Running head: *BRCA1* and *IGF1* genotype

Absence of the common *IGF1* 19 CA-repeat allele is more common among *BRCA1* mutation carriers than among non-carriers from *BRCA1* families

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ABSTRACT

BRCA1 mutations predispose to early-onset breast cancer. We previously reported an association between absence of the common *IGF1* 19CA-repeat allele (*IGF1*-19/-19) and being a *BRCA1* mutation carrier in young women from breast cancer high-risk families. Others have reported a four-fold risk of premenopausal breast cancer in women with a family history and the *IGF1*-19/-19 genotype. The aim of this study was to investigate whether the *IGF1*-19/-19 genotype was associated with being a *BRCA1* mutation carrier among women from *BRCA1* families. DNA was available from 268 women with known *BRCA1* status from the South Swedish Health Care Region. *IGF1* genotyping was successfully performed with fragment analysis in 211 women from 96 families. The *IGF1*-19/-19 genotype was significantly more common among *BRCA1* mutation carriers (14.2%) than among non-carriers (4.8%), OR 3.3 (95%CI 1.11-9.78, *P*=0.03) adjusted for family clustering. We confirmed our previous finding of an association between the *IGF1*-19/-19 genotype and *BRCA1* mutation status. Since the *IGF1*-19/-19 genotype in combination with OC use or multiparity confers an increased risk for early onset breast cancer in high-risk women and in women from the general population, future studies are needed to elucidate the importance of the *IGF1-19/-19* genotype concerning the variability in breast cancer risk among *BRCA1* mutation carriers.

Key words: *BRCA1*, breast cancer, genotype, *IGF1*

BRCA1 Breast cancer gene 1

BRCA2 Breast cancer gene 2

CA cytosine-adenine

CI confidence interval

DNA deoxyribonucleic acid

Ghr Growth hormone receptor

IGF1 Insulin-like growth factor-1 gene

IGF-1 Insulin-like growth factor-1

IGF-IR Insulin-like growth factor-1receptor

Igfals IGF acid labile subunit

Igfbp2 IGF binding protein 2

IRS-1 Insulin receptor substrate 1

OC oral contraceptive

OR odds ratio

BACKGROUND

In Western societies, breast cancer is the most common form of cancer among women. The lifetime risk in Sweden is 1 in 10 (data from the Swedish Cancer Registry). Approximately 10 % of breast cancers are hereditary and 15 – 20 % of these are associated with mutations in the *BRCA1* and *BRCA2* genes. The gene product of *BRCA1* participates in several biological pathways including cell growth and apoptosis, gene transcription and DNA damage repair (Wang et al., 2000). The lifetime risk of developing breast cancer for a carrier of a mutation in this tumour suppressor gene varies from about 60 to over 80% (Easton et al., 1995; Ford et al., 1994; Ford et al., 1998). The risk is not identical for all mutation carriers, but is also influenced by allelic heterogeneity, low-penetrance modifying genes and environmental and hormonal factors (Narod & Foulkes, 2004). Absence of the common 19-CA repeat allele of the *IGF1* (*IGF1-19/-19*) gene is associated with early-onset breast cancer after oral contraceptive (OC) use (Cleveland et al., 2006; Jernström et al., 2005) and in multiparous women (Bågeman et al., 2007) and may therefore be one such modifying gene.

Insulin-like growth factor-1 (IGF-1) is important for regulation of cell proliferation and apoptosis (Stewart & Rotwein, 1996) and plays an essential role in normal female breast development (Dickson & Russo, 2000). High levels of IGF-1 increase the risk for breast cancer among young, premenopausal women (Hankinson et al., 1998; Muti et al., 2002; Toniolo et al., 2000). Genetic variations account for up to 60% of the variability in IGF-1 levels (Harrela et al., 1996; Hong et al., 1996). The *IGF1* gene contains a dinucleotide cytosine-adenine (CA) repeat in the proximity of the promoter, 1kb upstream from the transcription start site (Weber & May, 1989). The CA repeat

sequence ranges from 11 to 24 repeats (Rosen et al., 1998; Schildkraut et al., 2005). The 19 CA-repeat allele is the most common in whites (Jernström et al., 2001a; Schildkraut et al., 2005). Studies of other genes indicate that the number of CA repeats in a promoter region is inversely correlated with transcription activity (Gebhardt et al., 1999; Tae et al., 1994). A non-significant trend of decreasing IGF-1 levels with increasing number of repeats has been reported (Figer et al., 2002). The level of IGF-1 is also influenced by other factors, such as oral contraceptive (OC) use (Jernström & Barrett-Connor, 1999; Jernström et al., 2001a; Jernström et al., 2001b; Narod et al., 2002). We have previously reported that the *IGF1*-19/-19 genotype was more common among known *BRCA1* mutation carriers than among other healthy women from high-risk breast cancer families of various types (i.e. *BRCA1*, *BRCA2*, *BRCAX* and untested families) (Jernström et al., 2005). The number of women with a known *BRCA1* mutation was fairly small and the comparison was made with all the other women from high-risk families. Another case-control study found that women with the *IGF1*-19/-19 genotype and a family history of breast cancer had a four-fold increased risk for premenopausal breast cancer compared with women with at least one *IGF1* 19 CA-repeat allele (*IGF1*+19 genotype) and no family history (Cleveland et al., 2006).

We hypothesized that the association between the *IGF1*-19/-19 genotype and premenopausal breast cancer in women with a family history of breast cancer may in part be explained by an association between the *IGF1*-19/-19 genotype and carrying a *BRCA1* mutation. The aim of this study was to investigate whether the *IGF1*-19/-19 genotype is associated with being a *BRCA1* mutation carrier among women belonging to *BRCA1* families.

MATERIALS AND METHODS

Study populations

This study's results are based on two study populations: a "new" study population of 268 women and part of the study population of our previous study of young healthy high-risk women, i.e. the 72 women from *BRCA1* families for whom the mutation status was known.

The new study population consisted of 268 women with known BRCA1 mutation status from the South Swedish Health Care Region. These women were born between 1911 and 1983 and belonged to 96 different families (between one and eight women in each family). Mutation testing was carried out between 1994 and 2006 by the Oncogenetic Clinic and the research laboratory of the Oncology Clinic of the Lund University Hospital, Sweden. All *BRCA1* mutation testing in the South Swedish Health Care Region is performed in our laboratory.

We also had access to the *IGF1* genotypes of 72 healthy 18 – 40 year-old women from *BRCA1* mutation families who constituted part of our previous study population (Jernström et al., 2005). A woman who was included in the previous study was not included in the new study population and no woman belonged to both of these study populations. Taken together, the two study populations thus represent all women with available DNA and known *BRCA1* mutation status who belong to *BRCA1* mutation families in this region. In total these women belonged to 99 different families. The Ethics Committee of the Lund University approved the study.

Questionnaire and cancer data

Comprehensive questionnaires are administered to women during the course of genetic counselling. These contain questions on reproductive factors, hormone intake,

smoking, height and weight etc. It is not required that a woman completes the questionnaire to obtain genetic testing. Questionnaire data was available for 114 of the 268 women in the new study population.

All 72 women from the previous study population completed a questionnaire administered during the course of that study, which also contained questions on reproductive factors, hormone intake and smoking. Their heights and weights were measured by a research nurse (Jernström et al., 2005).

Cancers among the women in the new study population were self reported. In the new study population of 268 women, 109 women had self-reported breast cancers and we were able to confirm the diagnosis in 84 of these through the Regional Tumour Registry. Breast cancers reported prior to 1958 are not included in the Regional Registry. Cancers diagnosed in women who were not residing in our region at the time of diagnosis are not included either in the Registry. Five of the 72 women in the previous study population have been diagnosed with breast cancer, and all five were confirmed.

Mutation testing

BRCA1 mutation status was obtained from clinical records. One hundred and fifty four women were *BRCA1* mutation carriers and 114 were non-carriers. In Sweden, *BRCA1* mutation testing is offered if an individual has at least three first-degree relatives with breast cancer and at least one was younger than 50 years at diagnosis; or if two first-degree relatives were diagnosed with breast cancer and at least one prior to age 40 years; or if one first-degree relative was diagnosed with breast cancer prior to age 30 years. The *BRCA1* mutation carriers included only those with confirmed deleterious

alterations, that is, non-sense or frameshift indel mutations that cause protein truncation, or known disease-associated missense mutations.

Genotyping of the IGF1 gene CA repeats

Blood or tumour DNA from the 268 women belonging to families where a disease-causing *BRCA1* mutation has been confirmed was available for *IGF1* genotyping. Genomic DNA was extracted from peripheral blood or tumour tissue using Wizard, Genomic DNA Purification Kit, (Promega, Madison, WI). The polymorphism in the *IGF1* gene is a tandem dinucleotide repeat, ranging in size from 11 to 24 repeats. PCR primers 5´-GCTAGCCAGCTGGTGTTATT and 5´-GTTTCTTACCACTCTGGGAGAAGGGTA were used, where the forward primer was fluorescently labelled with FAM (MWG-Biotech AG, Ebersberg, Germany). PCR was performed in 15 µl reactions using 25 ng DNA, 0.4 µM of each primer, 0.1 mM of each deoxynucleotide (Amersham Biosciences, Buckinghamshire, UK), 5% DMSO (Sigma, St Louis, MO), 2.5 mM $MgCl_2$ (Applied Biosystems, Foster City, CA), 1 X PCR Gold Buffer (Applied Biosystems, Foster City, CA) and 1 U AmpliTaq Gold (Applied Biosystems, Foster City, CA).

The PCR product was analyzed in an ABI3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) and the results were evaluated using Genescan software. The number of CA repeats was determined by sequencing samples of varying sizes (Big Dye, Terminator Cycle Sequencing, Applied Biosystems, Foster City, CA) and using them as standards in the fragment analysis. The results from the ABI3100 Genetic Analyzer have been manually evaluated, and each sample has been read in duplicates. Out of 169 samples analyzed with this method, genotyping was successful in 128 samples.

In April 2006, the system was upgraded to an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Ninety nine samples were run on the upgraded system ABI3130xl and 83 samples were successfully genotyped. The number of CA repeats were manually evaluated, as stated above, as well as automatically evaluated by the GeneMapper Software v.4.0. The number of CA repeats ranged from 12 to 23. The software could not determine repeat sizes shorter than 17 repeats, but these short alleles were very rare (0.7%). The concordance rate between the manual and automatic readings of samples with 17 to 23 repeats was 100%. For quality control, we attempted to re-analyze a set of samples (n = 72). Genotyping was successful in 57 samples and the concordance rate was 100%. We therefore did not perseverate in reanalyzing the remaining samples in the validation set.

In summary, microsatellite genotyping was successful in 211 out of the 268 samples. Of these 211 successful samples, 199 were analyzed in DNA extracted from blood, 11 were analyzed in DNA where we were unable to confirm the source, and one sample was analyzed in DNA from tumour tissue. We reanalyzed samples multiple times if the first attempt failed. Out of the 57 unsuccessful results, the DNA was extracted from tumours in 30 samples, from blood in 23 samples, and for the remaining four samples we were unable to confirm the source of the DNA.

Loss of heterozygosity may be a problem for genetic analyses from tumour tissue (Weber et al., 2006). We therefore examined the frequency of homozygosity for the 19 CA-repeat allele. Among all 211 women, 38% were homozygous for the 19 CA-repeat allele. In the 11 samples where the source of DNA was undetermined, four women were homozygous for the 19 CA-repeat allele (37 %). In the only sample where the *IGF1* genotyping worked from DNA known to be extracted from tumour tissue, the

woman was heterozygous (19/22). These results do not support the notion that loss of heterozygosity of the *IGF1* gene occurred at a significant frequency in the 11 DNA samples possibly derived from formalin-fixed paraffin-embedded tumour tissue.

We also had access to *IGF1* genotypes for 254 women from our previous study of young healthy high-risk women (Jernström et al., 2005). All the *IGF1* genotyping in this study was done on the ABI3100 Genetic analyzer as specified above. For quality control 23 samples were reanalyzed on the ABI Prism 3130xl Genetic Analyzer. Twenty two samples worked in the first attempt; the concordance rate was 100%. We therefore did not perseverate in reanalyzing the remaining sample in the validation set. From this study, we used the *IGF1* genotype data from the 72 women with known *BRCA1* status from *BRCA1* families.

Data analyses

The statistical software SPSS 13.0 and Stata 9.2 were used for statistical analyses. Odds ratio (OR) and 95% confidence intervals (CI) were calculated for the *IGF1*-19/-19 genotype in *BRCA1* carriers compared to non-carriers using logistic regression with adjustment for family clustering by using the cluster option of the regress command in Stata. A *P* - value of <0.05 was considered to be significant. All *P* - values were two-sided.

RESULTS

The characteristics of the 211 women with known *IGF1* genotypes are presented in table I. Only 114 women completed a study questionnaire and our data is therefore incomplete for several variables. Fifty percent were carriers of a *BRCA1* mutation. The *IGF1* CA-repeat alleles ranged in sizes from 12 to 23 repeats. Overall, 9.5% had the *IGF1*-19/-19 genotype.

Table II shows the distribution of the *IGF1* allele frequency by *BRCA1* mutation status for the 211 women. The *IGF1*-19/-19 genotype was significantly more common among *BRCA1* mutation carriers (14.2%) than among non-carriers (4.8%), odds ratio (OR) 3.30 (95% confidence interval (CI) 1.11-9.78, $P = 0.03$, adjusted for family clustering). As shown in table II, *BRCA1* carriers had a higher frequency of both the shorter 18 CA-repeat allele and the longer 21 CA-repeat allele compared with non-carriers. For the 69 index cases with known *IGF1* genotypes, the frequency of the *IGF1*-19/-19 genotype was 15.9 %. Among the women who did not have any reported cancers of any type at the time of *BRCA1* mutation testing (n=112), the *IGF1*-19/-19 genotype was still over three times as common among carriers compared with non-carriers OR 3.30 (95% CI 0.71 -15.5; $P = 0.13$ adjusted for family clustering), but the P - value was no longer significant due to lower numbers.

We then added the samples from 72 women from *BRCA1* families with known *BRCA1* mutation status from our previous study of women from high-risk breast cancer families (Jernström et al., 2005). These 72 samples, together with the new material of 211 samples, yielded 283 samples eligible for analysis. One hundred and twenty nine women (46%) carried a *BRCA1* mutation. Overall, 12% had the *IGF1*-19/-19 genotype.

Table III describes the distribution of the *IGF1* allele frequency by *BRCA1* mutation status for the two study populations combined. The results remained significant: the *IGF1*-19/-19 genotype was twice as common among carriers of a *BRCA1* mutation (16 %) than among non-carriers (8%), OR 2.3 (95% CI 1.08-4.91, *P* = 0.03, adjusted for family clustering). We also repeated the analyses after exclusion of women with any type of reported cancers at the time of mutation testing. The *IGF1*-19/-19 genotype was approximately three times as common in *BRCA1* mutation carriers compared with non-carriers OR 2.9 (95% CI 1.20 – 7.28; *P* = 0.02, adjusted for family clustering.)

Of the 42 *BRCA1* mutation carriers who were healthy at the time of mutation testing, nine had the *IGF1*-19/-19 genotype and three of these have since developed breast cancer, while three of the 33 women with the *IGF1*+19 genotype have developed breast cancer since mutation testing. Only one of these six incident cases belongs to the new study population. Four of these six incident cases were part of our previous report on incident breast cancer {Jernström, 2005 #1} and the sixth case was still healthy at the time of the previous report.

DISCUSSION

The main finding of this study was that the *IGF1*-19/-19 genotype was significantly more common among *BRCA1* mutation carriers than among non-mutation carriers of the same families. This finding confirms the results of our previous study where we reported that the *IGF1*-19/-19 genotype was more common among known *BRCA1* carriers than among other young healthy women from various types of breast cancer high-risk families (Jernström et al., 2005). The results remained significant after adding the data on the 72 women with known *BRCA1* mutation status from the previous study (Jernström et al., 2005) to the new material of 211 women. Taken together, these two study populations comprise all women in the South Swedish Health Care Region with known *BRCA1* mutation status for whom we could perform *IGF1* genotyping with our analytical methods.

The Long Island study reported a four-fold increase in breast cancer risk among women with a family history of breast cancer and the *IGF1*-19/-19 genotype (Cleveland et al., 2006). The proportion of Ashkenazi women is much higher on Long Island, New York, USA than in Sweden. It is well known that *BRCA1* mutations segregate at a high frequency among Ashkenazim 2-2.5 % (Warner et al., 1999). It is therefore possible that the association between the *IGF1*-19/-19 genotype and premenopausal breast cancer in women with a family history of breast cancer may in part be explained by an association between the *IGF1*-19/-19 genotype and carrying a *BRCA1* mutation. Another study also reported an association between *IGF1* CA-repeat allele sizes and breast cancer risk in Jewish women with and without *BRCA1/2* mutations (Figer et al., 2002). While the authors reported the risk of breast cancer as highest among women

with short allele sizes, they did not examine sporadic versus *BRCA1/2* associated breast cancers in relation to absence or presence of the 19 CA-repeat allele.

The women in the present study all belonged to *BRCA1* families with sufficiently high breast and ovarian cancer penetrance to qualify them for mutation testing according to our guidelines at the Oncogenetic Clinic. The penetrance of *BRCA1* and *BRCA2* mutation differs between series of mutation carriers from the general population and from high-risk families (Anglian Breast Cancer Study Group, 2000; Ford et al., 1998; Narod & Foulkes, 2004). It is therefore likely that other low penetrance genes that modify cancer risk, such as the *IGF1* gene, also segregate at a high frequency in high-risk families. However, the allele frequencies found in this study were comparable to those reported in other Caucasian populations (Cleveland et al., 2006; Frayling et al., 2002). One strength of the present study is that many women belonged to the same family. Our results remained significant after adjustment for family clustering. This minimizes the likelihood that our finding of a higher frequency of the *IGF1*-19/-19 genotype among mutation carriers than among non-carriers is simply due to differences in the *IGF1* genotypes of women belonging to different families.

Since the *IGF1*-19/-19 genotype was more common among *BRCA1* mutation carriers than among non-carriers from the same families, one may hypothesize that this combination confers some survival advantage, possibly during foetal development. *BRCA1* mutation carriers have been found to be significantly smaller for gestational age than their unaffected relatives (Jernström et al., 1998). If a *BRCA1* mutation inhibits foetal development, the loss of growth stimuli may be compensated for by elevated IGF-1 levels. Whether the IGF-1 level is higher in foetuses with the *IGF1*-19/-

19 genotype has to our knowledge not been studied. If so, *BRCA1* carriers with the *IGF1*-19/-19 genotype may then compensate for the negative effect of a *BRCA1* mutation on growth and development through higher IGF-1 levels during pregnancy. Data on gestational age, birth weight and *IGF1* genotype was available for 101 of 283 women. Women with the *IGF1*-19/-19 genotype were 276g lighter at birth compared with others ($P = 0.06$ adjusted for gestational age and *BRCA1* mutation status). In the 33 *BRCA1* carriers the effect was similar: women with the *IGF1*-19/-19 genotype were 287g lighter after adjustment for gestational age, which is in line with the findings of others (Vaessen et al., 2002).

The mitogenic actions of IGF-1 are mediated by the IGF-1 receptor. Associations have been reported between increased expression of the IGF-1 receptor and radioresistance, tumour recurrence and resistance to chemotherapeutic agents (Lu et al., 2001; Turner et al., 1997). The transcription of the IGF-1 receptor is under the inhibitory control of *BRCA1* through binding to Sp1, which prevents the zinc-finger protein from binding to and transactivating the IGF-1 receptor promoter (Abramovitch et al., 2003). A recent study indicates that the absence of BRCA1 results in increased expression of IRS-1, IGF-IR, Igfals, Igfbp2, and Ghr and increased levels of serum IGF-I in BRCA1-deficient mice, primary mammary tumors, and cultured human cells (Shukla et al Cancer Res 2007). The *IGF1* gene is one of the genes mapping to the PAH locus (12q23.2) which is a hotspot for genomic instability in the epithelium and stroma of *BRCA1/2*-related breast cancers (Weber et al., 2006). Because a small number of samples was derived from tumor tissue or an unknown source, we re-analyzed our data excluding these samples, the results became even stronger OR 3.70. Genomic instability at the *IGF1* locus is also associated with high T stage in *BRCA1/2* cancers

(Weber et al., 2006). The process of mammary differentiation requires penetration of the breast epithelium at the ductal end buds into the stroma. It is therefore possible that, as a result of defective *BRCA1/2* genes, the impaired DNA repair mechanism predisposes the highly susceptible proliferating mammary epithelium during pregnancy to carcinogenetic transformation. Russo *et al.* reported that the breast architectural pattern in parous women with a family history of breast cancer resembles that of nulliparous women and is different from that in the general population (Russo et al., 1994; Russo & Russo, 1997). We have previously reported that each pregnancy up to three confers an increased risk for breast cancer prior to age 40 years in *BRCA1/2* carriers (Jernström et al., 1999) and that the *IGF1*-19/-19 genotype in combination with multiparity predisposes to an early age at diagnosis among sporadic breast cancer patients (Bågeman et al., 2007). A loss of function of *BRCA1* in combination with the *IGF1*-19/-19 genotype may accordingly elevate the risk for developing early onset breast cancer in multiparous women.

In Jewish *BRCA1* and *BRCA2* mutation carriers, the breast cancer incidence by age 50 years has increased dramatically during the last century: among *BRCA1/2* mutation carriers born before 1940 the incidence was 24%, while the incidence was 67% among those born after 1940 (King et al., 2003). OC use was not discussed as a risk factor for early onset breast cancer in that study. We reported that incident breast cancers were more common in high-risk women who used OCs with the *IGF1*-19/-19 genotype (Jernström et al., 2005). A statistically significant interaction between OC use and the *IGF1*-19/-19 genotype was subsequently reported in a large study of sporadic breast cancers from Long Island, USA (Cleveland et al., 2006). In that study, the *IGF1*-19/-19 genotype conferred protection against early-onset breast cancer in women who had

never used OCs, while the risk of early-onset breast cancer was high in ever OC users with the same *IGF1* genotype (Cleveland et al., 2006). If it is confirmed that the *IGF1*-19/-19 genotype is more common among *BRCA1* carriers than among non-carriers it may be useful to test *IGF1* genotype in addition to *BRCA1* before prescribing OCs to young *BRCA1* carriers, because this genetic combination may be especially detrimental to breast tissue in combination with OC use. Since we lacked data on ever OC use for 45 % of the women in the new study population, and start ages and durations were missing for even more women, we were not able to properly address this question.

In conclusion, we confirmed our previous finding that the *IGF1*-19/-19 genotype is more common among *BRCA1* mutation carriers than non-carriers from *BRCA1* families. We have genotyped all tested women from *BRCA1* families in the South Swedish Health Care Region where the available DNA was of sufficient quality for microsatellite analyses. Our finding now needs to be confirmed in an independent population. The *IGF1*-19/-19 genotype in combination with OC use or multiparity confers an increased risk for early onset breast cancer, both in high-risk women and in women from the general population. A high proportion of *BRCA1* mutation carriers have this *IGF1* genotype. If our finding is confirmed, it may be useful to test *IGF1* genotype in *BRCA1* carriers to improve genetic counselling with respect to OC use, multiparity and appropriate age for initiation of breast cancer screening.

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Table 1. Characteristics of the subjects of the new study population and the new study population combined with women from our previous study published in BJC (Jernström *et al*., 2005). Only a subset of women in the new study had completed the questionnaire.

	New study population				New and previous study populations combined			
	BRCA1 carriers n=106		Non-carriers n=105		*BRCA1* carriers n=129		Non-carriers n=154	
	Median	(Range)	Median	(Range)	Median	(Range)	Median	(Range)
Year of birth	1951 n=106	(1916-1983)	1954 n=105	(1911-1983)	1954 n=129	(1916-1983)	1960 n=154	(1911-1983)
Age	49 n=104	(18-80)	46 n=104	(20-90)	45 n=127	(18-80)	39 n=153	(17-90)
Height*	167 n=49	(152-178)	165 n=65	(98*-179)	167 n=72	(152-181)	167 n=114	(98*-180)
Weight	69.5 n=52	(53-114)	65.5 n=66	(45-110)	69 n=75	(51-114)	66.2 n=115	(45-118)
Birth weight	3750 n=21	(1510-4670)	3395 n=42	(2100-5000)	3450 n=42	(1510-4670)	3350 n=89	(2100-5000)
Nulliparous, %	19 n=53		20 n=66		25 n=76		30 n=115	
Age at first full term pregnancy #	25 n=43	(17-33)	24 n=53	(17-35)	25 n=57	(17-34)	24 n=80	(16-36)
Ever use of oral Contraceptives, %	58 n=52		63 n=63		71 n=75		75 n=211	
Ever smoker, %	60 n=50		65 n=66		59 n=73		57 n=115	

* One woman was only 98 cm tall due to medical condition.   # In parous women

Table II. *IGF1* allele frequency distribution of polymorphic genetic variants by *BRCA1* mutation status for the new material. Please note that each woman carries two alleles. The percentage does not always total one hundred because the individual percentages were rounded off.

IGF1 genotype [CA]$_n$	All n = 211 No.	(%)	*BRCA1* carrier n = 106 No.	(%)	Non-carrier n = 105 No.	(%)
12	1	(0.2)	0	(0.0)	1	(0
17	1	(0.2)	0	(0.0)	1	(0.5)
18	22	(5.2)	14	(6.6)	8	(3.8)
19	271	(64.2)	131	(61.8)	140	(66.7)
20	93	(22.0)	48	(22.6)	45	(21.4)
21	23	(5.5)	14	(6.6)	9	(4.3)
22	10	(2.4)	4	(1.9)	6	(2.9)
23	1	(0.2)	1	(0.5)	0	(0.0)
IGF1 19-repeat allele						
Absent	20	(9.5)	15	(14.2)	5	(4.8)
Present	191	(90.5)	91	(85.8)	100	(95.2)

Absence of the 19 CA-repeat allele in *BRCA1* carriers OR 3.30 (95% CI 1.11-9.78, *P* = 0.03).

Table III. *IGF1* allele frequency distribution of polymorphic genetic variants by *BRCA1* mutation status for all *IGF1* genotyped women, with addition of the 72 women with known *BRCA1* mutation status from our previously published study of healthy young women from high-risk families (Jernström *et al*., 2005). Please note that each woman carries two alleles. The percentage does not always total one hundred because the individual percentages were rounded off.

IGF1 genotype [CA]$_n$	All n=283		*BRCA1* carrier n=129		Non-carrier n=154	
	No.	(%)	No.	(%)	No.	(%)
12	2	(0.4)	1	(0.4)	1	(0.3)
17	2	(0.4)	1	(0.4)	1	(0.3)
18	29	(5.1)	16	(6.2)	13	(4.2)
19	356	(62.9)	155	(60.0)	201	(65.3)
20	124	(21.9)	56	(21.7)	68	(22.1)
21	40	(7.1)	23	(8.9)	17	(5.5)
22	12	(2.1)	5	(1.9)	7	(2.3)
23	1	(0.2)	1	(0.4)	0	(0.0)

IGF1 19-repeat allele

	No.	(%)	No.	(%)	No.	(%)
Absent	33	(11.7)	21	(16.3)	12	(7.8)
Present	250	(88.3)	108	(83.7)	142	(92.2)

Absence of the 19 CA-repeat allele in *BRCA1* carriers OR 2.3 (95% CI 1.08-4.91, *P* = 0.03).