# IGF1 htSNPs in relation to IGF-1 levels in young women from high-risk breast cancer families: implications for early-onset breast cancer

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# *IGF1* htSNPs in relation to IGF-1 levels in young women from high-risk breast cancer families: implications for early-onset breast cancer

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Running head: *IGF1* polymorphisms in high-risk women

Abbrevations:

| | |
|---|---|
| IGF1 | insulin-like growth factor-1 |
| htSNPS | haplotype tagging single nucleotide polymorphisms |
| BRCA | breast cancer associated gene |
| ECR | evolutionary conserved regions |
| LD | linkage disequilibrium |
| IGF1R | type 1 IGF receptor |
| IRS1 | insulin receptor substrate 1 |

| | |
|---|---|
| IGFBP2 | IGF binding protein 2 |
| OCs | oral contraceptives |
| BMI | body mass index |

**Abstract**

High levels of insulin-like growth factor-1 (IGF-1) have been associated with increased risk of developing several types of cancer including breast cancer. A set of nine haplotype tagging SNPs (htSNPs) in the *IGF1* gene were associated with IGF-1 levels and prostate cancer in a Swedish population. We aimed to study the nine htSNPs in three haplotype blocks (block1: rs855211, rs35765, rs2162679; block2: rs1019731, rs7956547, rs5742632; and block3 rs2033178, rs7136446, rs6220) combined into diplotypes, and three additional SNPs (rs5742612, rs35765817, rs35455143) in relation to IGF-1 levels, *BRCA* status, the *IGF1* CA-repeat microsatellite, and breast cancer in a population of 325 Swedish women from breast cancer high-risk families. Questionnaire data and blood samples for IGF-1 and genetic analyses were obtained twice during the menstrual cycle from 269 women aged 40 years or younger. SNP analyses were also performed in 56 *BRCA1/2* mutation carriers. Women (n=14) with any rare variant block1 diplotype had higher odds to be *BRCA1* mutation carriers OR 4.1 (95% CI 1.4-12.2), to lack the common *IGF1* 19 CA-repeat allele OR 33.3 (95% CI 6.6-166.7), and were more likely to develop early-onset breast cancer (Log Rank $P<0.001$) than women with common block1 diplotypes. In the subgroup of *BRCA1* mutation carriers, block1 rare diplotypes were associated with earlier diagnosis (Log Rank $P=0.031$). No association was found between IGF-1 levels and individual SNPs or diplotypes. If confirmed, these rare diplotypes may identify women with particularly high risk for early-onset breast cancer and this group should be included in forthcoming studies.

## Introduction

Insulin-like growth factor-1 (IGF-1) is important for normal breast development [1]. It stimulates cell proliferation and decreases apoptosis [2]. High levels of circulating IGF-1 have repeatedly been associated with several types of cancer, including prostate and premenopausal breast cancer [3-5]. The levels of circulating IGF-1 are highly influenced by genetic factors [6], and several polymorphic variants including single nucleotide polymorphisms (SNPs) and microsatellites have been associated with both IGF-1 levels and breast cancer risk [7-10]. SNPs (rs35765817, rs35455143, and rs3839984) located in evolutionary conserved regions (ECR) close to binding sites for a transcription factor that is important for IGF-1 expression were associated with circulating IGF-1 levels in a study of Caucasian women [10]. Another SNP (rs5742612) was found to be in linkage disequilibrium (LD) with variations in a microsatellite 969 bp upstream from the *IGF1* transcription start site among Singapore Chinese women [11].

Haplotype tagging SNPs (htSNPs) can be used as markers that cover the majority of genetic diversity of several individual SNPs. In the *IGF1* gene, a set of nine htSNPs (rs855211, rs35765, rs2162679, rs1019731, rs7956547, rs5742632, rs2033178, rs7136446, and rs6220) in three haplotype blocks with three htSNPs in each was studied in relation to prostate cancer risk in a Swedish population. One haplotype (TCC in block three) in the 3' region of the gene was associated with increased risk of developing prostate cancer [12].

Genetic variations in genes other than *IGF1* are likely to also influence IGF-1 levels. BRCA1 has been shown to interact with IGF signaling. In Brca1-deficient mice, the expression of IGF1R, IRS1, and IGFBP2 was increased and so were the serum levels of IGF-1 compared with those of control mice expressing Brca1 [13]. BRCA1 supresses IGF1R promoter activity

in MCF-7 breast cancer cell lines [14]. In one study, the IGF1R levels were higher in tumors from *BRCA1* mutation carriers compared with non-carriers [15]. IGF-1 is also important for intrauterine growth and *BRCA1* mutation carriers have been reported to be smaller than non-carriers from *BRCA1* mutation families [16], which further supports a link between *BRCA1* and IGF-1. Moreover, we have previously reported an association between absence of the common *IGF1* 19 CA-repeat polymorphism and *BRCA1* mutation status [17]. Absence of the 19 CA-repeat has been associated with increased premenopausal breast cancer risk [7, 8]. *BRCA1* and *BRCA2* mutation carriers have a substantially increased risk of developing breast cancer. However, the lifetime risk estimates range from 20 to 80% [18], a range that suggests that risk modifying genetic and non-genetic factors play an important role in carcinogenesis. We hypothesized that other genetic variations in the *IGF1* gene may, by altering IGF-1 levels, contribute to the variation in breast cancer susceptibility between and within families with a high risk of developing breast cancer.

The first aim of our study was to determine the frequencies of the SNP in LD with the *IGF1* microsatellite (rs5742612), two of the SNPs in the ECR (rs35765817 and rs35455143), and the nine individual htSNPs in the *IGF1* gene identified in the Swedish prostate cancer study within a population of women from Swedish breast cancer high-risk families. The second aim was to determine whether circulating IGF-1 levels were associated with the SNPs and diplotypes. The third aim was to determine diplotype frequencies in relation to *BRCA* status, *IGF1* CA-repeat genotypes and breast cancer risk.

## Materials and Methods

*Study Population*

The study population consisted of 325 ethnic Swedish women from 194 breast cancer high-risk families, from the Lund Oncogenetic Clinic. The original population included 269 young healthy women with varying *BRCA* mutation status from 161 families. These women were 40 years or younger at the time of enrollment between 1996 and 2006. They were all menstruating, had no history of cancer, and no prophylactic mastectomy or bilateral oophorectomy. Blood samples, taken twice during the menstrual cycle on day 5-10 and again on day 18-23 (5-10 days before the predicted onset of the next menstrual period), were used for genetic and hormonal analyses. Women were asked to call back with the date of the first day of their next menstrual period. Plasma and blood cells were separated and frozen at minus 70 degrees Celsius at the laboratory of the Department of Oncology, Lund. Body measurements were taken at both visits when blood samples were collected. Each woman also filled out a questionnaire including questions on lifestyle and reproductive factors such as use of oral contraceptives. Written informed consent was obtained from all women. IGF-1 levels were analyzed in 258 women and 267 consented for genetic analyses. *BRCA1/2* mutation testing was not performed as a part of this study but data on mutation status was obtained from the Lund Oncogenetic Clinic.

In addition, 56 women (40 *BRCA1* and 16 *BRCA2* mutation carriers from the South Swedish Health Care Region born between 1950 and 1988), were included irrespective of cancer status and whether they had undergone prophylactic mastectomies or oophorectomies. No hormone analyses were performed on these blood samples but the women had consented to genetic analyses. Questionnaire data was available for some, but not all of these women.
The local ethics committee at Lund University approved the study.

*Genotyping of IGF1 htSNPs*

Genomic DNA Purification Kit (Promega, Madison, WI, USA) was used to extract DNA from 300 uL of peripheral blood. All SNPs were genotyped at Region Skåne Competence Centre (RSKC Malmö), Malmö University Hospital, Malmö, Sweden.The SEQUENOM massARRAY® designer software was used for multiplex SNP analysis design. The analyses 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. Three SNPs did not work well on iPLEX. The SNPs (rs855211, rs6220, and rs2033178) were genotyped using a Taqman SNP allelic discrimination assay in 384-well format on ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). For quality control 40 samples were run in duplicate. The concordance was 100% for all SNPs. The call rates were 90% for rs855211 and over 97% for the remaining SNPs.

*Diplotype construction*

HtSNPs can be used to construct haplotypes and since we have two copies of *IGF1*, we constructed paired haplotypes called diplotypes. By crosstabulation of each htSNP in one block against the other SNPs in the same block, all possible linkage combinations were identified. Sometimes several combinations were equally likely, see figure 1. For statistical analyses, diplotypes with less than 10 carriers were grouped into a composite category of "rare diplotypes". In block 1 the "rare variant" diplotype consisted of 14 women with six or seven different diplotypes (six genotypes, one with two possible combinations). Block 2 "rare diplotypes" included 22 women with six or seven different diplotypes and block 3 included 29 women with six different diplotypes.

*Hormone analysis*

IGF-1 was analyzed in EDTA-plasma taken during cycle day 5-10 and day 18-23 from 258 women of the original study population. The analyses were performed at the Endocrinological Laboratory of Karolinska Hospital, Stockholm, Sweden, using a radioimmunoassay (RIA) method as previously described by Bang *et al.* [19]. IGF-1 was separated from IGFBPs by acid ethanol extraction followed by cryo-precipitation. Recombinant human IGF-1 (Kabi-Pharmacia, Stockholm, Sweden) was used as standard. The international IGF-1 standard was delivered by the National Institute of Biological Standards and Control, London, Great Britain. The intra assay variation was 4% and the inter assay variation was 11%. The limit of detection was 6 μg/L. IGF-1 levels were missing during cycle day 5-10 for one woman. For several of the OC users, the samples obtained during cycle day 5-10 were collected during their pill-free week.

*Follow-up*

Women were considered at risk for breast cancer from age 20 and were followed until the development of a first breast cancer according to the Regional Cancer Registry, until the date of a self-reported prophylactic mastectomy or oophorectomy, or until May 31 2009, whichever came first. The women in the study who were considered to have a high-risk of developing breast cancer were offered clinical follow-up including annual mammograms, ultrasounds or magnetic resonance imaging and physical examination of the breasts. The report rate of the Swedish cancer registries is close to 100 percent.

*Data analyses*

All statistical analyses were performed using the statistical software PASW 17.0 (formerly known as SPSS). Women who were currently breast feeding (n=4) or using hormonal contraceptives other than combined OCs (n=19), or both (n=1), were excluded from analyses of IGF-1 levels. Two women did not report the onset of their next menstrual period. Non-standardized and standardized means and 95% CIs were calculated for OC users and non-users combined as well as stratified according to current OC use. Non-standardized means for IGF-1 levels according to diplotypes were obtained using one-sample t-tests. For standardized levels, multivariate linear regression analyses were performed for each single SNP variant with the ancestral variant as reference and the hetero- and homozygous variant alleles coded as dummy variables in the analyses. Each diplotype was entered in separate multivariate analyses. IGF-1 levels were standardized at age 29 years, weight 67 kgs, day 7 or 7 days until the next period (i.e. day 21 in most women). When analyses of OC users and non-users were combined, the IGF-1 levels were standardized to non-use. The diplotype distribution among women from different *BRCA*-family categories was studied using Chi-square tests. Breast cancer free survival rates (from 20 years of age) in relation to different genotypes were assessed using Kaplan-Meier log-rank test and Cox regression models. Due to the large number of individual SNPs tested, we considered a *P*-value of <0.001 as statistically significant when analyzing IGF-1 levels in relation to individual SNPs. Nominal *P*-values are presented, All P-values are two-sided.

## Results

Table 1 shows the characteristics of all 325 women and of the subgroup of 231 women included in the IGF-1 analyses. Genotype frequencies for each SNP, block compositions and frequencies of most likely diplotypes are shown in figure 1. Based on cross-tabulations, we found that the combination of rs2033178 CC, rs7136446 TC and rs6220 TC in block 3, most likely generates a mix of the two possible diplotypes CCC/CTT and CCT/CTC and we therefore present the genotypes as CC/CT/TC.

### *IGF-1 levels and IGF1 SNPs*

Standardized levels of IGF-1 during cycle days 5-10 and 18-23 were calculated for all 231 women as well as for the 143 non-users and the 88 current OC users separately (table 2). IGF-1 levels did not differ significantly according to individual htSNPs or the three additional SNPs among all women, or when stratifying according to current OC status. No trend was observed for increasing or decreasing IGF-1 levels for any of the SNP variants.

### *IGF-1 levels and IGF1 diplotypes*

We examined IGF-1 levels in relation to diplotypes in all women, current OC users and non-users during cycle days 5-10 and 18-23, figures 2a and 2b. Only the composite group of rare variant diplotypes in block 1 had lower levels during cycle day 5-10 compared with the women with other diplotypes in all women ($P$=0.006) and non-users ($P$=0.03), adjusted for age, weight, menstrual cycle day, and OC use. No association between rare variant diplotype and IGF-1 levels was seen in current OC users.

*Diplotype distribution according to BRCA family status*

The diplotype frequencies were examined according to *BRCA* family status for all women with available diplotypes belonging to either a family with a confirmed *BRCA1* or a *BRCA2* mutation, or to a family where no mutation has been identified (”*BRCAX*”). Women from untested families were excluded. No significant differences in diplotype distribution was observed. Including only the first assigned woman from each family did not change the result.

*Diplotype distribution according to BRCA mutation status*

*BRCA1* mutation carriers more often had a rare diplotype in block 1, OR 4.1 (95% CI 1.4 - 12.2; *P*=0.007) compared with other women. *BRCA1* mutation carriers had also more often a rare diplotype in block 2 compared with other women OR 2.8 (95% CI 1.1 - 6.8; *P*=0.021). Moreover, *BRCA1* mutation carriers had higher odds of having one of the variants of a htSNPs in block 1, rs2162679 GG, OR 11.2 (95% CI 1.2 - 109.8, *P*=0.009) than other women. Figures 3a, 3b, 3c.

*IGF1 CA-repeats in relation to diplotypes*

The *IGF1* CA-repeat allele was successfully genotyped for 260 women and diplotypes in block 1 were available for 232 of these. Women with a rare diplotype in block 1 were more likely than the other women to lack the common *IGF1* 19 CA-repeat allele OR 33.3 (95% CI 6.6-166.7; *P*<0.001). IGF-1 measurements were only available for two women with rare diplotypes in the group of current OC users.

The rs5742612 SNP was successfully genotyped for 259 women. In line with a previous study in Singapore Chinese women [11], we also found LD between the two polymorphisms. The 21 CA-repeat was found in 9% of the women with the rs5742612 TT genotype (n=235), 52%

of the women with the TC genotype (n=23) and the only woman with the CC genotype was homozygous for the 21 CA-repeat allele, (100%). This SNP was not associated with IGF-1 or breast cancer.

*Diplotypes in relation to breast cancer*

After inclusion of the 56 additional *BRCA* mutation carriers, there were 23 women diagnosed with breast cancer. Having a rare variant diplotype in block 1 was significantly associated with a higher risk of developing breast cancer (Log Rank $P<0.001$), figure 4a. The number of women in this group was very small (n=14) and the rare variant diplotype group consisted of several different diplotypes. Four women with rare diplotypes in block 1 had been diagnosed with breast cancer, and they had three different diplotypes. No other diplotype was associated with breast cancer risk.

Since women with rare diplotypes in block 1 were more likely to have been diagnosed with breast cancer and also more likely to be *BRCA1* mutation carriers, the hazard analysis was also restricted to *BRCA1* mutation carriers, figure 4b. Having a rare diplotype in block 1 was not only associated with a higher risk of developing breast cancer but was also associated with earlier diagnosis of a first breast cancer among *BRCA1* mutation carriers (Log Rank $P=0.031$).

## Discussion

Minor allele frequencies of the nine htSNPs were essentially in line with the findings of a previous study of a healthy Swedish male population [12]. Only the composite group of rare diplotypes in block 1 was associated with IGF-1 levels. Stratification on OC use did not indicate effect modification of OC use on IGF-1 levels for any of the diplotypes. The odds of having a rare diplotype in block 1 was higher in *BRCA1* mutation carriers and in women lacking the common *IGF1* 19 CA-repeat allele. The rare diplotype group of block 1 was associated with an increased hazard of a first breast cancer and with a younger age at diagnosis in *BRCA1* mutation carriers.

### *SNPs in relation to IGF-1 levels and cancer risk and risk factors*

The SNPs in the present study were chosen based on previous studies by Johansson *et al.* who studied *IGF1* htSNPs in relation to prostate cancer and IGF-1 levels in Swedish populations. They found that the TCC haplotype of block 3 was associated with an increased risk for prostate cancer [12]. They also reported that the TCC haplotype, and two of the three htSNPs tagging this haplotype, were associated with increased levels of IGF-1 in men [20]. In the later study they did not study block 1 or 2 in relation to IGF-1 levels.

In the present study, none of the three SNPs included in the TCC haplotype was associated with IGF-1 levels. The group of women with the diplotype potentially containing the TCC haplotype had non-significantly higher IGF-1 levels when all women were included in the analysis, as well as when the analysis was restricted to women not currently using OCs. Major differences in age and in gender as well as potential differences in lifestyle and diet/nutrition status may account for the differing results between this study and Johansson's *et al.*'s study. A large study assessing some of the htSNPs included in the present study (rs5742612, rs1019731, rs7956547, and rs2033178) as well as a number of other SNPs in the

*IGF1* gene found associations between diplotypes and circulating IGF-1 levels [21]. However, the study did not take into account age, BMI, OC use, parity, or cycle day of blood draw[22]. We measured IGF-1 levels in two blood samples from each woman and estimated values standardized for age, weight, and menstrual cycle day. We also stratified according to OC status. The stratifications resulted in smaller groups than would otherwise have been the case. A larger study population would be beneficial given that the current study already includes the majority of the healthy women from high-risk breast cancer families in the South Swedish health care region, a larger study population would have to include women from other areas of Sweden or other countries. IGF-1 levels measured in several blood samples during a menstrual cycle for each woman could have improved the estimates. Nutrition status influences circulating IGF-1 levels [23], but we lacked the information required to be able to adjust for this variable in the current study. However, fasting status at the time of blood draw did not affect the results (data not shown).

Rs1019731 has been associated with increased risk for ovarian cancer [24]. Another large study of IGF-1 polymorphisms, IGF-1 levels and breast cancer risk reported no association between this SNP and circulating IGF-1 levels or breast cancer risk [25]. We did not study ovarian cancer in the present study.

In one study of IGF-1 levels and breast density, the three htSNPs in block 3 (rs2033178, rs7136446 and rs6220) were shown to be associated with elevated IGF-1 levels, but the relationship with breast density was indecisive [26]. Another study of *IGF1* SNPs and breast density among postmenopausal women included a haplotype of rs6220 and rs2162679 that was associated with breast density but the association did not remain significant after stratification by use of hormone replacement therapy [27]. Tamimi *et al*. found *IGF1* SNPs

associated with mammographic density but only one of the SNPs in the current study was included (rs1019731) [28]. We did not study breast density in the present study.

Rs 2033178, rs1019731, rs2162679 and rs35765 (among others) were studied in relation to age at menarche in Caucasian women. These SNPs were not associated with age at menarche while others were[29].

*Diplotypes*

We studied the htSNPs combined as diplotypes since we believe that diplotypes better represent the genetic composition of each woman. The use of diplotypes results in smaller subgroups, since women without complete SNP genotyping are excluded and more combinations are possible than for individual SNPs. In the analyses of diplotypes in relation to IGF-1 levels, diplotypes with less than 10 carriers were grouped into the composite category of ”rare diplotypes”. Johansson *et al.* excluded such rare haplotypes from the analyses [12, 21], and we were therefore unable to compare our results with their data. We found that the rare block 1 diplotypes were associated with absence of the *IGF1* 19 CA-repeat allele, *BRCA1* mutation status and with an increased risk of developing early-onset breast cancer. These rare diplotypes could hypothetically be due to genotyping errors, but we do not consider such an explanation likely since the validation samples showed 100 percent concordance. Rather, these rare diplotypes may indicate genetic conditions in the *IGF1* gene that are evolutionary unfavorable, thus explaining why they are not very common. They may also tag for abnormalities in the *IGF1* gene that may contribute to the risk of developing breast cancer since four of the nine women included in the group of rare diplotypes of block 1 were diagnosed with breast cancer. Since our women were selected from families with high penetrance of breast cancer, it is likely that other low penetrance polymorphisms, associated

with breast cancer, segregate at a higher frequency in this population than in the general population [30]. However, as stated above, we were unable to compare our data to Johansson *et al.* [12, 21].

Abscence of the *IGF1* 19 CA-repeat allele has been associated with a four-fold increased risk of developing breast cancer among women with a family history of breast cancer and even higher risk if the woman had ever used OCs [8]. The majority (>90%) of the women included in the present study had used or were current users of OCs. Therefore, we believe that the higher proportion of breast cancer cases among the women with rare diplotypes in block 1 may partly be driven by the lack of the *IGF1* 19 CA-repeat allele. A previous study based on results from a subset of the current cohort's population showed that women lacking the *IGF1* 19 CA-repeat allele had higher IGF-1 levels during OC use[7]. Since the block 1 rare variant group was associated with absence of 19 CA-reapeat allele, and use of OCs among women with abscence of the 19 CA-repeat allele is associated with increased breast cancer risk, the higher frequency of breast cancer in the women with rare block 1 diplotypes was not surprising. However, since IGF-1 measurements were only available for two women with rare diplotypes in the group of current OC users, we could not investigate the relationship between IGF-1 levels and block 1 rare variant diplotypes in OC users. We do not know whether the increased risk for earlier onset breast cancer was driven by the absence of the common 19 CA-repeat allele or by the rare variant diplotype. However, these rare *IGF1* genotype variants were more common in *BRCA1* carriers and confirmed an increased risk for very early-onset breast cancer among *BRCA1* mutation carriers. Since almost all women had used OCs at some point, we hypothesized that rare *IGF1* genotypes in combination with OCs are especially detrimental in high-risk women.

One study of *IGF1* htSNPs among *BRCA1* and *BRCA2* mutation carriers did not see any association between diplotypes and breast cancer [31]. However, Neuhausen *et al's*. study differs from the present study in a number of ways. Their htSNPs were not selected specifically for a Caucasian population even though they studied a Caucasian cohort and D'Alosio et al has shown the importance of ethnic specfic tag SNPs in *IGF1* [21]. Aside from SNP rs6220, Neuhausen *et al.* investigated different htSNPs. Moreover, they constructed haplotypes instead of diplotypes, which makes less biological sense, since *IGF1* is expressed on two alleles. In our study, rare diplotypes in block 1 and 2 were more common in *BRCA1* mutation carriers than in other women. We hypothesize that the rare diplotype variants may confer some form of survival advantage during fetal life among *BRCA1* mutation carriers, maybe through crosstalk between the genes[15].

In conclusion, neither the individual SNPs, nor the htSNPs combined as diplotypes were associated with IGF-1 levels in this study. These findings do not exclude the possibility that these SNPs or diplotypes maybe useful markers for breast cancer risk. A high proportion of women diagnosed with breast cancer had rare diplotype variants in block 1. Having a rare variant was also associated with two other genotypes associated with an increased risk of developing breast cancer, absence of the *IGF1* 19 CA-repeat allele and *BRCA1* mutation, and predicted early-onset breast cancer beyond the effect of a *BRCA1* mutation. These rare diplotypes may identify women with particularly high risk for early-onset breast cancer and data on rare variant diplotypes should be included in forthcoming studies. However, our study warrants confirmation in independent population.

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**Figure legends**

Figure 1. The SNP distribution in the *IGF1* gene and allele and diplotype frequencies. Genotypes and minor allele frequencies (MAF) are presented for each htSNP. Possible combinations of the htSNPs are listed together with the number of carriers and the diplotype(s) that can result from each block combination. A diplotype seen in less than 10 women was grouped into a composite group of rare diplotypes.

Figure 2 Non-standardized and standardized IGF-1 levels, days 5-10 (a) and days 18-23 (b), according to diplotypes in all blocks, in all women (n=231), in non-users of OC (n=143), and in current OC users (n=88). The y-axis cuts the x-axis at the mean level of each of the three groups, the broken line represents the mean of the 231 women. The shaded span around the y-axis represents the standard error of the mean (SEM). Diamonds represents mean levels for each genotype group and horisontal lines represents SEM. Computed values standardized at age 29 years, 67 ksg and cycle day 7 (a) or 21 (b) are indicated by smaller dots.

Figure 3 *IGF1* diplotype distribution for block 1 (a), block 2 (b) and block 3 (c) in relation to *BRCA* mutation status (*BRCA1* mutation carriers (*BRCA1+*) *BRCA1* non-carriers (*BRCA-*), *BRCA2* mutation carriers (*BRCA2+*) *BRCA2* non carriers (*BRCA2-*), and women where no *BRCA1/2* mutation had been found in the family (*BRCAX*). The number of women included in each *BRCA* group is indicated under the x-axis. Each diplotype group is represented as their percentage of contribution to the bar for women in the respective *BRCA* group.

Figure 4 Influence of rare *IGF1* diplotypes in block 1 on cumulative hazard of a first breast cancer among a) all women and (Log rank $P<0.001$) b) *BRCA1* mutation carriers (Log Rank $P=0.031$). The number of women at each follow-up time is indicated below the graphs.

Table 1. Characteristics for all women and for the women included in the analyses of IGF-1 levels

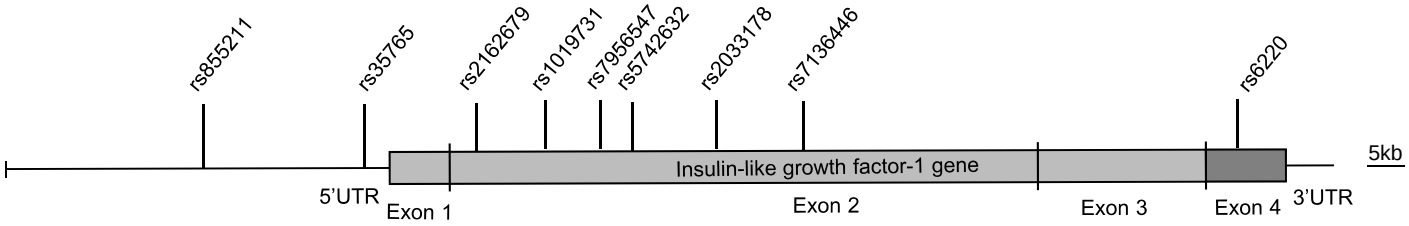
| | All women | | Included in IGF-1 analyses | |
|---|---|---|---|---|
| | Median (IQR) [1], % or n | n | Median (IQR) [1], % or n | n |
| Medians (IQR) | | | | |
| Age (years) | 30 (25-36) | 325 | 29 (24-35) | 231 |
| Year of birth | 1969 (1964-1975) | 291 | 1969 (1964-1976) | 231 |
| Birthweight (g) | 3410 (3070-3720) | 291 | 3405 (3070-3700) | 224 |
| Weight (kg) | 64 (58-74) | 269 | 64 (58-73) | 231 |
| Height (cm) | 168 (164-172) | 269 | 168 (164-172) | 231 |
| BMI (kg/m$^2$) | 22.8 (20.0-25.6) | 269 | 22.6 (20.8-25.1) (25.2/233) | 231 |
| Waist-to-hip ratio | 0.76 (0.73-0.80) | 269 | 0.76 (0.73-0.80) | 231 |
| Age at menarche (years) | 13 (12-14) | 312 | 13 (12-14) | 230 |
| Precentages (%) | | | | |
| Parous | 54.4 | 305 | 47.2 | 231 |
| Ever use of oral contraception | 92.2 | 246 | 91.3 | 231 |
| Current use of combined oral contraception | 34.2 | 269 | 37.7 | 231 (233) |
| Current use of progestin-only pills | 4.5 | 269 | 0 | 231 |
| Current use of other hormonal contraception or IUD* | 3.3 | 269 | 0 | 231 |
| Ever use of hormone replacement therapy | 5.4 | 268 | 0 | 231 |
| Ever smoker | 41.8 | 268 | 43.1 | 232 |
| Current smoker | 22.8 | 269 | 23.3 | 232 |
| Number of women (n) | | | | |
| BRCA1 family | 134 | 325 | 76 | 233 |
| Mutation carrier | 70 | | 23 | |
| Negative | 49 | | 44 | |
| Untested | 15 | | 9 | |
| BRCA2 family | 34 | 325 | 14 | 233 |
| Mutation carrier | 23 | | 7 | |
| Negative | 7 | | 5 | |
| Untested | 4 | | 2 | |
| BRCAX family | 110 | 325 | 102 | 233 |
| Untested family | 45 | 325 | 41 | 233 |

* Intra Uterine Device

# Table 2. Standardized IGF-1 levels in relation to *IGF1* SNP genotypes

| | | Menstrual cycle days 5-10 | | | | | | | | | Menstrual cycle days 18-23 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | All women | | | No OC use | | | Current OC | | | All women | | | No OC use | | | Current OC | | |
| SNP | Genotype | N | IGF-1 (ug/L) | *P*-value | N | IGF-1 (ug/L) | *P*-value | N | IGF-1 (ug/L) | *P*-value | N | IGF-1 (ug/L) | *P*-value | N | IGF-1 (ug/L) | *P*-value | N | IGF-1 (ug/L) | *P*-value |
| **Block 1** | | | | | | | | | | | | | | | | | | | |
| rs855211 | GG | 149 | 212 | | 93 | 209 | | 56 | 215 | | 149 | 214 | | 93 | 211 | | 56 | 194 | |
| | GA | 53 | 224 | 0,211 | 36 | 233 | 0,035 | 17 | 209 | 0,718 | 53 | 231 | 0,049 | 36 | 241 | 0,008 | 17 | 184 | 0,334 |
| | AA | 5 | 158 | 0,048 | 5 | 162 | 0,079 | 0 | | | 5 | 196 | 0,447 | 5 | 201 | 0,69 | 0 | | |
| rs35765 | CC | 188 | 212 | | 114 | 209 | | 74 | 212 | | 188 | 216 | | 114 | 215 | | 74 | 183 | |
| | CA | 43 | 209 | 0,754 | 30 | 215 | 0,608 | 13 | 195 | 0,348 | 43 | 219 | 0,761 | 30 | 227 | 0,313 | 13 | 170 | 0,33 |
| | AA | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| rs2162679 | AA | 174 | 213 | | 106 | 209 | | 68 | 215 | | 174 | 217 | | 106 | 215 | | 68 | 186 | |
| | AG | 53 | 210 | 0,732 | 35 | 220 | 0,356 | 18 | 192 | 0,157 | 53 | 216 | 0,851 | 35 | 227 | 0,281 | 18 | 164 | 0,05 |
| | GG | 3 | 157 | 0,109 | 3 | 160 | 0,15 | 0 | | | 3 | 217 | 0,988 | 3 | 221 | 0,846 | 0 | | |
| Block 2 | | | | | | | | | | | | | | | | | | | |
| rs1019731 | CC | 168 | 209 | | 104 | 210 | | 64 | 202 | | 168 | 214 | | 104 | 215 | | 64 | 175 | |
| | CA | 53 | 216 | 0,47 | 35 | 205 | 0,622 | 18 | 231 | 0,083 | 53 | 225 | 0,199 | 35 | 221 | 0,62 | 18 | 196 | 0,064 |
| | AA | 9 | 229 | 0,322 | 5 | 251 | 0,124 | 4 | 193 | 0,781 | 9 | 233 | 0,28 | 5 | 245 | 0,271 | 4 | 176 | 0,979 |
| rs7956547 | AA | 127 | 211 | | 79 | 211 | | 48 | 205 | | 127 | 220 | | 79 | 221 | | 48 | 181 | |
| | AG | 77 | 216 | 0,529 | 48 | 212 | 0,974 | 29 | 224 | 0,183 | 77 | 214 | 0,462 | 48 | 214 | 0,496 | 29 | 181 | 0,95 |
| | GG | 26 | 201 | 0,456 | 17 | 203 | 0,603 | 9 | 193 | 0,599 | 26 | 212 | 0,503 | 17 | 212 | 0,558 | 9 | 175 | 0,701 |
| rs5742632 | AA | 129 | 211 | | 82 | 212 | | 47 | 206 | | 129 | 220 | | 82 | 222 | | 47 | 182 | |
| | AG | 77 | 212 | 0,896 | 47 | 210 | 0,857 | 30 | 214 | 0,548 | 77 | 212 | 0,272 | 47 | 211 | 0,324 | 30 | 177 | 0,593 |
| | GG | 24 | 209 | 0,902 | 15 | 208 | 0,816 | 9 | 209 | 0,883 | 24 | 216 | 0,735 | 15 | 216 | 0,749 | 9 | 180 | 0,883 |
| Block 3 | | | | | | | | | | | | | | | | | | | |
| rs2033178 | CC | 197 | 211 | | 124 | 208 | | 73 | 215 | | 197 | 216 | | 124 | 215 | | 73 | 182 | |
| | CT | 30 | 211 | 0,995 | 20 | 227 | 0,189 | 10 | 182 | 0,123 | 30 | 222 | 0,531 | 20 | 233 | 0,2 | 10 | 169 | 0,366 |
| | TT | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| rs7136446 | TT | 85 | 215 | | 52 | 216 | | 33 | 214 | | 85 | 221 | | 52 | 226 | | 33 | 181 | |
| | TC | 102 | 213 | 0,816 | 65 | 209 | 0,507 | 37 | 216 | 0,897 | 102 | 216 | 0,517 | 65 | 213 | 0,228 | 37 | 186 | 0,611 |
| | CC | 38 | 197 | 0,129 | 23 | 198 | 0,228 | 15 | 195 | 0,32 | 38 | 210 | 0,32 | 23 | 213 | 0,369 | 15 | 172 | 0,484 |
| rs6220 | TT | 113 | 210 | | 71 | 209 | | 42 | 210 | | 113 | 219 | | 71 | 219 | | 42 | 185 | |
| | TC | 88 | 218 | 0,393 | 56 | 218 | 0,344 | 32 | 215 | 0,739 | 88 | 217 | 0,862 | 56 | 219 | 0,988 | 32 | 180 | 0,621 |
| | CC | 24 | 202 | 0,517 | 14 | 202 | 0,713 | 10 | 203 | 0,736 | 24 | 214 | 0,705 | 14 | 219 | 0,959 | 10 | 172 | 0,395 |
| rs5742612 | TT | 210 | 211 | | 129 | 211 | | 81 | 209 | | 210 | 216 | | 129 | 217 | | 81 | 182 | |
| | CT | 19 | 214 | 0,854 | 14 | 214 | 0,827 | 5 | 212 | 0,914 | 19 | 219 | 0,802 | 14 | 225 | 0,63 | 5 | 170 | 0,533 |
| | CC | 1 | 222 | 0,853 | 1 | 235 | 0,68 | 0 | | | 1 | 322 | 0,046 | 1 | 328 | 0,057 | 0 | | |
| rs35765817 | CC | 201 | 211 | | 126 | 210 | | 75 | 210 | | 201 | 214 | | 126 | 216 | | 75 | 179 | |
| | CT | 28 | 213 | 0,841 | 16 | 213 | 0,844 | 12 | 202 | 0,677 | 28 | 229 | 0,18 | 16 | 227 | 0,447 | 12 | 190 | 0,399 |
| | TT | 2 | 240 | 0,486 | 2 | 241 | 0,464 | 0 | | | 2 | 274 | 0,113 | 2 | 274 | 0,162 | 0 | | |
| rs35455143 | CC | 208 | 211 | | 130 | 210 | | 78 | 210 | | 208 | 215 | | 130 | 216 | | 78 | 179 | |
| | CT | 21 | 211 | 0,983 | 12 | 213 | 0,883 | 9 | 201 | 0,688 | 21 | 232 | 0,151 | 12 | 232 | 0,355 | 9 | 193 | 0,346 |
| | TT | 2 | 240 | 0,49 | 2 | 241 | 0,465 | 0 | | | 2 | 274 | 0,113 | 2 | 274 | 0,162 | 0 | | |

Figure 1.




| Block | SNP | Genotype | MAF | | Combined genotypes (N) | | Diplotypes |
|---|---|---|---|---|---|---|---|
| Block 1 | rs85521 | GG | 0.1672 | | GG/CC/AA | 198 | GCA/GCA |
| | | GA | | | GA/CA/AG | 52 | GCA/AAG |
| | | AA | | | GA/CC/AG | 14 | GCA/ACG |
| | rs35765 | CC | 0.1068 | | GA/CC/AA | 11 | GCA/ACA |
| | | CA | | | GG/CC/AG | 3 | GCA/GCG |
| | rs2162679 | AA | 0.1429 | | GA/CA/AA | 3 | GCA/AAA |
| | | AG | | | AA/CA/GG | 3 | ACG/AAG |
| | | | | rare | AA/CA/AG | 3 | AAG/AGA or ACG/AAA |
| | | GG | | | AA/CC/GG | 1 | ACG/ACG |
| | | | | | AA/CC/AG | 1 | ACG/ACA |
| | | | | | | N=289 | |
| Block 2 | rs1019731 | CC | 0.1227 | | CC/AA/AA | 103 | CAA/CAA |
| | | CA | | | CC/AG/AG | 85 | CAA/CGG |
| | | AA | | | CA/AA/AA | 46 | CAA/AAA |
| | rs7956547 | AA | 0.2888 | | CC/GG/GG | 25 | CGG/CGG |
| | | AG | | | CA/AG/AG | 20 | CGG/AAA |
| | | GG | | | CC/GA/AA | 11 | CAA/CGA |
| | rs5742632 | AA | 0.2764 | | AA/AA/AA | 10 | AAA/AAA |
| | | AG | | | CC/AA/AG | 8 | CAA/CAG |
| | | GG | | | CC/GG/AG | 6 | CGA/CGG |
| | | | | | CC/AG/GG | 4 | CAG/CGG |
| | | | | rare | CA/AG/AA | 2 | CGA/AAA |
| | | | | | CC/GG/AA | 1 | CGA/CGA |
| | | | | | CA/AA/AG | 1 | CAG/AAA or CAA/AAG |
| | | | | | | N=322 | |
| Block 3 | rs2033178 | CC | 0.0714 | | CC/TT/TT | 98 | CTT/CTT |
| | | CT | | | CC/CT/TC | 58 | CCC/CTT or CCT/CTC |
| | | TT | | | CC/CT/TT | 41 | CCT/CTT |
| | rs7136446 | TT | 0.3943 | | CT/CT/TC | 23 | CTT/TCC |
| | | TC | | | CC/CC/TC | 19 | CCT/CCC |
| | | CC | | | CC/TT/TC | 16 | CTT/CTC |
| | rs6220 | TT | 0.3119 | | CC/CC/CC | 15 | CCC/CCC |
| | | TC | | | CT/CC/CC | 9 | CCC/TCC |
| | | CC | | | CC/CC/TT | 6 | CCT/CCT |
| | | | | rare | CT/CC/TC | 5 | CCT/TCC |
| | | | | | CC/CT/CC | 4 | CCC/CTC |
| | | | | | CT/CT/CC | 3 | CTC/TCC |
| | | | | | CC/TT/CC | 2 | CTC/CTC |
| | | | | | | N=299 | |

Figure 2a. IGF-1 levels during menstrual cycle days 5-10

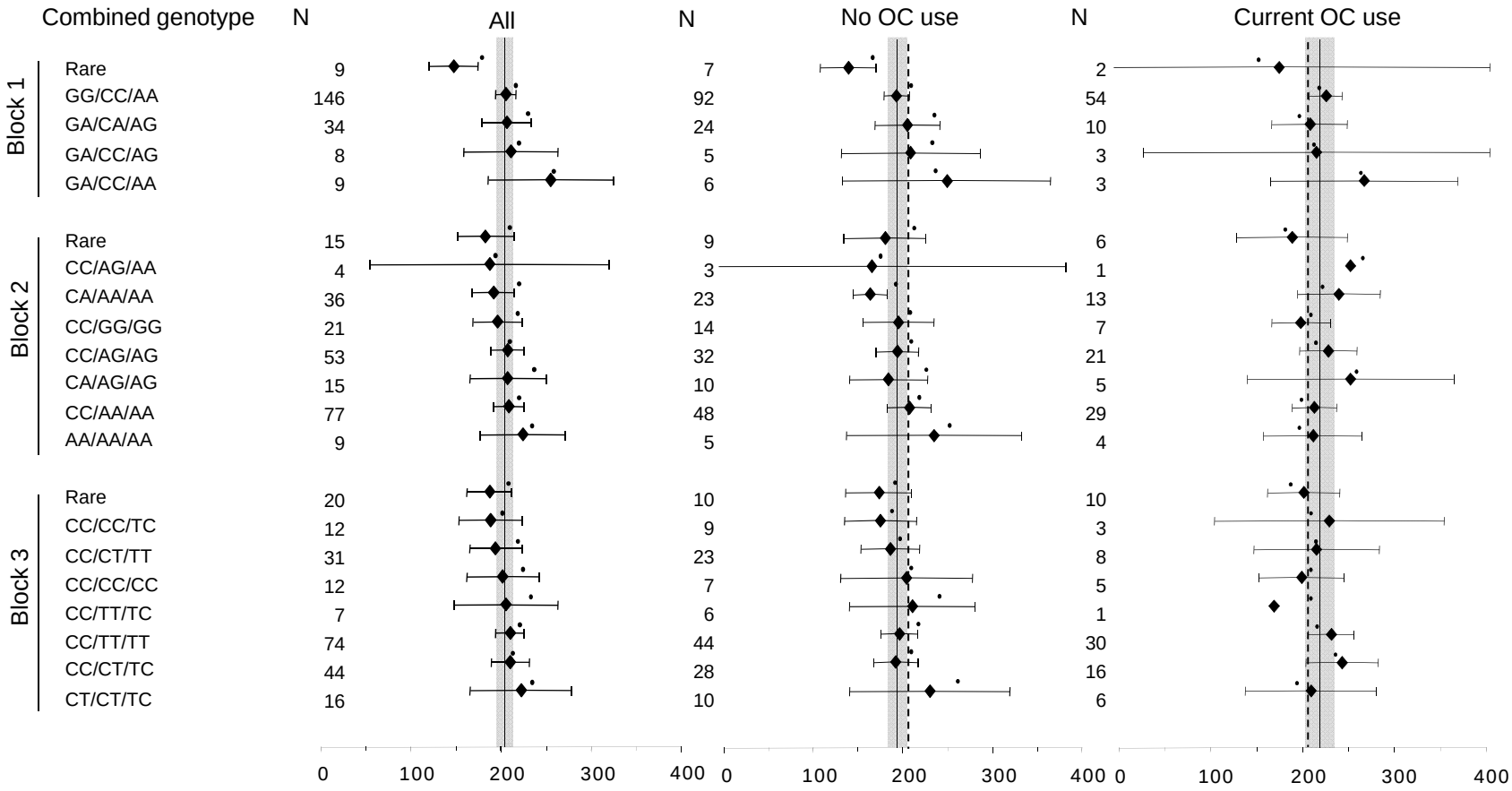


Figure 2b. IGF-1 levels during menstrual cycle days 18-23

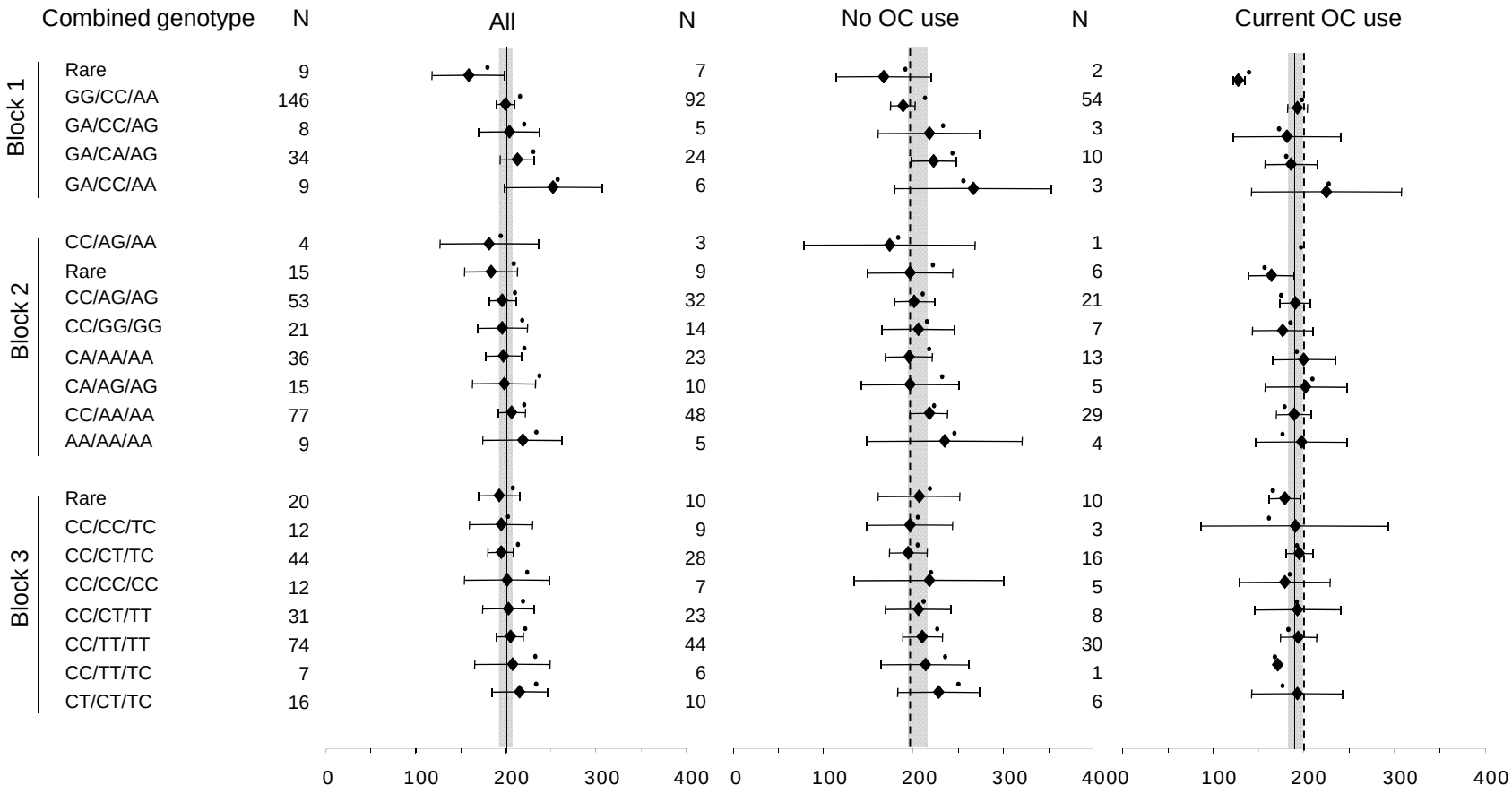
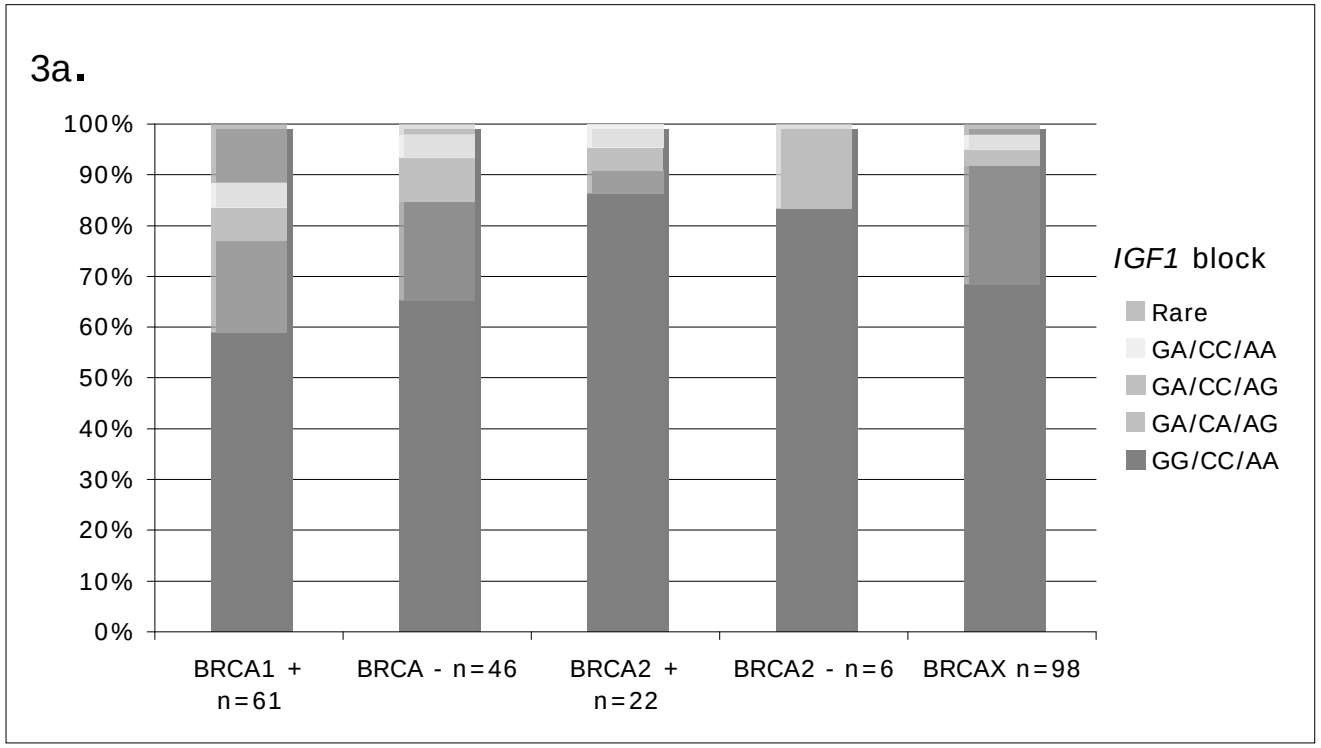
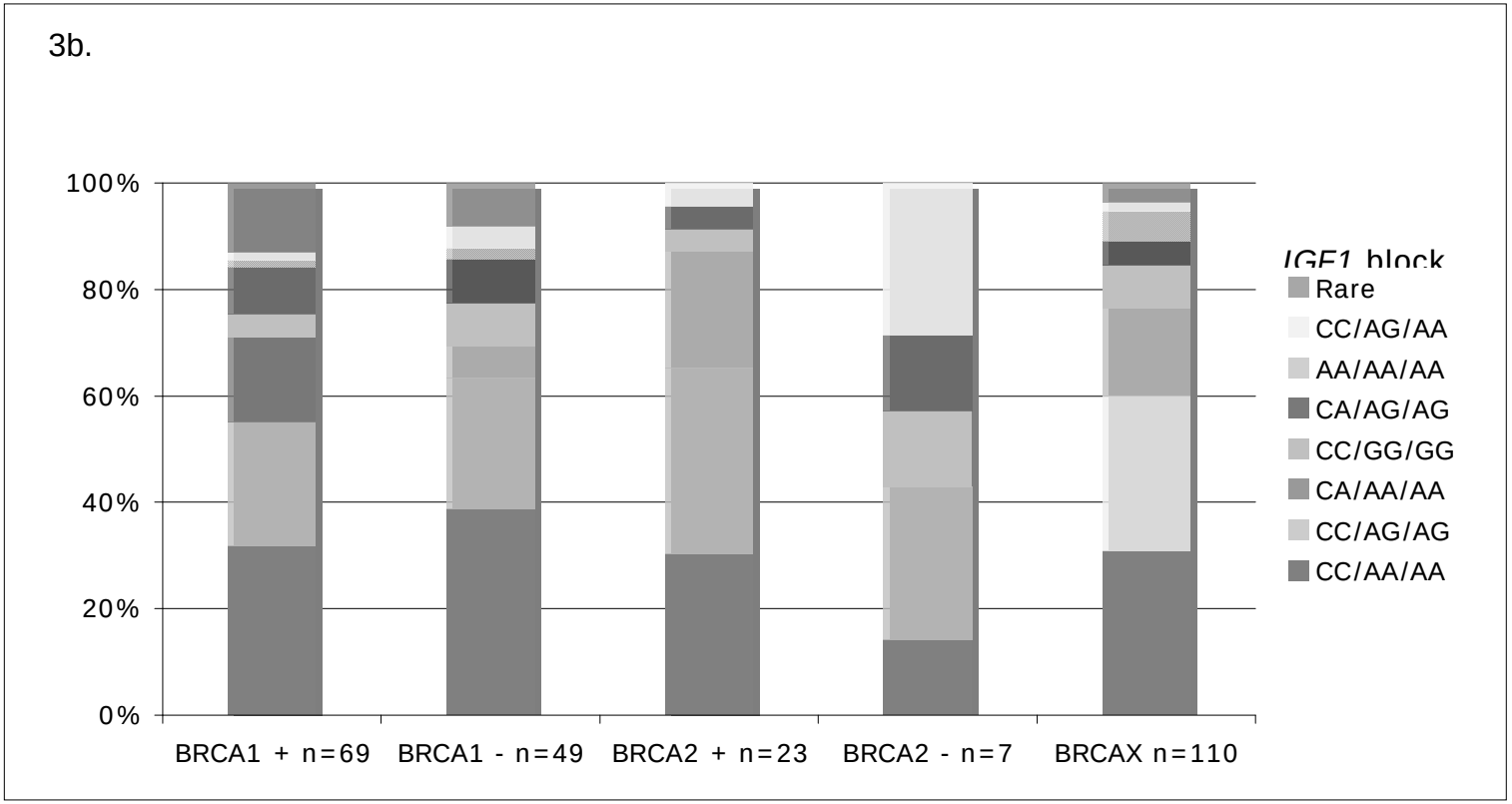
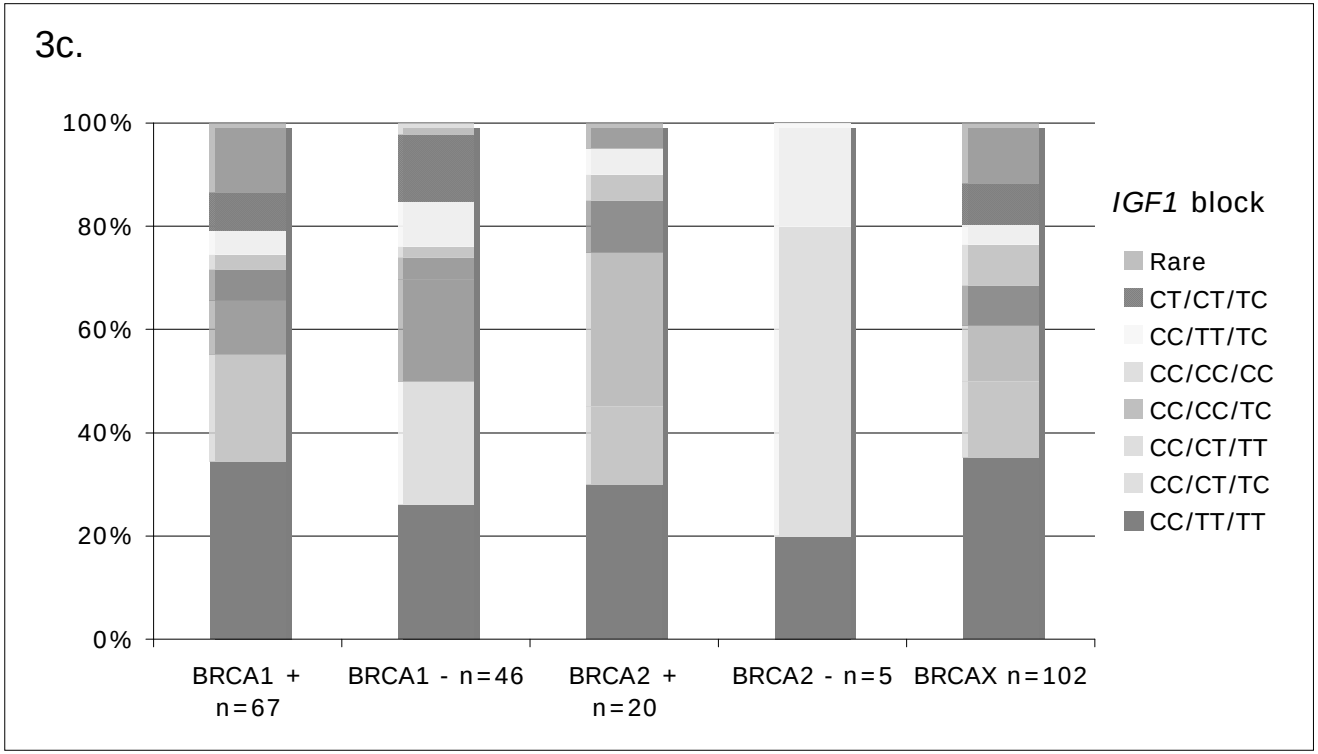


Figure 3. IGF1 diplotype distribution in relation to BRCA status

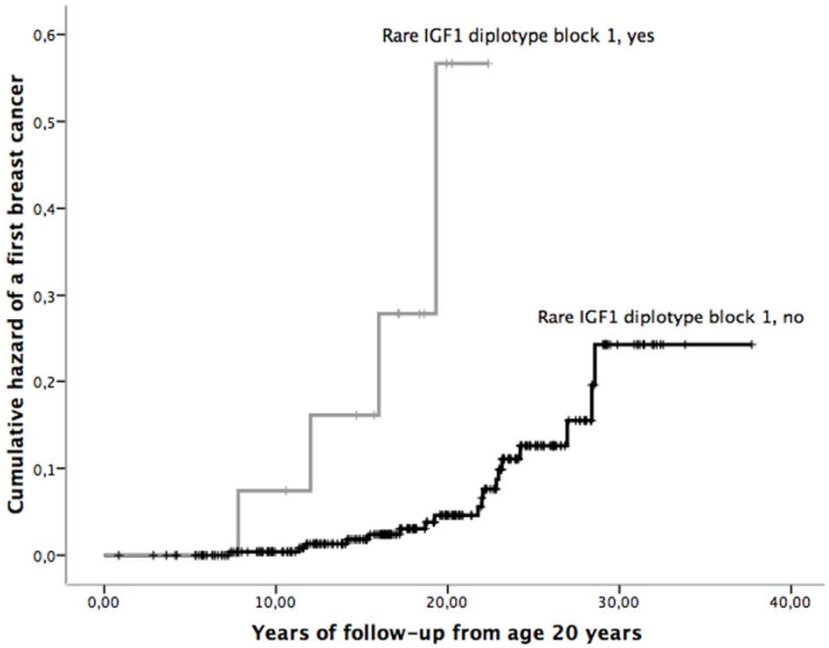


3b.

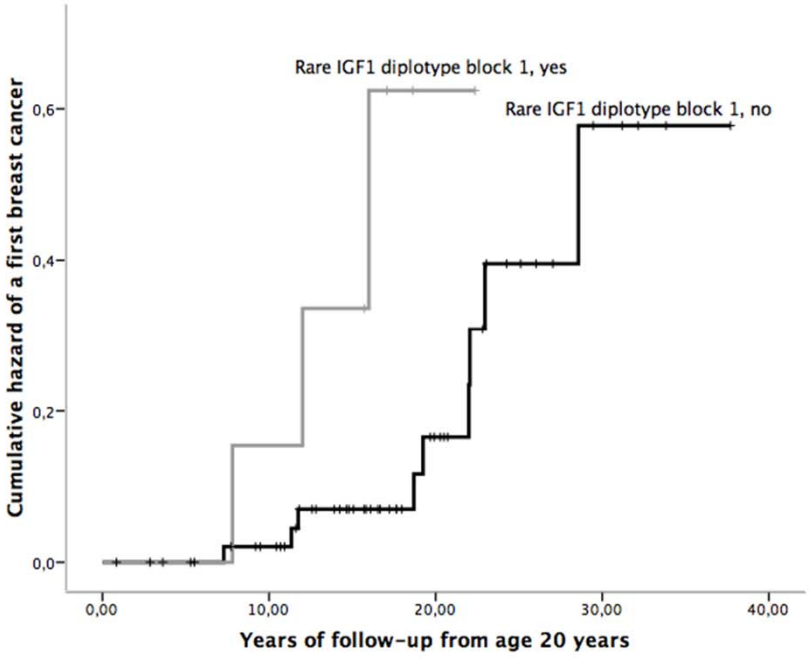

100%
80%
60%
40%
20%
0%
BRCA1 + n=69
BRCA1 - n=49
BRCA2 + n=23
BRCA2 - n=7
BRCAX n=110
IGF1 block
Rare
CC/AG/AA
AA/AA/AA
CA/AG/AG
CC/GG/GG
CA/AA/AA
CC/AG/AG
CC/AA/AA


3c.
100%
80%
60%
40%
20%
0%
BRCA1 + n=67
BRCA1 - n=46
BRCA2 + n=20
BRCA2 - n=5
BRCAX n=102
IGF1 block
Rare
CT/CT/TC
CC/TT/TC
CC/CC/CC
CC/CC/TC
CC/CT/TT
CC/CT/TC
CC/TT/TT

a) All women




b) BRCA1 mutation carriers




| Rare IGF1 diplotype in block 1 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Yes, N=14 | 13 | 2 | 0 | 0 | N=7 | 6 | 1 | 0 | 0 |
| No, N=275 | 231 | 117 | 13 | 0 | N=54 | 45 | 30 | 4 | 0 |