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Androgen receptor genotypes predict response to endocrine treatment in breast cancer patients

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Running title: *AR* genotypes in breast cancer

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Summary

Background: The androgen receptor (AR) is frequently expressed in breast cancers. The *AR* genotype may affect disease-free survival and response to endocrine therapy.

Methods: 634 women undergoing breast cancer surgery between 2002 and 2008 were followed until June 30, 2010. Six htSNPs in the *AR*, and the resulting *AR* diplotypes, were examined in relation to breast cancer patient characteristics, tumour characteristics, disease-free survival, and response to endocrine treatment.

Results: Five common *AR* diplotypes were found. Seventeen rare variants were combined into a composite group. The resulting six *AR* diplotype groups clustered into two subgroups, groups A (n=128) and B (n=499), with three diplotypes in each. Patients in group B had larger total breast volume (P=0.024), higher BMI (P=0.050), more axillary lymph node involvement (P_{trend}=0.020), and higher histological grade (P_{trend}=0.031). There were 59 breast cancer events in the 569 patients with invasive cancers and no preoperative treatment. Patients in group B also had shorter disease-free survival (P=0.037) than patients in group A. Among patients in group B with oestrogen receptor-alpha positive tumours, tamoxifen treatment was associated with longer disease-free survival (P=0.008), while treatment with aromatase inhibitors was not (P=0.94). Response to endocrine treatment could not be predicted based on BMI, suggesting that the effect of *AR* diplotypes went beyond that of a higher BMI.

Conclusions: A marker for a group of patients who responded to tamoxifen, but not to aromatase inhibitors, was identified. If this finding is confirmed, *AR* genotyping may provide useful information for selection of endocrine treatment of breast cancer patients.

Keywords: Androgen receptor, Breast cancer, Oestrogen receptor α, Genotype, Single nucleotide polymorphism

Introduction

Breast cancer is the most common malignancy among women in Sweden. Over 7000 women are diagnosed with breast cancer every year (The National Board of Health and Welfare, (www.socialstyrelsen.se)). Polymorphisms in genes regulating hormone and growth factor levels have been associated with disease progression and therapeutic outcome in several cancers arising from tissues under hormonal influence (Giwercman *et al*, 2004; Piersma *et al*, 2007; Sissung *et al*, 2011).

Both oestrogens and androgens are important for normal breast development (Dimitrakakis & Bondy, 2009). The balance between the stimulatory effects of oestrogens and the inhibitory effects of androgens functions as a critical factor that regulates mammary cell proliferation, in normal as well as in cancer tissues. Androgens exert their effect in the mammary epithelial cell via two separate pathways, either directly via the androgen receptor (AR), or indirectly through aromatisation to oestrogen. Results from preclinical studies suggest that testosterone may function as a natural, endogenous protector of the breast and limit mitogenic and cancer promoting effects of oestrogens on mammary epithelium, (reviewed by Somboonporn & Davis, 2004). However, in postmenopausal women, who have low levels of circulating oestrogens and increased aromatase activity, higher androgen levels have been associated with a small increase in breast cancer risk.

The AR functions as a transcription factor, which regulates the activity of other genes (Gao *et al*, 2005). It is expressed in approximately 70 – 90% of primary breast tumours, closely reflecting the frequency of oestrogen receptor α (ER) expression (Birrell *et al*, 2007; Hanley *et al*, 2008; Hu *et al*, 2011; Park *et al*, 2010), and in 75% of breast cancer metastases (Birrell *et al*, 2007). Low AR expression in ER positive breast cancer has been associated with significantly reduced relapse-free and overall survival (Peters *et al*, 2009).

Endocrine treatment options for patients with ER positive breast cancers currently include tamoxifen (TAM) and various aromatase inhibitors (AIs) (Bardia & Stearns, 2010; Burstein *et al*, 2010; Colleoni & Giobbie-Hurder, 2010; Hackshaw *et al*, 2011). In randomised trials of unselected patients, AIs were shown to have a better effect than TAM (Baum *et al*, 2003; Burstein *et al*, 2010). Since adjuvant therapy does not work as intended for a considerable number of patients (Colleoni & Giobbie-Hurder, 2010; Dowsett *et al*, 2010; Early Breast Cancer trialists´Collaborative Group (EBCTGC), 2005), there is a need to identify markers for better selection of endocrine treatment. It has been suggested that response to TAM treatment depends on the *CYP2D6* genotype, although the results are widely heterogeneous (Dunn *et al*, 2010; Higgins & Stearns, 2011; Lash *et al*, 2009; Punglia *et al*, 2008). *CYP2D6* genotyping prior to TAM treatment is currently not recommended (Burstein *et al*, 2010; Lash *et al*, 2009). Response to AI treatment may depend on the *CYP19A1* genotype (Colomer *et al*, 2008; Fasching *et al*, 2008), but is currently not carried out prior to selection of endocrine treatment.

The efficacy of TAM has been shown to be equal in obese and non-obese patients (Dignam *et al*, 2003), while a high body mass index (BMI) has been associated with worse response to AI treatment (Pfeiler *et al*, 2011). In women, the density of the AR appears to be higher in visceral than in subcutaneous tissue and the effects of androgens are associated with body fat composition (Bjorntorp, 1997). AR overexpression may enhance TAM's agonistic properties in breast cancer and contribute to resistance (De Amicis *et al*, 2010). Conversely, increased androgens and AR expression following AI treatment may contribute to reduced tumour cell proliferation (Chanplakorn *et al*, 2011). AR signalling may also be dependent on the AR genotype (Rodriguez-Gonzalez *et al*, 2009). Body constitution may therefore impact on the relationship between androgens, AR and endocrine treatment response.

The present study focussed on a set of six haplotype-tagging single nucleotide polymorphisms (htSNP) in the *AR* (Fig.1). These SNPs were previously identified to capture 95% of the haplotypes found in Swedish men. The haplotypes were associated with prostate cancer risk (Lindstrom *et al*, 2006). Since the *AR* gene is located on chromosome X and women carry two copies, we investigated diplotypes rather than haplotypes. To our knowledge, there is only one study published on androgen levels in women in relation to these *AR* diplotypes (Hietala *et al*, 2011). The study reported a weak correlation between diplotype and plasma androgen levels in pre-menopausal women. The correlation was modulated by exogenous hormone use.

The aims of the present study were to compare the frequency of the *AR* diplotypes in a cohort of women diagnosed with breast cancer with the frequencies found by Hietala *et al.* (Hietala *et al*, 2011), and to investigate whether any of the *AR* diplotypes were associated with patient characteristics, specifically BMI and waist-hip-ratio (WHR), and tumour characteristics. In addition, the study aimed to elucidate whether *AR* diplotypes were associated with breast cancer-free survival independent of treatment, or predicted response to endocrine therapy in patients with ER positive tumours.

Subjects and Methods

Breast cancer-patients

Women assessed pre-operatively at Lund University Hospital in Southern Sweden for a first breast cancer were invited to take part in an ongoing study regarding genetic and non-genetic factors that could be associated with breast cancer prognosis and treatment response. Patients were included between October 2002 and October 2008. Women were invited to participate regardless of ethnic background, age and stage. The vast majority of women included were ethnic Swedes. Patients with a previous breast cancer or who had been diagnosed and treated

for another type of cancer within the past 10 years were not eligible to participate. The Ethics Committee of Lund University approved the study. Written informed consent was obtained from all patients. A total of 634 women were included in the study.

During the preoperative visit, a trained research nurse collected blood samples and measured body weight, height, waist and hip circumferences, and breast volume. 'Breast volume' was defined as the sum of the volumes of the right and left breasts. The volume of each breast was measured using plastic cups employed by plastic surgeons doing breast reductions and reconstructions. These cups come in 11 sizes ranging from 200 to 2000 mL as previously described (Ringberg *et al*, 2006).

All patients filled out a preoperative questionnaire including questions on reproductive history, use of exogenous hormones, and concomitant medications during the past week. Follow-up questionnaires were completed at three to six months, and one, two, three, five and seven years postoperatively. Information including type of surgery, adjuvant treatment, sentinel node biopsy and axillary node dissection was obtained from each patient's chart. Tumour size, histological type and grade, axillary node involvement, signs of distant metastases, ER and progesterone receptor (PR) status were obtained from each patient's pathology report. ER and PR status were determined by immunohistochemistry using the Dako LSAB kit system (Dako) and the antibodies M7047 (ER) and M3569 (PR). Tumours with >10% positive nuclear staining were considered ER positive or PR positive (Bågeman *et al*, 2008). All tumours were analysed at the Department of Pathology of Skåne University Hospital in Lund. Date of death was obtained from the Swedish Population Registry.

Breast cancer surgery is performed at seven different hospitals in the South Swedish Health Care Region, with Skåne University Hospital in Lund serving almost 300'000 inhabitants. Since breast cancer patients are not referred to other hospitals for surgery, this study is population-based. According to data obtained from the Regional Tumour Registry,

1139 women with breast cancer were registered in Lund between October 2002 and October 2008, and 1090 received surgical treatment. Six-hundred and thirty-four patients (58%) were included in the present study. The majority of the non-participating patients did not decline participation, but were missed due to lack of available research nurses. Approximately five percent of patients were missed due to unverified diagnosis at the time of surgery. The median age at surgery for all operated patients was 60.1 years. ER status was positive in 84.6% of patients and PR status was positive in 68.1%. The follow-up rates for the breast cancer patients (without pre-operative interstitial laser thermotherapy or neo-adjuvant treatment) who were alive and recurrence free at each visit were as follows for the 1-year, 2-year, 3-year, 5-year and 7-year follow-up visits: 98.5%, 95.1%, 92.4%, 94.6%, and 90.8%, respectively.

SNP genotyping

Genomic DNA was extracted from 300 ml of peripheral blood using the Wizard genomic DNA purification kit (Promega, Madison, WI, USA). Genotyping of the AR htSNPs, was performed at Region Skåne Competence Centre, Malmö University Hospital, Malmö, Sweden. The genotyping of *AR* rs 1337080 was done according to the manufacturer's protocol with TaqMan ® assay by allelic discrimination based on real-time PCR on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Analyses of *AR* rs17302090, rs6152, rs7061037, rs5031002, and rs5964607, as well as CYP19A1 rs4646, were performed on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry on a Sequenom MassARRAY® platform (Sequenom, San Diego, CA, USA), using iPLEX reagents according to the manufacturers' protocol. For quality control, over 10% of the samples were run in duplicate. The concordance was 100% for the validated samples. The call rates varied between 96.2% and 100%.

AR diplotype construction

Each SNP was cross-tabulated against the other five SNPs. This procedure showed that certain combinations did not exist or were very rare. We therefore constructed the haplotypes and diplotypes based on the most likely combinations. Diplotype variants present in less than one per cent of the patients were classified as rare variants and combined into a single group termed “rare diplotypes”. Rs17302090 analysis failed for one patient. Based on the other SNPs, this SNP could be imputed. In 24 patients, results for rs6152 were missing, of which 23 could be imputed based on the results of rs7061037, since the R2 between these two SNPs was 0.963. One of these resulted in two copies of the common minor haplotypes, thus resulting in a rare diplotype, while the other 22 were all common variants. For the remaining subject, the diplotype could not be determined, although it could be assigned to the rare diplotype-group, since all possible variants at this position resulted in a rare diplotype. For rs1337080, analysis failed for 21 patients. For 20 patients, the diplotype could be imputed. The remaining subject could be assigned to the rare diplotype-group. Two of the patients with missing results for rs1337080 also had missing results for rs6152 but could be imputed for both positions, thus resulting in two common diplotypes. SNP analysis failed for rs5031002 in two patients, but the diplotypes could be be imputed. Ten patients had missing results for rs5964607, of which two were imputed and one was assigned to the rare diplotype-group. For the remaining seven patients, the SNP data could not be imputed. Thus, a total of four subjects were included in the rare-diplotype group, although complete diplotypes were missing for three of them.

Data analysis

Statistical analyses were performed using the statistical software SPSS Statistics 19 (IBM, Chicago, Illinois, USA). BMI, WHR, and total breast volume were not normally distributed

and were transformed using the natural logarithm (ln). Age, BMI and WHR were also dichotomized according to the following: age <50 *vs*. ≥50 years, BMI <25 *vs*. ≥25, and WHR ≤0.85 *vs*. >0.85. Chi-square was calculated for dichotomized variables. We also examined whether any of the *AR* diplotypes clustered together with respect to BMI, WHR, and disease-free survival. Mann-Whitney U-test was used for comparison of non-parametric variables.

Breast cancer-free survival was calculated from inclusion to diagnosis of a breast cancer event, the last study follow-up, or death due to a non-breast cancer related cause, whichever came first, prior to July 1, 2010. A breast cancer event was defined as local or regional recurrence, new breast cancer, or distant metastasis. Patients who had received preoperative treatment (n=41, plus one patient with missing information regarding interstitial laser thermotherapy), patients with *in situ* carcinoma (n=14), and patients diagnosed with a breast cancer event within three months from inclusion (n=3) were excluded from the survival analyses. One patient with early metastatic spread had also received preoperative treatment (Fig. 2). Kaplan-Meier was used to calculate disease-free survival. Cox regression was used to obtain adjusted hazard ratios (HR), adjusting for age (continuous), axillary node involvement (yes/no), tumour size (pT2+: yes/no), grade (grade III: yes/no), TAM treatment (yes/no), and AI treatment (yes/no). Adjuvant treatment reported in the chart after last follow-up or breast cancer event was not considered. Since this was a hypothesis driven exploratory study, no adjustments for multiple testing were performed (Bender & Lange, 2001). Nominal *P*-values are presented. All *P*-values were two-tailed and regarded as significant at $P<0.05$.

Results

AR diplotypes

AR htSNP analyses were performed on 634 patients. A total of 22 different complete genotypes were found in the cohort (n=627). The genotype was missing for seven women.

(Fig 1). An additional three patients had incomplete genotypes, but could be identified as carriers of rare genotypes. Five common diplotype variants were found. Almost 63% of the women were homozygous carriers of the wild-type variant GGAAGC. The other four common diplotypes were all composed of one wild-type allele and one minor allele. These were present in 4.4 – 9.6% of the women. Seventeen different rare diplotypes were found among 36 patients and were combined into a composite group termed rare diplotypes. An additional three patients had rare diplotypes, although the exact genotype was unknown.

Patient characteristics

The study included 634 female breast cancer patients, ranging in age from 25 to 99 years, with a median age of 59.6 years. Preoperative patient characteristics are presented in Table 1, and did not differ significantly between the *AR* htSNPs or between the six *AR* diplotype groups when the most common GGAAGC/GGAAGC variant was used as a reference. With respect to BMI, the six diplotypes appeared to cluster in two groups. As shown in table 1, patients with one of the three diplotypes GGAAGC/AAGAGT, GGAAGC/GGAAAC, or rare diplotypes had a lower median BMI of 23.83 kg/m^2 (group A) compared to patients with the remaining diplotypes GGAAGC/GGAAGC, GGAAGC/GAGGGT or GGAAGC/GGAAGT (group B) where the median BMI was 24.77 kg/m^2 (P=0.050). There were no significant differences in WHR. Group B also had larger total breast volume (P=0.024),

Tumour characteristics

The tumour characteristics of the 592 patients who did not receive neo-adjuvant therapy or preoperative interstitial laser thermotherapy (Fig. 2) are presented in Table 2. Individual *AR* htSNPs were not significantly associated with tumour characteristics. When comparing the different diplotypes, a trend towards less axillary node involvement (0 *vs*. 1-3 *vs*. 4+) was

seen for the GGAAGC/AAGAGT diplotype, compared to all other diplotypes, (P_{trend}=0.037). Group A had less axillary node involvement (P_{trend}=0.020) and lower histological grade (P_{trend}=0.031) compared to group B.

A total of 107 patients had received postoperative chemotherapy. No significant differences in any of the treatment distributions were seen between patients carrying the different *AR* diplotypes.

Breast cancer-free survival and *AR* diplotypes

After exclusion of breast cancer events detected on the postoperative metastasis screen, 59 breast cancer events were reported, of which 38 were distant metastases. Breast cancer-free survival in relation to *AR* diplotypes was thus analysed in the 569 patients with invasive cancers and without preoperative treatment (Fig. 2). The median total follow-up time was 3.03 years (interquartile range 2.01 – 4.97). Three diplotypes were associated with longer disease-free survival, while the other three, including the homozygous wild type variant, were associated with shorter disease-free survival (Fig 3A). Overall, there was no significant difference in disease-free survival between the six diplotype groups. However, the three diplotypes with longer disease-free survival were the same as those in group A. The three diplotypes with shorter disease-free survival were the same as those in group B. Group B was associated with a statistically significantly shorter disease-free survival compared to group A (Log rank *P*=0.037), (HR: 2.38, 95% CI: 1.02 – 5.55) (Fig. 3B). The results became slightly weaker after stratification according to BMI ≥25 (*P*=0.056). However, Group B had shorter disease-free survival in both strata of BMI. Stratification according to BMI ≥25 alone did not yield any significant differences with respect to disease-free survival. The results remained essentially the same when stratifying according to previous use of hormone therapy.

Among these 569 patients with invasive cancers and no preoperative treatment, group B also presented with a larger total breast volume (*P*=0.007), a higher BMI (*P*=0.011), more axillary lymph node involvement (*P*=0.045) and larger tumours (*P*=0.049) compared to group A. No significant differences in WHR or in endocrine treatment duration were found between groups A and B.

After adjusting for age, tumour characteristics, TAM and AI treatment, the difference in breast cancer-free survival between groups A and B was no longer statistically significant, although the HR remained approximately the same (adjusted HR: 2.22, 95% CI: 0.95 – 5.21; *P*=0.065). The results remained essentially the same when using the last disease-free follow-up date or date at diagnosis of a breast cancer event as endpoints in the follow-up.

AR diplotype groups and endocrine treatment response

Disease-free survival in relation to *AR* diplotype groups and endocrine treatment was then estimated in patients with ER positive tumours who had not received chemotherapy (n=436) (Fig. 2). In patients belonging to group A, neither treatment with TAM nor treatment with AI seemed to have any significant effect on breast cancer-free survival. There were only four breast cancer events in group A - one in a patient with no endocrine therapy, three in patients who had received TAM, and none in the patients who had received AI with or without TAM.

Patients treated with TAM in group B had significantly longer breast cancer-free survival compared to patients who had not received TAM (Log rank *P*=0.008), (HR: 0.40, 95% CI: 0.20 – 0.81) (Fig. 4A). In contrast, treatment with AI seemed to have no effect on breast cancer-free survival (Log rank *P*=0.94) (Fig. 4B). Since some patients had received both TAM and AI while others had received either monotherapy or no endocrine treatment, we first excluded patients who had not received any endocrine therapy. TAM-treatment was still significantly associated with longer disease-free survival (Log rank *P*=0.012), while AI

treatment was not. We then excluded patients who had received both TAM and AI. Once again TAM treatment was significantly associated with longer disease-free survival (Log rank *P*=0.036), while AI treatment was not. The results for both TAM and AI were essentially the same after stratifications or adjustments for axillary node involvement, tumour size and BMI. A multivariate model including age, BMI, tumour size, axillary node involvement, grade, as well as both AI and TAM showed that TAM was significantly associated with longer disease-free survival (adjusted HR: 0.29, 95% CI: 0.14 – 0.64; *P*=0.002), but AI was not (*P*=0.27).

Since AI response may be dependent on the *CYP19A1* SNP rs4646 (Colomer *et al*, 2008; Darabi *et al*), the rs4646 allele distribution was assessed. The rs4646 distribution did not significantly differ between groups A and B (results not shown). Further adjustment for rs4646 in the multivariate model did not alter the results.

Similar results were obtained when using the last disease-free follow-up date or date at diagnosis of a breast cancer event as endpoints in the total follow-up, or after inclusion of patients who had received neo-adjuvant therapy or adjuvant chemotherapy.

Discussion

The main finding of the present study was that adjuvant therapy with TAM, but not with AI, was significantly associated with longer breast cancer-free survival in patients carrying certain *AR* diplotype variants (group B) compared to patients not treated with TAM.

In this study, *AR* diplotypes were analysed in relation to patient and tumour characteristics, endocrine treatment, and disease-free survival. The study included 58% of all breast cancer patients who had received surgery during the same time period. The patients included in the study were comparable to all operated patients with respect to age, ER and PR status as reported by the Regional Tumour Registry. The frequencies of the five most common *AR* diplotypes were also similar to those found in young Swedish women from high-

risk breast cancer families (Hietala *et al*, 2011). No significant differences in patient or tumour characteristics were seen between the different *AR* htSNPs or the *AR* diplotypes, except for the GGAAGC/AAGAGT diplotype, which was associated with less axillary node involvement.

The three *AR* diplotypes in group B were associated with shorter disease-free survival compared to the *AR* diplotypes in group A. Patients in group B also presented with higher BMI, larger total breast volume, larger tumours and a higher frequency of axillary lymph node involvement. However, none of these variables alone could explain the shorter disease-free survival in group B. Moreover, WHR did not differ between groups A and B. Thus, android type obesity or overweight could not explain the shorter disease-free survival in group B (Qiao & Nyamdorj, 2010).

In patients belonging to group B, adjuvant therapy with TAM was significantly associated with longer breast cancer-free survival, compared to patients not treated with TAM. In contrast, adjuvant AI treatment had no effect on breast cancer-free survival in this group of patients, a finding suggestive of AI resistance. Therefore, we analysed the allele distribution of the *CYP19A1* rs4646, which has been suggested to be involved in AI resistance (Colomer *et al*, 2008; Darabi *et al*, 2011). The difference in AI and TAM treatment response in group B was independent of the *CYP19A1* rs4646 variant.

Among patients in group A, neither TAM nor AI treatment had any significant effect on the breast cancer-free survival. However, none of the four breast cancer events in this group were found among the 29 patients who had received AI treatment. This might indicate a better effect on breast cancer-free survival for AI in this *AR* diplotype group, but needs to be confirmed in a larger, independent cohort with longer follow-up.

The mechanisms behind the results remain to be elucidated. The *AR* genotype is associated with AR signalling (Rodriguez-Gonzalez *et al*, 2009). However, the *AR* diplotypes

did not tag for CAG or GGC repeat length polymorphisms in the previous study (Hietala *et al*, 2011). Others have shown that the AR is a direct repressor of ERα signalling in breast cancer cells (Panet-Raymond *et al*, 2000; Peters *et al*, 2009) due to an association between AR and the oestrogen response elements (Peters *et al*, 2009). It is possible that the *AR* variants constituting group B lead to a reduced ability of the AR to repress the ERα-signalling. The ER is blocked by TAM, thereby inhibiting oestrogen dependent cell proliferation. Treatment with AI, on the other hand, seemed to have no effect on the disease-free survival in group B. Aromatase activity is found in adipose tissues, including the breast, and breast tumour tissue expresses aromatase activity (Miller, 2006). In postmenopausal women, aromatase levels are known to increase with increasing BMI, and it has been suggested that the levels reached may exceed the amount that can be successfully inhibited by AIs (Goodwin & Pritchard, 2010). In the present study, patients in group B had higher BMI and larger total breast volume compared to group A, and therefore potentially higher aromatase levels, whereby AI treatment may be less effective. However, endocrine treatment response could not be predicted based on BMI, thus suggesting that the effect of *AR* diplotypes went beyond that of a high BMI.

AIB1 (amplified in breast cancer-1) is a known cofactor for both AR and ERα (Gojis *et al*, 2010; Zhou *et al*, 2010). AIB1 expression is frequently increased in breast cancer tissue and has been associated with markers of more aggressive disease. Several *AR* mutations found in prostate cancer tissue have been found to increase the binding affinity to AIB1 (Zhou *et al*, 2010). Moreover, high levels of AIB1 were associated with better response to the AI examestane (Yamashita *et al*, 2009). Thus, ARs in group B may have altered binding affinity for AIB1, possibly affecting the response to AI treatment.

AR expression analysis was not included in the routine analyses, and we were therefore unable to compare *AR* diplotype data with AR expression levels in the tumour.

Likewise, information regarding HER-2/neu status and Ki-67 expression was lacking for the majority of patients included in the study. HER-2/neu status has been routinely analysed as of November 2005 and Ki-67 expression as of March 2009. A recent study showed that AR expression was associated with molecular subtypes of breast cancer (Collins *et al*, 2011) and was most frequent in luminal A and B. As the breast tumours were not routinely classified according to molecular subtypes in Lund, we were unable to investigate whether the *AR* diplotypes were associated with any of the molecular subtypes.

Since this was an exploratory study, we presented nominal *P*-values without adjustment for multiple testing (Bender & Lange, 2001). In the present exploratory setting, we feel that Bonferroni correction is too stringent and decreases power. Since there is a risk for false positive findings, the results require confirmation in an independent patient population.

In conclusion, we found a marker for a group of patients who responded to TAM but not to AI treatment. These results warrant confirmation in an independent cohort, preferably with patients who have been randomised to different treatment arms. If confirmed, *AR* diplotype profiling of patients may be useful for selection of endocrine therapy.

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Legends to Figures and Tables

Figure 1. Frequencies of *AR* SNPs and diplotypes among 627 women diagnosed with breast cancer. Genotypes and frequencies are presented for each SNP. Diplotypes present in less than 1% of the patients were clustered together into a composite group of rare diplotypes. Seven patients were missing due to failed SNP analysis.

Figure 2. Flow-chart displaying patient selection for the different analyses.

Figure 3. A Kaplan-Meier survival analysis of disease-free survival with respect to *AR* diplotypes. **B** Disease-free survival in group A and group B. Group A includes patients with *AR* diplotypes GGAAGC/AAGAGT, GGAAGC/GGAAAC or rare diplotypes. Group B includes patients with *AR* diplotypes GGAAGC/GGAAGC, GGAAGC/GAGGGT or GGAAGC/GGAAGT.

Figure 4. Disease-free survival in group B patients who had ER positive tumours and who had not received chemotherapy. **A**: Patients treated with TAM; **B**: patients treated with AI.

Table 1. Background characteristics for the whole cohort and the six different *AR* diplotype groups. Figures are presented as medians with interquartile ranges or as frequencies.

Table 2. Tumour characteristics for patients who did not receive neoadjuvant therapy (n=30) or pre-operative interstitial laser thermotherapy (n=11, and missing information for one patient) prior to the surgery. Data presented for all patients (n=592) and for the six different *AR* diplotype groups (n=585, diplotype information missing for 7 patients).

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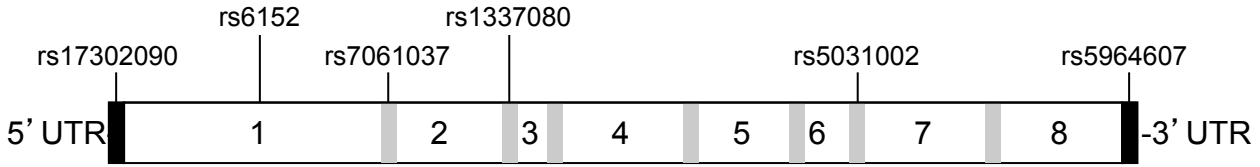
Figure 1



SNP	*Genotype*	*N*	*%*
rs17302090	GG	555	87.5
	GA	78	12.3
	AA	1	0.2
rs6152	GG	480	75.7
	GA	141	22.2
	AA	12	1.9
rs7061037	AA	480	75.7
	AG	143	22.6
	GG	11	1.7
rs1337080	AA	552	87.1
	AG	76	12.0
	GG	5	0.8
rs5031002	GG	597	94.2
	GA	37	5.8
	AA	0	0.0
rs5964607	CC	430	67.8
	CT	178	28.1
	TT	18	2.8

	Combined genotypes	*N (%)*	*Diplotypes*
	GG/GG/AA/AA/GG/CC	398 (62.8)	GGAAGC/GGAAGC
	GA/GA/AG/AA/GG/CT	61 (9.6)	GGAAGC/AA GAGT
	GG/GA/AG/AG/GG/CT	60 (9.5)	GGAAGC/GAGGGT
	GG/GG/AA/AA/GG/CT	41 (6.5)	GGAAGC/GGAAGT
	GG/GG/AA/AA/GA/CC	28 (4.4)	GGAAGC/GGAAAC
Rare	GA/AA/GG/AG/GG/TT	5 (0.8)	
	GG/GA/AG/AG/GG/ TT	5 (0.8)	
	GA/GA/AG/AA/GG/TT	4 (0.6)	
	GA/GA/AG/AA/GA/CT	3 (0.5)	
	GG/GA/AG/AG/GA/CT	3 (0.5)	
	GG/GG/AA/AA/GA/CT	3 (0.5)	
	GG/AA/GG/GG/GG/TT	2 (0.3)	GAGGGT/GAGGGT
	GG/AA/GG/GG/GG/CT	2 (0.3)	GAGGGC/GAGGGT
	AA/AA/GG/AA/GG/TT	1 (0.2)	AAGAGT/AAGAGT
	GG/GA/AG/AG/GG/CC	1 (0.2)	
	GA/GA/AG/AA/GG/CC	1 (0.2)	
	GG/GG/AA/AA/GG/TT	1 (0.2)	GGAAGT/GGAAGT
	GA/GA/AA/AA/GG/CT	1 (0.2)	
	GA/AA/AG/AA/GG/CT	1 (0.2)	
	GG/GA/AA/AA/GG/CT	1 (0.2)	
	GG/GG/AG/AG/GG/CT	1 (0.2)	
	GA/GG/AG/AA/GG/CT	1 (0.2)	
	GG/GG/AA/GG/GG/XX	1 (0.2)	
	GA/GA/AG/XX/GG/CC	1 (0.2)	
	GG/XX/AG/AG/GG/CC	1 (0.2)	

Figure 2

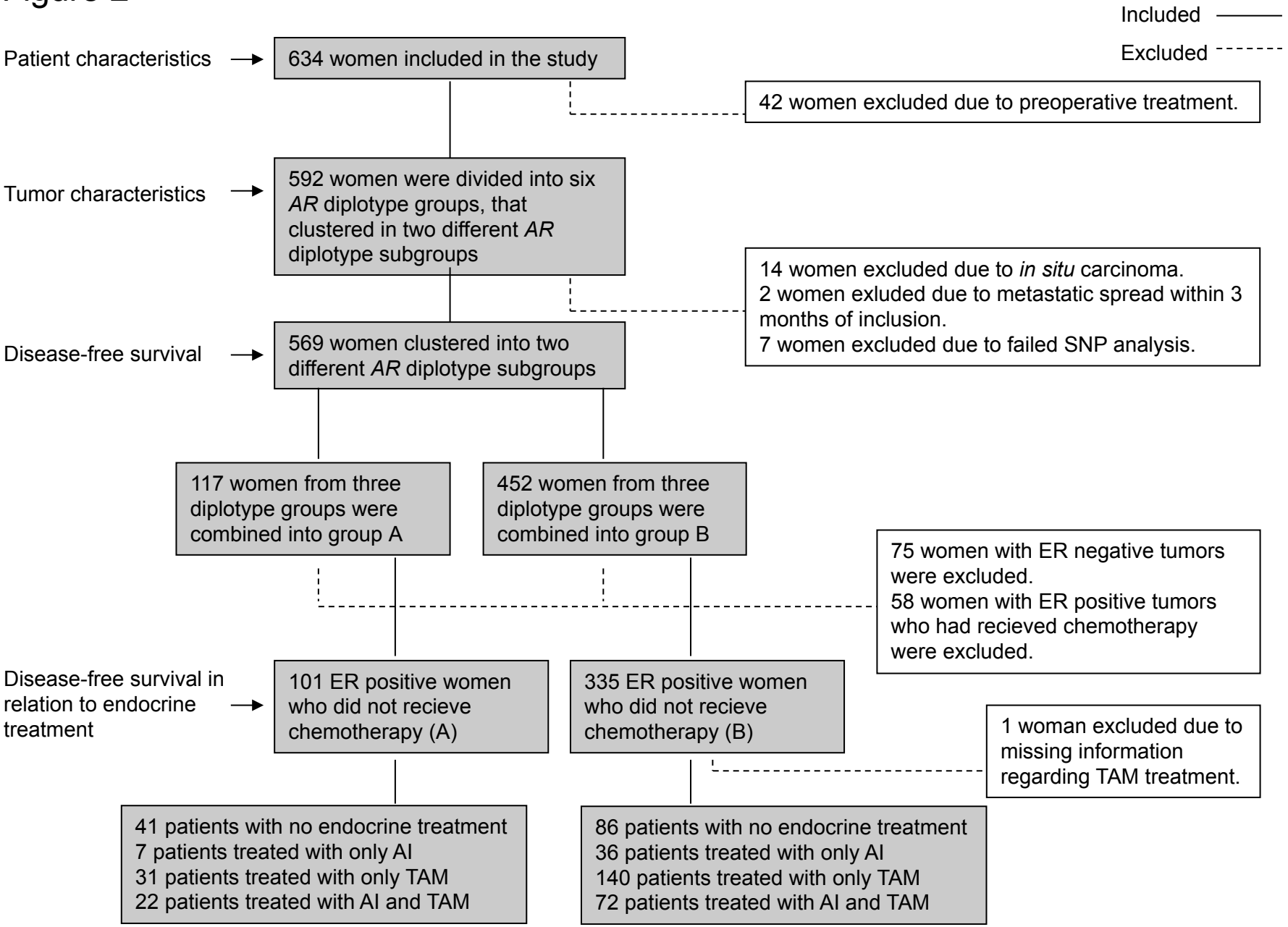
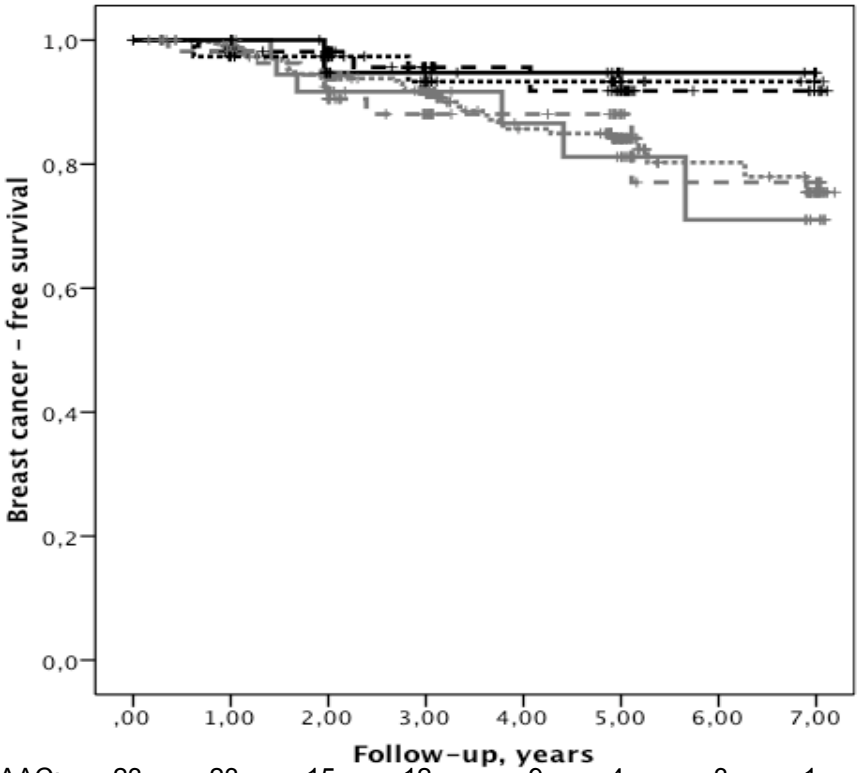
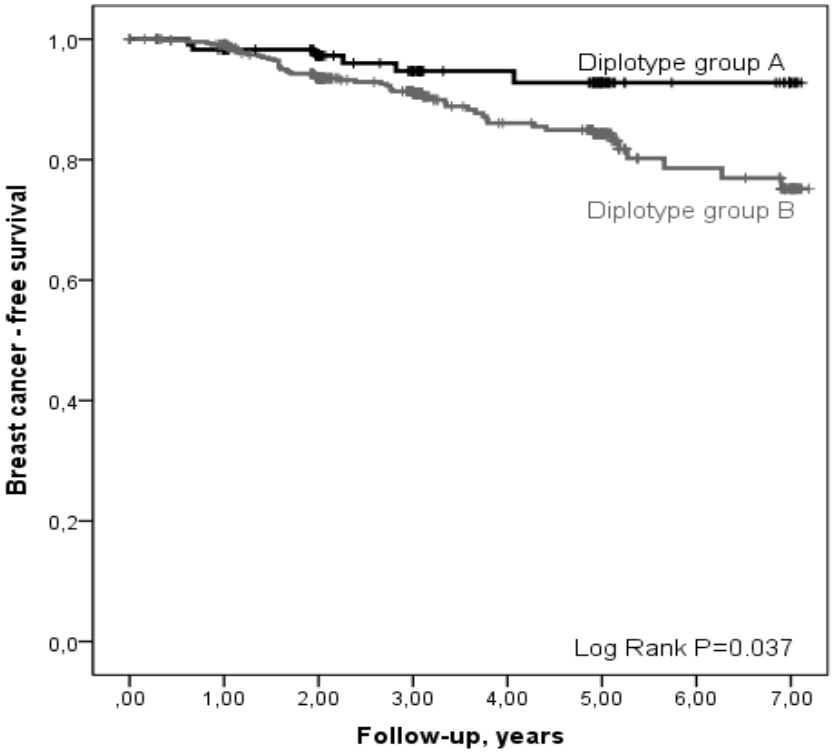

Included
Excluded
Patient characteristics
634 women included in the study
42 women excluded due to preoperative treatment.
Tumor characteristics
592 women were divided into six *AR* diplotype groups, that clustered in two different *AR* diplotype subgroups
14 women excluded due to *in situ* carcinoma.
2 women exluded due to metastatic spread within 3 months of inclusion.
7 women excluded due to failed SNP analysis.
Disease-free survival
569 women clustered into two different *AR* diplotype subgroups
117 women from three diplotype groups were combined into group A
452 women from three diplotype groups were combined into group B
75 women with ER negative tumors were excluded.
58 women with ER positive tumors who had recieved chemotherapy were excluded.
Disease-free survival in relation to endocrine treatment
101 ER positive women who did not recieve chemotherapy (A)
335 ER positive women who did not recieve chemotherapy (B)
1 woman excluded due to missing information regarding TAM treatment.
41 patients with no endocrine treatment
7 patients treated with only AI
31 patients treated with only TAM
22 patients treated with AI and TAM
86 patients with no endocrine treatment
36 patients treated with only AI
140 patients treated with only TAM
72 patients treated with AI and TAM

Figure 3 A



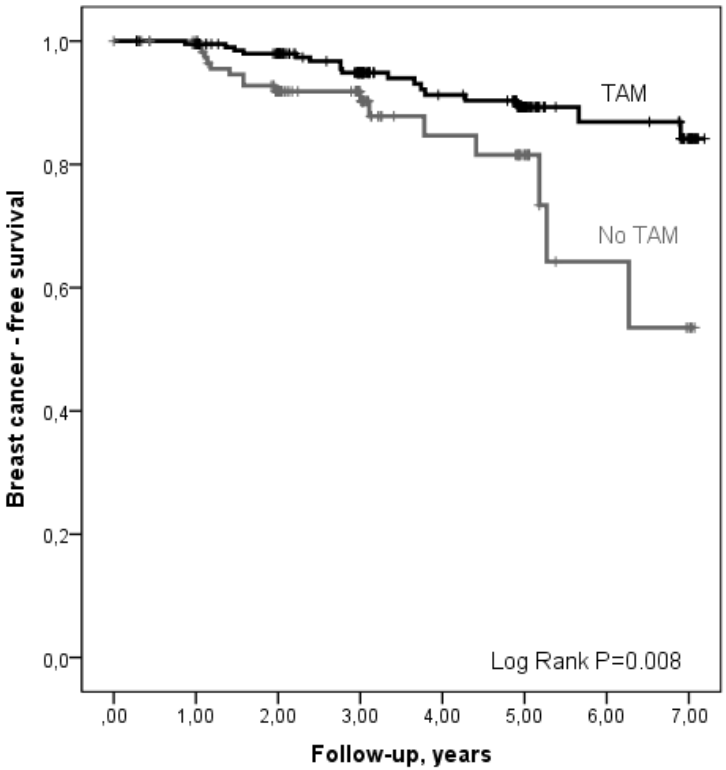
GGAAGC_GGAAAC:	23	23	15	12	9	4	3	1
Rare:	38	36	31	20	15	8	4	1
GGAAGC_AAGAGT:	56	51	46	33	25	19	7	4
GGAAGC_GAGGGT:	55	54	45	31	20	11	6	6
GGAAGC_GGAAGC:	357	342	283	196	117	76	35	15
GGAAGC_GGAAGT:	40	38	32	26	16	14	7	4

3 B



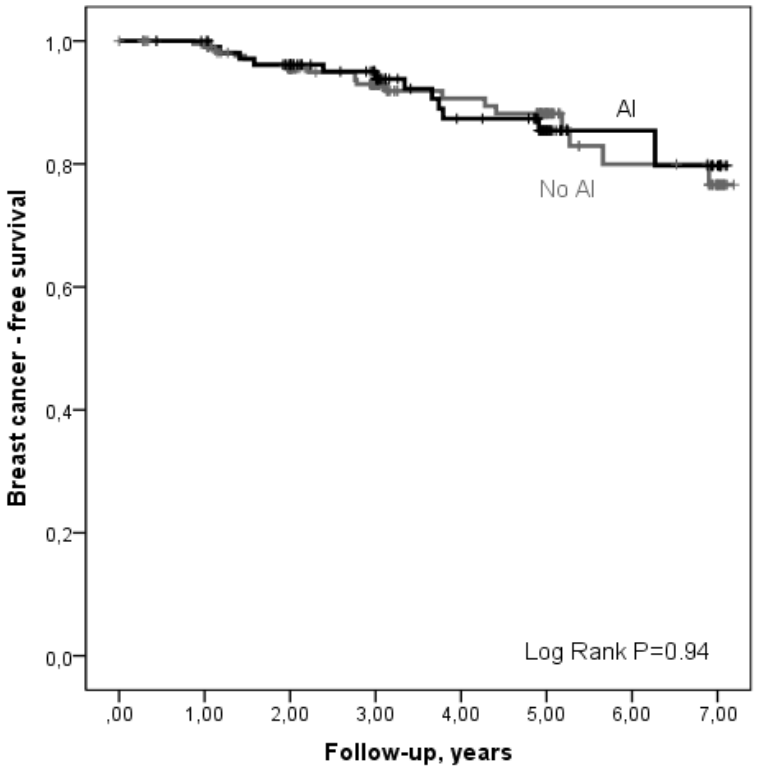
A:	117	110	92	65	49	31	14	6
B:	452	434	360	253	153	101	48	25

4A



TAM:	212	206	182	136	100	64	36	20
No TAM:	122	116	91	58	27	18	6	3

4B



AI:	108	106	97	78	53	31	15	9
No AI:	227	217	177	116	74	51	27	14

Table 1.	*All n=634*		*GGAAGC/ GGAAGC (B) n=398*	*GGAAGC/ AAGAGT (A) n=61*	*GGAAGC/ GAGGGT (B) n=60*	*GGAAGC/ GGAAGT (B) n=41*	*GGAAGC/ GGAAAC (A) n=28*	*Rare diplotypes (A) n=39*
	Median (IQR) or %	*n*	*Median (IQR) or %*	*Median (IQR) or %*	*Median (IQR) or %*	*Median (IQR) or %*	*Median (IQR) or %*	*Median (IQR) or %*
Age at diagnosis, yrs	59.6 (51.1 - 66.1)	634	59.7 (50.4 - 66.5)	58.7 (52.8 - 67.5)	60.6 (51.8 - 66.3)	59.6 (53.3 - 65.3)	59.4 (52.1 - 64.7)	58.1 (51.3 - 63.0)
Weight, kgs	68.0 (61.0 - 76.2)	632	68.0 (60.1 - 78.5)	65.0 (59.0 - 79.4)	69.2 (65.0 - 75.8)	68.6 (63.0 - 76.7)	69.5 (59.3 - 75.0)	66.0 (58.0 - 73.0)
Height, m	1.66 (1.62 - 1.70)	633	1.66 (1.61 - 1.70)	1.65 (1.62 - 1.69)	1.67 (1.61 - 1.70)	1.67 (1.62 - 1.70)	1.65 (1.60 - 1.72)	1.65 (1.63 - 1.68)
BMI, kgs/m^2	24.6 (22.3 - 27.8)	631	24.8 (22.4 - 28.0)	23.9 (21.1 - 28.3)	24.8 (22.8 - 28.9)	24.8 (22.6 - 27.7)	23.9 (21.5 - 27.5)	23.2 (21.7 - 26.7)
Waist-Hip ratio	0.84 (0.78 - 0.89)	627	0.84 (0.79 - 0.89)	0.84 (0.78 - 0.89)	0.84 (0.79 - 0.92)	0.83 (0.76 - 0.87)	0.84 (0.78 - 0.87)	0.83 (0.79 - 0.86)
Total breast volume[a], mL	1000 (625 - 1450)	546	1000 (625 - 1600)	900 (700 - 1300)	1025 (700 - 1500)	825 (600 - 1325)	700 (500 - 1250)	650 (500 - 1300)
Age at menarche, yrs	13.0 (12.0 - 14.0)	629	13.0 (12.0 - 14.0)	13.0 (13.0 - 14.0)	13.0 (12.0 - 14.0)	13.0 (12.0 - 14.0)	13.0 (13.0 - 14.0)	13.0 (12.0 - 14.0)
Parous, %	84.7	634	83.4	90.2	91.7	92.7	78.6	76.9
Age at first full term pregnancy, yrs	25.0 (22.0 - 28.0)	534	25.0 (22.0 - 28.0)	25.0 (21.0 - 29.3)	25.0 (22.0 - 27.0)	25.0 (22.0 - 28.0)	24.5 (22.8 - 29.3)	24.0 (22.0 - 27.0)
Ever use of hormone replacement therapy, %	45.3	633	44.0	36.0	47.0	56.0	43.0	56.0

[a] Eighty breast cancer patients were excluded due to previous breast surgery.

Table 2.	*All n=634 n (%)*	*GGAAGC/ GGAAGC (B) n=398, n (%)*	*GGAAGC/ AAGAGT (A) n=61, n (%)*	*GGAAGC/ GAGGGT (B) n=60, n (%)*	*GGAAGC/ GGAAGT (B) n=41, n (%)*	*GGAAGC/ GGAAAC (A) n=28, n (%)*	*Rare diplotypes (A) n=39, n (%)*
Neoadjuvant therapy	30 (4.7)	21 (5.3)	3 (4.9)	3 (5.0)	1 (2.4)	2 (7.1)	0
Pre-operative interstitial laser thermotherapy	11[a] (1.7)	7[a] (1.8)	0	2 (3.3)	0	2 (7.1)	0
No pre-operative treatment	***n=592***	***n=369***	***n=58***	***n=55***	***n=40***	***n=24***	***n=39***
pT							
In situ	14 (2.4)	10 (2.7)	2 (3.4)	0	0	1 (4.2)	1 (2.6)
1	424 (71.6)	261 (70.7)	45 (77.6)	34 (61.8)	30 (75.0)	17 (70.8)	31 (79.5)
2	144 (24.3)	91 (24.7)	11 (19.0)	20 (36.4)	9 (22.5)	6 (25.0)	6 (15.4)
3	9 (1.5)	6 (1.6)	0	1 (1.8)	1 (2.5)	0	1 (2.6)
4	1 (0.2)	1 (0.3)	0	0	0	0	0
Missing	0	0	0	0	0	0	0
Histological grade							
I	157 (26.6)	86 (23.4)	25 (43.1)	15 (27.3)	7 (17.5)	9 (37.5)	13 (33.3)
II	308 (52.1)	204 (55.4)	22 (37.9)	30 (54.5)	23 (57.5)	9 (37.5)	17 (43.6)
III	126 (21.3)	78 (21.2)	11 (19.0)	10 (18.2)	10 (25.0)	6 (25.0)	9 (23.1)
Missing	1	1	0	0	0	0	0
Hormone receptor status							
ER+	502 (86.7)	304 (84.4)	53 (94.6)	46 (85.2)	37 (92.5)	22 (95.7)	33 (84.6)
ER-	77 (13.3)	56 (15.6)	3 (5.4)	8 (14.8)	3 (7.5)	1 (4.3)	6 (15.4)
PR+	402 (69.4)	255 (70.8)	39 (69.6)	33 (61.1)	28 (70.0)	13 (56.5)	28 (71.8)
PR-	177 (30.6)	105 (29.2)	17 (30.4)	21 (38.9)	12 (30.0)	10 (43.5)	11 (28.2)
Missing	13	9	2	1	0	1	0
Axillary node involvement							
0	368 (62.4)	227 (61.9)	42 (72.4)	27 (49.1)	26 (65.0)	15 (62.5)	26 (66.7)
1-3	167 (28.3)	101 (27.5)	15 (25.9)	19 (34.5)	11 (27.5)	8 (33.3)	11 (28.2)
4+	55 (9.3)	39 (10.6)	1 (1.7)	9 (16.4)	3 (7.5)	1 (4.2)	2 (5.1)
Missing	2	2	0	0	0	0	0

[a] Information about pre-operative interstitial laser thermotherapy was missing for one patient.