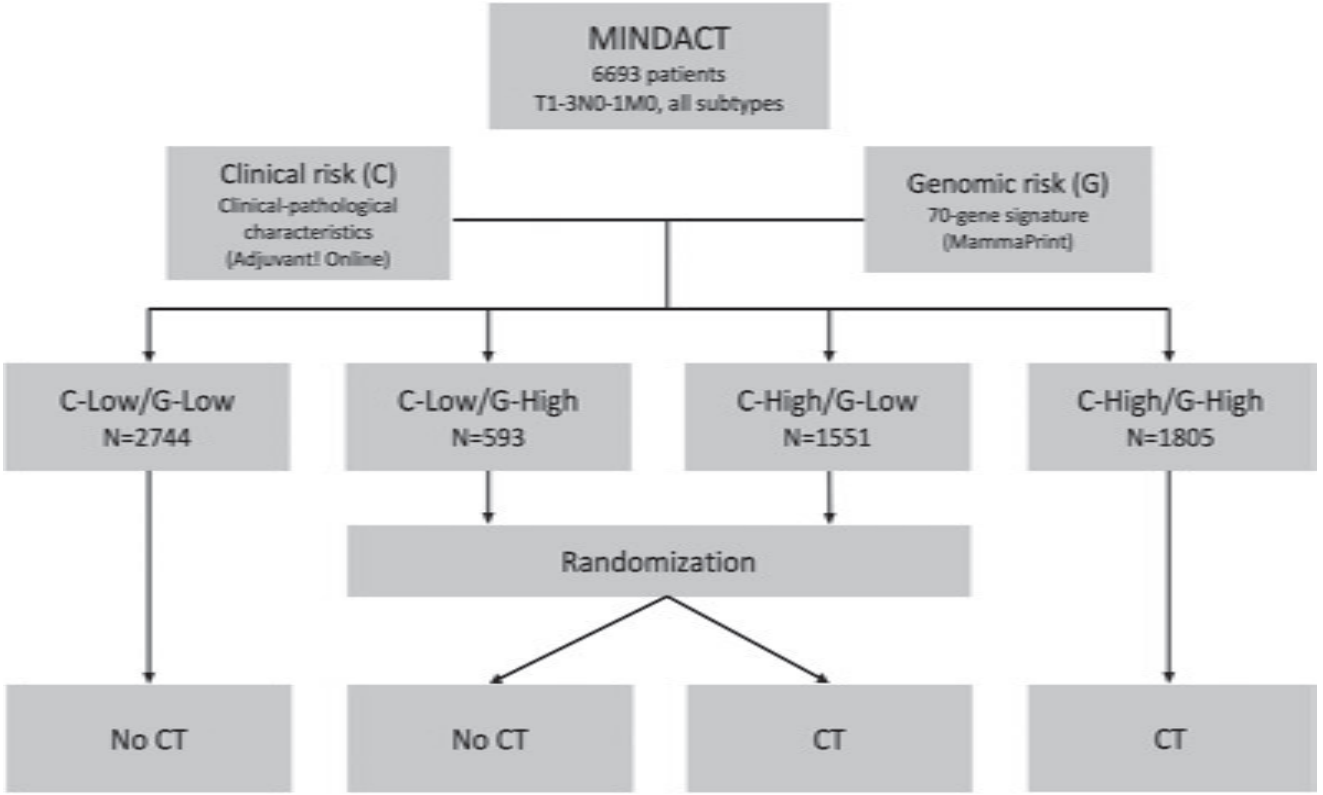
ER, estrogen receptor; FFPE, formalin fixed paraffin embedded; HER2, human epidermal growth factor receptor 2; LN, lymph node; PR, progesterone receptor

CLINICAL UTILITY OF BREAST CANCER GENE EXPRESSION ASSAYS: THREE LANDMARK TRIALS

The clinical application of gene expression assays is to use them to identify patients with a low risk of developing distant metastases, despite unfavorable clinical-pathological characteristics, who may be spared unnecessary adjuvant systemic treatment, also known as de-escalation of treatment. However, evidence of the clinical utility of a new test needs to be established first. Currently only two gene expression assays, the 70-gene signature (MammaPrint) and the 21-gene recurrence score (OncotypeDX), have provided evidence of level 1 clinical utility, through large prospective phase 3 studies, MINDACT for the 70-gene signature and TAILORx and RXponder for the 21-gene recurrence score.

MINDACT included women aged 18-70 years, with operable invasive breast cancer (T1, T2 or operable T3), with negative or 1-3 positive lymph nodes (N0-1), and no distant metastases.[27] In MINDACT allocation to adjuvant systemic treatment was based on a combination of the genomic risk, assessed by the 70-gene signature, and the clinical risk based on clinical-pathological characteristics assessed by the online decision-making tool Adjuvant! Online. Patients with a concordant low risk were allocated to receive no chemotherapy, whereas patients with a concordant high risk received chemotherapy. Patients with a discordant risk were randomized to follow either the clinical or the genomic risk to receive chemotherapy or not (Figure 1).[27] In total 6693 patients were enrolled in MINDACT. Of the women with a high clinical risk according to clinical-pathological characteristics, 46% had a low genomic risk. This group was the focus for the primary analysis, to test if the lower boundary of the 95% confidence interval (CI) of the 5-year distant metastasis free survival (DMFS) rate of patients who received no chemotherapy exceeded 92%. The primary endpoint was met in 2016, with a 5-year DMFS rate of 94.7% (95% CI 92.5-96.2) in patients with a high clinical and low genomic risk who received no chemotherapy. In a secondary analysis in the clinical high risk and genomic low risk population, the absolute difference in 5-year DMFS between patients who received chemotherapy or not was 1.5%.[27]

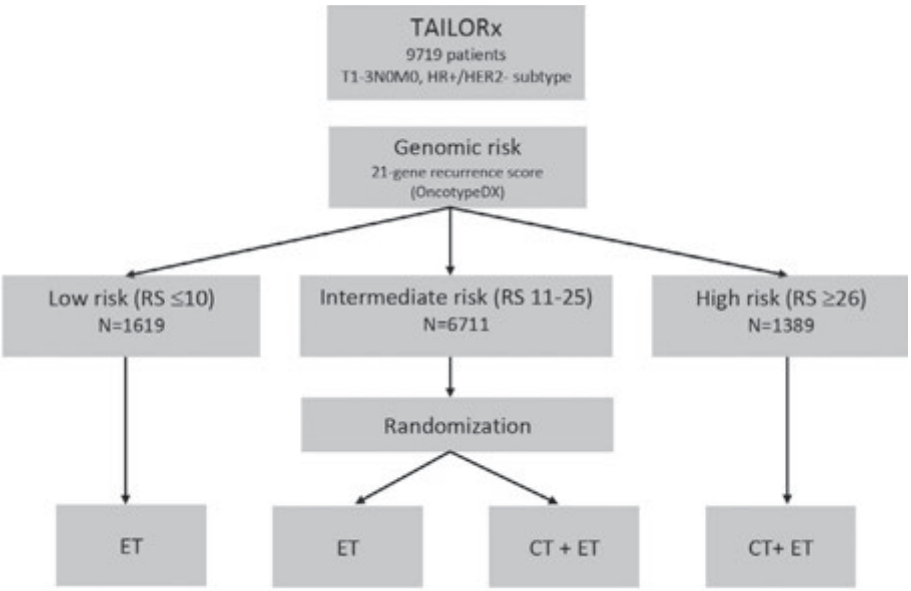
Figure 1 MINDACT trial design



CT, chemotherapy

TAILORx included women aged 18-75, with hormone receptor-positive (HR+), HER2- and lymph node negative (N0) breast cancers and no distant metastases.[36] Allocation to adjuvant systemic treatment was based only on the genomic risk, assessed by the 21-gene recurrence score (RS). Patients with a low risk (RS ≤10) received endocrine therapy, patients with an intermediate risk (RS 11-25) were randomized to receive chemoendocrine therapy or endocrine therapy alone, and patients with a high risk (RS ≥26) received chemoendocrine therapy (Figure 2).[36] Of the 9719 patients included in the main analysis, 69% had an intermediate risk. In patients with an intermediate risk, endocrine therapy was non-inferior to chemoendocrine therapy for invasive disease-free survival (HR 1.08, 95% CI 0.94-1.24). The absolute difference in 9-year distant recurrence-free interval in intermediate risk patients receiving endocrine therapy alone or chemoendocrine therapy was 0.5%. Furthermore, they found a difference in benefit from chemotherapy in the intermediate risk patients according to age; women aged 50 years or younger with an RS 16-25 seemed to have some benefit from chemotherapy.[36]

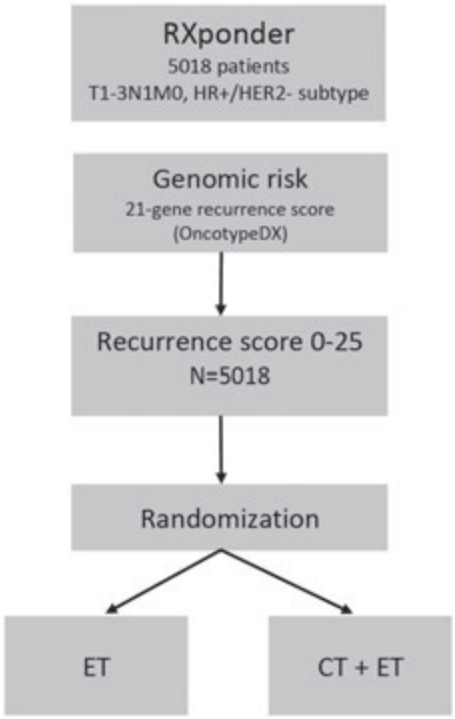
Figure 2 TAILORx trial design



ET, endocrine therapy; CT, chemotherapy; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; RS, recurrence score

RXponder included women older than 18 years, with HR+, HER2- breast cancers (T1-3), with 1-3 positive lymph nodes (N1) and no distant metastases.[37] For all patients the 21-gene recurrence score was assessed, patients with a RS ≥26 were not eligible, patients with a RS 0-25 were randomized to endocrine therapy alone or chemoendocrine therapy (Figure 3).[37] A total of 5018 patients participated in the trial. Results were analyzed according to menopausal status (33% premenopausal, 67% postmenopausal). No chemotherapy benefit was seen in postmenopausal patients for invasive disease-free survival (HR 1.02, 95% CI 0.82-1.26), with an absolute difference at 5 years of 0.6% and no difference in DMFS at 5 years. In premenopausal patients, a chemotherapy benefit was seen with an absolute difference in 5-year invasive disease-free survival of 4.9% (HR 0.60, 95% CI 0.43-0.83) and an absolute difference in 5-year DMFS of 3.3% (HR 0.58, 95% CI 0.39-0.87).[37]

Figure 3 RXponder trial design



ET, endocrine therapy; CT, chemotherapy

Although all three trials use a gene expression assay to select patients eligible for de-escalation of chemotherapy, some important differences are present between them. First, MINDACT included women with all subtypes of breast cancer, including women with triple negative and HER2+ breast cancer, whereas TAILORx and RXponder only included patients with HR+, HER2- breast cancers. The majority of the triple negative and HER2+ patients in MINDACT were clinical and genomic high risk, therefore the proportion of these patients randomized was small, leading to inconclusive evidence in these subgroups.[27] Second, clinical risk was not included as a factor for allocation of treatment in TAILORx and RXponder. As a result, the TAILORx population is predominantly clinical low-risk, 74% of the patients with an intermediate risk had a low clinical risk, and 26% had a high clinical risk.[36] If treatment was allocated according to the MINDACT protocol, only patients with a high clinical risk would have been randomized to receive chemotherapy or not, whereas the clinical low-risk patients in this group would not have been considered for chemotherapy. In an additional analysis with the TAILORx data, they showed that clinical risk provided additional prognostic information to the 21-gene recurrence score about the risk of distant recurrence.[38] As also shown in MINDACT, a combination of clinical and genomic risk provides a more accurate estimation of prognosis than provided by either clinical or genomic risk alone. In RXponder, only patients with lymph node positive disease were included, nevertheless, 18% of patients had a low clinical risk.[37] Lastly, there are differences in the type of chemotherapy regimens between the three trials, and where all patients in TAILORx and RXponder received endocrine therapy by trial design, in MINDACT endocrine therapy was administered according to local guidelines.[27,36,37] Despite the differences in trial design, these three are landmark trials that have shown the value and clinical utility of gene signatures for de-escalation of chemotherapy and have changed clinical practice for treatment of early breast cancer.

RATIONALE AND OUTLINE OF THIS THESIS

The aim of this thesis is to evaluate whether the use of the 70-gene signature can be improved by taking into account other factors to better define subgroups of patients who are at risk of overtreatment, and for whom adjuvant systemic treatment (chemotherapy and/or endocrine therapy) could be safely omitted. We evaluated the influence of the 70-gene signature on risk assessment and chemotherapy recommendation in the years after MINDACT. Furthermore, we evaluated the association between the 70-gene signature and a polygenic risk score (PRS) for breast cancer, as well as the associations of this PRS with tumor characteristics and survival.

As follow-up information continued to be collected for patients included in MINDACT after publication of the first results in 2016, the primary endpoint could be re-evaluated with longer follow-up and now >90% of patients having at least 5 years of follow-up. In **chapter 2** the updated analyses of the MINDACT trial are presented, including an analysis by age to assess if similar differences in survival were seen for pre- versus postmenopausal patients as in TAILORx. In the years since the introduction of the 70-gene signature and the emerging evidence of its applicability and clinical utility, it has been increasingly employed in the clinic to guide decisions on chemotherapy. A previous study among 12 oncologists showed a modest improvement in agreement when the 70-gene signature was added to clinical-pathological characteristics. In **chapter 3** we evaluated the agreement on risk assessment and chemotherapy recommendation among a larger group of breast cancer specialists before and after providing information on the 70-gene signature result, and at different points in time to assess any changes over time.

Using the long-term follow-up data from the MINDACT trial, we were able to explore different subgroups of patients who may be at risk of overtreatment. In **chapter 4** we assessed the outcomes of stage I ER+/HER2- breast cancer patients who did not receive any adjuvant systemic treatment, and compared them to a matched group of patients with similar clinical-pathological characteristics who received endocrine therapy. Method of detection has shown to be associated with prognosis of breast cancer, independent of clinical-pathological characteristics. Screen-detected cancers were also found to have a more favorable tumor biology compared to interval cancers, in the cohort of Dutch patients included in MINDACT, with higher proportions of 70-gene signature low and ultralow risk tumors. In **chapter 5** we further evaluated whether this observed difference in tumor biology also results in a difference in survival. For the 70-gene signature a threshold was established within the low-risk category to identify patients with an ultralow risk of distant recurrence. These ultralow risk tumors are thought to be indolent cancers, and identifying patients with ultralow risk tumors could help to avoid overtreatment. Patients with 70-gene signature ultralow risk tumors had excellent long-term survival in historic cohorts, but the number of ultralow risk patients in these studies was small. In **chapter 6** we evaluated the outcomes of patients with an ultralow risk 70-gene signature in the MINDACT trial, the largest cohort to date.

The development of breast cancer is multifactorial and is influenced by family history, breast density, life style factors and genetic factors, among others. There are several well-known germline-mutations that are associated with breast cancer risk (BRCA1/2, CHEK2, etc.), but large genome-wide association studies (GWAS) have identified hundreds of common genetic variants (mostly single nucleotide polymorphisms (SNPs)) that are associated with breast cancer risk. Individual SNPs have a small effect on risk, but their joint effects can be substantial. A PRS consisting of 313 SNPs (PRS_{313}) is associated with the risk of developing breast cancer. One of the most promising clinical applications for PRS is to provide a personalized risk assessment in order to individualize breast cancer screening. For a subgroup of patients in MINDACT, the PRS_{313} was available. In **chapter 7** we investigated the association of the PRS_{313} with clinical-pathological characteristics and survival of breast cancer in the large database of the Breast Cancer Association Consortium. In the subgroup of MINDACT patients, we also explored the association of the PRS_{313} with the 70-gene signature.

This thesis concludes with a general discussion and future prospects in **chapter 8** and a summary of results in **chapter 9**.

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