Research article

Genetic variation in insulin-like growth factor signaling genes and breast cancer risk among BRCA1 and BRCA2 carriers

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Abstract

Introduction Women who carry mutations in BRCA1 and BRCA2 have a substantially increased risk of developing breast cancer as compared with the general population. However, risk estimates range from 20 to 80%, suggesting the presence of genetic and/or environmental risk modifiers. Based on extensive in vivo and in vitro studies, one important pathway for breast cancer pathogenesis may be the insulin-like growth factor (IGF) signaling pathway, which regulates both cellular proliferation and apoptosis. BRCA1 has been shown to directly interact with IGF signaling such that variants in this pathway may modify risk of cancer in women carrying BRCA mutations. In this study, we investigate the association of variants in genes involved in IGF signaling and risk of breast cancer in women who carry deleterious BRCA1 and BRCA2 mutations.

Methods A cohort of 1,665 adult, female mutation carriers, including 1,122 BRCA1 carriers (433 cases) and 543 BRCA2 carriers (238 cases) were genotyped for SNPs in IGF1, IGF1 receptor (IGF1R), IGF1 binding protein (IGFBP1), IGF2 binding protein (IGFBP2),
IGFBP5), and IGF receptor substrate 1 (IRS1). Cox proportional hazards regression was used to model time from birth to diagnosis of breast cancer for BRCA1 and BRCA2 carriers separately. For linkage disequilibrium (LD) blocks with multiple SNPs, an additive genetic model was assumed; and for single SNP analyses, no additivity assumptions were made.

**Results** Among BRCA1 carriers, significant associations were found between risk of breast cancer and LD blocks in IGFR1 (global \( P = 0.011 \) for LD block 2 and global \( P = 0.012 \) for LD block 11). Among BRCA2 carriers, an LD block in IGFBP2 (global \( P = 0.0145 \)) was found to be associated with the time to breast cancer diagnosis. No significant LD block associations were found for the other investigated genes among BRCA1 and BRCA2 carriers.

**Conclusions** This is the first study to investigate the role of genetic variation in IGF signaling and breast cancer risk in women carrying deleterious mutations in BRCA1 and BRCA2.

Among BRCA1 carriers, significant associations were found between risk of breast cancer and LD blocks in IGFR1 (global \( P = 0.011 \) for LD block 2 and global \( P = 0.012 \) for LD block 11). Among BRCA2 carriers, an LD block in IGFBP2 (global \( P = 0.0145 \)) was found to be associated with the time to breast cancer diagnosis. No significant LD block associations were found for the other investigated genes among BRCA1 and BRCA2 carriers.

**Materials and methods**

**Participants**

Women with germline, deleterious mutations in BRCA1 and BRCA2 were identified in 14 centers in the US, one center in Canada, and one center in Austria, which recruited from the Medical University in Vienna, Creighton University, Dana Farber, Fox Chase Cancer Center, Georgetown University, the Mayo Clinic, Medical University of Vienna, North Shore University Health System in Chicago, University of California, Los Angeles, University of California, Irvine, University of Chicago, University of Pennsylvania, and Women’s College Hospital. The majority of subjects were recruited from the Medical University in Vienna, Creighton University, Dana Farber, Fox Chase Cancer Center, and the University of California, Irvine (previously at the University of Utah). All centers are part of the Modifiers and Genetics in Cancer consortium. All participants were enrolled under Institutional Review Boards or ethics committee approval at each participating site.

Women were participating in research studies or were either physician- or self-referred to risk evaluation clinics for genetic testing, generally because of a strong family history of breast cancer and/or ovarian cancer. The current study is composed of a total of 1,665 adult, female mutation carriers, including...
The 47 SNPs had been selected and genotyped in a previous SNP genotyping study of African-American women. Briefly, a minimal set of informative SNPs (tagging SNPs) had been chosen across each gene to mark the common genetic variation and to minimize the genotyping costs. Tagging SNP sets were selected using the TagSNPs program [21] from genotype data, downloaded directly from the National Institute of Environmental Health Sciences Environmental Genome Project [22]. For the data available at the time, it was not possible to select tagging SNPs for just a Caucasian population.

Genotyping was performed by the MGB Taqman probe Assay from Applied Biosystems Inc. (Foster City, CA USA) or the MGB Eclipse™ probe assay from Nanogen Inc. (San Diego, CA USA) for all SNPs. Primer and probe sequences are available from the authors on request.

Specifically, for the MGB Taqman probe assays, the reaction mix in a final volume of 5 μl included 10 ng genomic DNA, 4.5 pmol each primer, 1.25 pmol each probe, 1 × PCR reaction buffer (Qiagen, Gaithersburg, MD USA), 2 × Q solution (Qiagen), 500 pmol dNTP, and 0.15 units Qiagen DNA polymerase. PCR cycling included 55 cycles of a two-step PCR (95°C for 10 seconds, 58°C for 20 seconds and 72°C for 20 seconds) after an initial 2 minutes at 95°C.

After completion of PCR, endpoint dissociation melting curves were generated on the ABI PRISM 7900 HT instrument by monitoring the fluorescence while heating the reactions from 30°C to 80°C at a 10% rate. An EclipseMeltMacro_v2.328 program (Nanogen Inc., San Diego, CA USA) was employed to assign the genotype from the dissociation curve data. Duplicates of 22 DNA samples and water controls were genotyped for quality control. The laboratory technician was blinded as to whether samples were duplicates, cases, or controls. The order of the DNA samples on 384-well plates was randomized in order to ensure balance in study conditions across covariates. Genotyping call rates ranged from 95% to 99% and duplicate concordance rates were higher than 99%.

Determination of linkage disequilibrium blocks
We recalculated the linkage disequilibrium (LD) blocks for this study, primarily because we wanted the LD groups to reflect this predominantly non-Hispanic Caucasian cohort rather than the mixed sample from which the tagging SNPs were originally identified. SNPs were grouped according to their adjacent pairwise LD coefficient (D'). The coefficient was computed between all adjacent marker pairs within each candidate gene. In order to account for within-family correlation, multiple output [23] was used to estimate D'. In this case, a single member from each family was randomly sampled to create a single bootstrap sample, from which D' was computed. This process was repeated to obtain 200 bootstrap samples, yielding an empirical distribution of D'. An LD block was defined as a set of contiguous SNPs having D' values exceeding 0.90 between each contiguous pair of SNPs. The boundary of an LD block would be defined by a marker pair with D' ≤ 0.9. The LD blocks for the SNPs within each gene are shown in Additional data file 1.

Statistical analysis
Breast cancer rates were calculated as the observed number of breast cancers per total patient time at risk, and were standardized to the age distribution of the study cohort at the time of interview [24]. Subjects were considered at risk for breast cancer from birth until the first occurrence of breast cancer diagnosis, death, or loss to follow-up. In addition, subjects were censored in the event that they underwent a bilateral prophylactic surgery of the breasts more than 1 year preceding the diagnosis of breast cancer. Bilateral prophylactic surgery of the breasts occurring within 1 year of breast cancer was considered an event in order to avoid potential biases resulting from informative censoring.

Covariates that vary with time (ovarian cancer and prophylactic ovarian surgery) were treated as time dependent in the calculation of rates. A subject who was diagnosed with ovarian cancer therefore contributed time at risk in the non-ovarian cancer...
group prior to the diagnosis and then time at risk in the ovarian cancer group following the diagnosis. Because subjects were ascertained primarily from high-risk clinics, there was an over-sampling of cases. In order to account for potential bias in cumulative risk estimates due to nonrandom sampling from the general population, Kaplan-Meier estimates of the cumulative risk were calculated. A total of 1,122 women were included in the BRCA1 analysis, with 433 cases resulting in an incidence rate of 26.94 per 1,000 women per year (95% confidence interval: 19.79, 34.10). For the BRCA2 cohort, 543 women were included, with 238 cases for an incidence rate of 25.03 per 1,000 women per year (95% confidence interval: 18.71, 31.36).

Table 1

| Characteristic                              | BRCA1 |          |               | BRCA2 |          |               |
|---------------------------------------------|-------|----------|---------------|-------|----------|---------------|
|                                             | n     | Cases    | Incidence rate | n     | Cases    | Incidence rate |
| Total                                       | 1,122 | 433      | 26.94 (19.79, 34.10) | 543   | 238      | 25.03 (18.71, 31.36) |
| Raceb                                       |       |          |               |       |          |               |
| Caucasian (non-Jewish, non-Hispanic)        | 774   | 283      | 26.72 (19.58, 33.86) | 381   | 176      | 27.70 (20.63, 34.77) |
| African American                            | 29    | 14       | 39.37 (28.13, 50.61) | 13    | 6        | 25.28 (18.47, 32.09) |
| Jewish                                      | 245   | 98       | 24.08 (17.95, 30.22) | 119   | 43       | 17.39 (13.19, 21.60) |
| Caucasian Hispanic                          | 35    | 17       | 43.97 (30.46, 57.47) | 9     | 5        | 33.07 (22.50, 43.65) |
| Other                                       | 31    | 16       | 38.66 (28.28, 49.05) | 19    | 6        | 20.68 (15.61, 25.74) |
| Ovarian cancer                              |       |          |               |       |          |               |
| Yes                                         | 128   | 26       | 20.86 (11.70, 30.01) | 30    | 5        | 18.90 (8.84, 28.96) |
| No                                          | 994   | 407      | 27.78 (20.46, 35.10) | 513   | 233      | 25.43 (19.10, 31.77) |
| Prophylactic ovarian surgery                 |       |          |               |       |          |               |
| Yes before breast cancer                     | 282   | 44       | 24.88 (17.53, 32.23) | 108   | 18       | 27.64 (19.07, 36.21) |
| Yes after breast cancer                      | 167   | 167      | --             | 99    | 99       | --             |
| No bilateral prophylactic oophorectomy      | 671   | 221      | 28.07 (20.72, 35.42) | 336   | 121      | 25.29 (18.93, 31.64) |
| Clinic Site                                 |       |          |               |       |          |               |
| Medical University Vienna                   | 204   | 84       | 39.26 (29.31, 49.21) | 62    | 37       | 28.19 (20.83, 35.55) |
| Beth Israel                                 | 8     | 4        | 30.58 (22.39, 38.76) | 15    | 6        | 125.51 (35.71, 215.31) |
| Baylor University Medical Center -Dallas    | 14    | 10       | 69.46 (50.29, 88.63) | 1     | 1        | 14.59 (10.21, 18.97) |
| City of Hope                                | 56    | 25       | 43.30 (31.79, 54.81) | 28    | 17       | 54.17 (40.17, 68.18) |
| Creighton                                   | 155   | 65       | 28.49 (21.39, 35.59) | 40    | 23       | 55.87 (42.83, 68.91) |
| Dana Farber                                 | 88    | 41       | 36.22 (27.27, 45.18) | 32    | 11       | 27.20 (21.04, 33.37) |
| NorthShore University Health System         | 35    | 16       | 26.55 (19.62, 33.47) | 21    | 9        | 17.77 (13.57, 21.97) |
| Fox Chase Cancer Center                     | 40    | 10       | 14.41 (9.97, 18.86)  | 28    | 9        | 18.18 (13.32, 23.04) |
| Georgetown University                       | 42    | 13       | 21.35 (16.23, 26.47) | 16    | 3        | 21.64 (16.24, 27.05) |
| University of California, Los Angeles       | 43    | 18       | 36.95 (25.09, 48.81) | 17    | 7        | 13.62 (10.03, 17.21) |
| Mayo Clinic                                 | 60    | 17       | 18.57 (14.53, 22.61) | 31    | 10       | 30.38 (24.01, 36.75) |
| University of Texas Health Science Center at San Antonio | 35    | 17       | 40.18 (27.33, 53.02) | 32    | 13       | 11.67 (9.00, 14.34) |
| University of Chicago                       | 34    | 15       | 52.84 (36.66, 69.02) | 18    | 9        | 18.73 (14.47, 22.98) |
| University of Pennsylvania                  | 147   | 56       | 24.08 (17.37, 30.78) | 92    | 44       | 51.62 (43.58, 59.66) |
| University of Utahc                         | 115   | 30       | 14.85 (10.70, 19.00) | 87    | 27       | 27.54 (19.36, 35.72) |
| Women's College Hospital, Toronto           | 46    | 12       | 16.24 (11.72, 20.76) | 23    | 12       | 44.56 (33.11, 56.02) |

*aData presented as incidence per 1,000 women per year (95% confidence interval). Rates have been externally standardized to the age distribution of the study cohort at the time of genetic testing. bFive subjects missing race information. cNow at University of California, Irvine. dData presented as mean ± standard deviation.
A two-step approximation was used to compute posterior estimates of
regression models for analyzing case-control data and was
similar approach has previously been applied to logistic
weight the individual's contribution to the partial likelihood. A
their known genotype, and these probabilities were used to
the probability of all potential haplotypes for a subject given
uncertainty in haplotype analysis, we used a two-step approx-
gle SNP, however, a general genetic model making no additiv-
group for comparisons. When an LD block consisted of a sin-
multiple SNPs, an additive haplotype effect was assumed
probability of diagnosis for two individuals, differing only by a
tative risk or hazard ratio (HR) is then interpreted for each cov-
are weighted to account for oversampling of cases to controls [1].
probability of breast cancer diagnosis were computed using
Cox proportional hazards regression was used to model the
time from birth to diagnosis of breast cancer. In this model, the
hazard or instantaneous probability of breast cancer diagnosis is
modeled as a function of the predictor covariates. The rela-
tive risk or hazard ratio (HR) is then interpreted for each cov-
ariate as the proportionate change in the instantaneous
probability of diagnosis for two individuals, differing only by a
single unit of that covariate. When analyzing LD blocks with
multiple SNPs, an additive haplotype effect was assumed
where the most common haplotype was used as the referent
group for comparisons. When an LD block consisted of a sin-
gle SNP, however, a general genetic model making no additiv-
ity assumption was used. In order to account for phase
uncertainty in haplotype analysis, we used a two-step approx-
imation to the semiparametric maximum likelihood estimator of
Lin and Zeng [25]. Using this method, the expectation-maximi-
ization algorithm was used to compute posterior estimates of
the probability of all potential haplotypes for a subject given
their known genotype, and these probabilities were used to
weight the individual’s contribution to the partial likelihood. A
similar approach has previously been applied to logistic
regression models for analyzing case-control data and was
shown to provide robust inference for relatively common hap-
lootypes with little phase ambiguity [26]. In order to account for
hierarchical clustering at the individual level (multiple records
per individual were analyzed according to the number of
potential diplotypes consistent with the individual’s genotype)
and at the family level (matched controls were often selected
from the family of a case), the sandwich estimator of Lin and
Wei [27] was used in combination with multiple outputation
[23] to obtain robust variance estimates of haplotype
associations.

All estimates were adjusted for birth cohort (to account for
frequency matching of cases and controls), race/ethnicity, parity,
and region of center (North American (US) vs. European).
Ashkenazi Jewish individuals were considered a separate eth-
nicity because the carriers only had one of three founder
mutations. Parity, prophylactic oophorectomy, and ovarian cancer
status were treated as time-dependent covariates in the anal-
ysis, with these covariates updated at the time of childbirth.
Beyond adjustment for birth cohort, no additional weighting for
selection was employed. For LD blocks exhibiting significant
associations with the time to breast cancer diagnosis, second-
ary analyses of individual SNPs making up the LD block were
carried out. No significant departures from the proportional
hazards assumption were observed.

In total, the current analysis involves testing of 48 LD blocks,
which is likely to result in an inflation of the family-wise type I
error rate for the study if unadjusted critical values are used for
assessing LD block significance. Noting that this analysis rep-
resents a first-stage in identifying variants in the IGF pathway
that are associated with time to breast cancer diagnosis, we
sought to control the family-wise type error rate at 15% in
order to minimize the type II error rate, limiting the possibility of
ruling out potentially important LD blocks from future investiga-
tion. Simulation was used to estimate the family-wise type I
error rate, assuming a correlation of 0.75 across tests was
assumed. Based upon 100,000 simulations it was estimated
that an adjusted \( P \) value of 0.016 on any individual LD block
test would result in a family-wise type I error rate of 15% for
the study. An adjusted \( P < 0.016 \) was interpreted as a signifi-
cant association.

**Results**

The characteristics of the cases and the sites, and the
observed incidence rate (per 1,000 women per year) of breast
cancer diagnosis stratified by BRCA status are presented in
Table 1. The presented rates have been externally standard-
dized to the age distribution of the study cohort at the time of
genetic testing. The study included 1,222 BRCA1 carriers
(433 diagnosed with breast cancer) and 543 BRCA2 carriers
(238 diagnosed with breast cancer). The age-standardized

![Weighted Kaplan Meier Plot](image-url)

Kaplan-Meier estimates of the cumulative probability of breast cancer
diagnosis by BRCA status. Statistics in the lower portion of the plot
represent the number of patients at risk (cumulative number of diag-
noses) at each decade of life, ranging from 20 to 80 years. Estimates
are weighted to account for oversampling of cases to controls [1].
incidence rate of breast cancer diagnosis was estimated to be 26.94 per 1,000 per year in \textit{BRCA1} carriers (95% confidence interval (CI) = 19.79, 34.10) compared with 25.03 per 1,000 per year in \textit{BRCA2} carriers (95% CI = 18.71, 31.36). The majority of study subjects in both strata were White Caucasian (non-Jewish, non-Hispanic). Of the study subjects, 9.5% underwent bilateral prophylactic mastectomy (107/1,122 among \textit{BRCA1} carriers and 40/543 among \textit{BRCA2} carriers) and 39.4% underwent prophylactic bilateral salpingo-oophorectomy (449/1,122 among \textit{BRCA1} carriers and 207/543 among \textit{BRCA2} carriers). Figure 1 shows the estimated cumulative probabilities of breast cancer diagnosis in \textit{BRCA1} and \textit{BRCA2} carriers observed in the study. The median age at diagnosis was estimated to be 57.0 years (95% CI = 54.1, 62.2) among \textit{BRCA1} carriers and was 70.5 years (95% CI = 67.7, INF) among \textit{BRCA2} carriers.

**IGF binding proteins IGFBP1, IGFBP2, and IGFBP5**

Figure 2 presents the estimated HR for time to diagnosis by LD block within each of the IGFBPs, and the BRCA status after adjustment for covariates (described in Materials and methods). For \textit{BRCA1} carriers, no significant associations were observed for the three IGF binding genes. Among \textit{BRCA2} carriers, one LD block in IGFBP2 showed significance in the hazard for diagnosis. For IGFBP2 LD block 2 (defined by a single SNP rs9341134), women with at least one variant allele were estimated to experience a 41% lower risk of diagnosis when compared with women with no variant allele.
Haplotype presence for insulin-like growth factor receptor substrate 1 and insulin-like growth factor 1. Estimated hazard ratios (Est HR) associated with haplotype presence for (a) insulin-like growth factor receptor substrate 1 (IRS1) and (b) insulin-like growth factor 1 (IGF1). Linkage blocks were defined as in Figure 2 (pairwise linkage disequilibrium coefficient $D' \geq 0.90$). Estimates were stratified by BRCA status (left column, BRCA1; right column, BRCA2) and adjusted for birth cohort and ethnicity as well as first pregnancy, prophylactic oophorectomy, and diagnosis of ovarian cancer as time-dependent covariates. LD Grp, linkage disequilibrium group; Geno/Haplo, genotype/haplotype; Freq, frequency.

alleles (HR = 0.59; 95% CI = 0.39, 0.90; unadjusted global $P = 0.0145$). For IGFBP5 LD block 2 (defined by a single SNP rs2241193), women with at least one variant allele were estimated to experience a 29% lower risk of diagnosis when compared with women with no variant alleles (HR = 0.71; 95% CI = 0.53, 0.96; unadjusted global $P = 0.0242$).

**Insulin-like growth factor receptor substrate 1 and insulin-like growth factor 1**

Estimated HRs for the haplotypes of IRS1 are shown in Figure 3a. Among BRCA1 carriers, the global LD block test for IRS1 was not significant (unadjusted global $P = 0.0551$). Relative to the referent haplotype, however, individuals with haplotypes homozygous for the common variant (excluding haplotypes 001 and 100) were estimated to have a 43% (CI = 1.06, 1.95; $P = 0.02$) higher risk of breast cancer diagnosis.

We then investigated the HRs for the three SNPs within the LD block to determine whether the observed haplotype associations were attributable to particular SNPs (Table 2). For SNPs rs13306465 and rs1801123, individuals carrying at least one variant allele experienced a 44% (HR = 1.44; 95% CI = 1.07, 1.94; unadjusted $P = 0.0165$) and 37% (HR = 1.37; 95% CI = 1.11, 1.69; unadjusted $P = 0.0033$) higher risk of breast cancer relative to wild-type carriers, respectively. There was no individual association of the rs1801278 (G972R) SNP and risk. For the single IRS1 LD block, a similar, but nonsignificant HR of 1.52 (95% CI = 0.99, 2.32; unadjusted $P = 0.055$) was observed in BRCA2 carriers.

For IGF1, no significant associations were found for either BRCA1 or BRCA2 carriers (Figure 3b).

**Insulin-like growth factor-1 receptor**

Figure 4 shows HR estimates for the 12 LD blocks genotyped in IGF1R. For BRCA1 carriers, significant associations were found between LD block 2 (SNP rs2715415) and LD block 11 and the risk of breast cancer diagnosis (unadjusted global $P$ values corresponding to a test of homogeneity of risk within the LD blocks were 0.011 for LD block 2 and 0.012 for LD block 11). While qualitatively consistent associations were also observed among BRCA2 carriers, they were not significant. After investigation in BRCA1 carriers of the individual SNPs within LD block 11 (Table 2), the only SNP that was significantly associated with risk was rs8038415 - in which individuals homozygous for the variant allele were estimated to
experience a 40% higher risk of breast cancer diagnosis (unadjusted $P = 0.014$, with $P$ for trend = 0.015).

**Discussion**

The IGF pathway plays essential roles in regulating cell proliferation, differentiation, and apoptosis. It is a key factor in the development and progression of breast cancer, based on evidence from more than 1,100 published papers, ranging from in vivo and in vitro studies in humans and mice to epidemiologic studies (reviewed in [14-16]). This is the first study to investigate the role of genetic variants in IGF signaling as modifiers of breast cancer risk in women who carry deleterious mutations in \( BRCA1 \) and \( BRCA2 \). We investigated only a small number of the genes involved in IGF signaling. We found significant HRs associated with genetic variants in \( IGF1R \) and \( IRS1 \) in \( BRCA1 \) carriers, and in \( IGFBP2 \) in \( BRCA2 \) carriers. No other significant associations in the studied genes were identified.

There have been a limited number of epidemiologic studies of the association of sporadic breast cancer risk and genetic variation in genes in the IGF pathway. For IGF1, the primary ligand for IGF1R, there have been inconsistent reports of associations with breast cancer risk with reports showing significant associations [28,29] and no associations [30-35]. The inconsistent results may be due to differences in genetic variants examined in the genes and/or in study design (for example, restriction to postmenopausal or premenopausal breast cancers). Several studies of SNPs in \( IGFBP1 \) reported no association with breast cancer, similar to what we observed for \( BRCA1 \) and \( BRCA2 \) mutation carriers [28,35,36]. The IGFBPs serve as growth modulators, both independently and as regulators of IGFs [37-39]. IGFBP5 and IGFBP2 are overexpressed in breast cancer tissues [40,41], and are involved in apoptosis [42-44]. In a study of African Americans, with replication in Nigerians, we reported significant associations of SNPs within the \( IGFBP2 \) to \( IGFBP5 \) region and the risk of breast cancer [45]. These two genes are in a tail-to-tail configuration separated by only 10 kb on chromosome 2q, so it is possible the same underlying causal variation results in association with both genes. In the present study, we report a significant association of \( IGFBP2 \) SNP rs9341134, also observed in the previous study [45], and marginally significant associations with variants in \( IGFBP5 \). Resequencing is needed to try to identify the actual causal variant. Another piece of evidence that this region may be associated with breast cancer is the association of SNP rs13387042 with a 1.2-fold increased risk in breast cancer, reported in a deCODE genome-wide association study [46] - with replication by the Cancer Genetic Markers of Susceptibility project (odds ratio = 1.2) [47], by the Breast Cancer Association Consortium (odds ratio = 1.14) [48], and by the Consortium of Investigators of Modifiers of \( BRCA1 \) and \( BRCA2 \) (HR = 1.14 and HR = 1.18 for \( BRCA1 \) and \( BRCA2 \) carriers, respectively) [49]. It is hypothesized that this SNP may act as a long-range regulatory element on expression of IGFBP2 or IGFBP5 [46].

Of the genes examined, only genetic variants in IGF1R and its adaptor protein IRS1 were associated with risk of breast cancer in \( BRCA1 \) carriers. IGF1R has both mitogenic and anti-apoptotic roles in tumor development via signaling through the
phosphatidylinositol-3-kinase and mitogen-activated protein
kinase pathways [50], with its adaptor protein IRS1 critical in
activating the downstream pathways. Both IGF1R overexpres-
sion and IRS1 overexpression have been associated with
breast cancer development, and IGF1R is overexpressed in a
majority of breast tumors [51]. Interestingly, BRCA1 directly
affects IGF1 signaling. In multiple experimental systems
including primary mammary tumors, cultured human cells, and
Brca1-deficient mice, Shukla and colleagues showed that
BRCA1 deficiency resulted in increased expression of IRS1,
IGF1R and IGFBP2, and increased levels of serum IGF1 [19].
In another study investigating IGF1R levels in breast tumors,
there were significantly higher levels of IGF1R in tumors from
BRCA1 mutation carriers as compared with noncarriers [20].

In a series of experiments co-transfecting cell lines with IGF1R
promoter constructs driving luciferase reporter genes, and a
BRCA1 expression vector, it was shown that BRCA1 sup-
pressed IGF1R promoter activity in a dose-dependent manner
[52], through preventing binding of Sp1 to the IGF1R pro-
moter, thus reducing transcription [52,53]. As demonstrated
using western blots, wild-type BRCA1 was able to induce a
large reduction in endogenous IGF1R levels [20]. In addition
to its interaction with the IGF1R, BRCA1 interacts directly
with the IRS1 promoter to inhibit its activity [19]. With induc-
tion of BRCA1, the authors observed a twofold and threefold
decrease of IRS1 mRNA and protein levels, respectively, as
well as a decrease in the phosphorylation level of AKT, a
downstream target of IGF1R and IRS1 [19].

Figure 4

IGF1R

Haplotype presence for insulin-like growth factor-1 receptor. Estimated hazard ratios (Est HR) associated with haplotype presence for insulin-like
growth factor-1 receptor (IGF1R). Linkage blocks were defined as in Figure 2 (pairwise linkage disequilibrium coefficient $D' \geq 0.90$). Estimates were
stratified by BRCA status (left column, BRCA1; right column, BRCA2) and adjusted for birth cohort and ethnicity as well as first pregnancy, prophyl-
lactic oophorectomy, and diagnosis of ovarian cancer as time-dependent covariates. LD Grp, linkage disequilibrium group; Geno/Haplo, genotype/
haplotype; Freq, frequency.
Based on these experiments, there is strong evidence that mutant forms of \textit{BRCA1} cause increased IGF1R activation, leading to a decrease in apoptosis and a concomitant increased survival of malignant cells, which then can proliferate. There is therefore a strong rationale for why genetic variation in IGF1R and IRS1 would be important in breast cancer risk. The present study is the first to investigate the role of genetic variation in IGF1R and breast cancer risk in women who carry deleterious mutations in \textit{BRCA1} and \textit{BRCA2}. While the study does provide an important first step in identifying potential genetic modifiers of risk among \textit{BRCA1} and \textit{BRCA2} carriers, it does suffer some limitations. First, although the IGF pathway was hypothesized \textit{a priori} as a source for potential modifiers, multiple LD blocks were considered for association testing and such testing could lead to inflation of the overall type I error rate for the study. With this said, we only studied a small number of the genes in IGF signaling that we deemed \textit{a priori} would potentially play a role in the time to diagnosis. Further, the goal of the current research was to generate hypotheses based upon the results from this well-defined set of genes, and it is our intention to further validate these results using an independent sample. As with all observational studies, there is the potential for selection bias and unmeasured confounding. We have, however, adjusted for those environmental factors that previous research has shown to most highly influence the risk of breast cancer diagnosis within this cohort, thus lowering the potential for unadjusted confounding.

We and others have investigated putative risk factors, and a number of published studies have implicated candidate genes (for example, \textit{AB1} in \textit{BRCA1}, \textit{RAD51} in \textit{BRCA2}) and SNPs in \textit{FGFR2}, \textit{MAP3K1}, \textit{TNRC9}, \textit{LSP1}, and \textit{2q35} previously identified from genome-wide association studies of breast cancer as modifiers of breast cancer or ovarian cancer penetrance in women who carry germline \textit{BRCA1} or \textit{BRCA2} mutations [9-13,49]. Our results suggest that variation in genes in IGF signaling also modify breast cancer penetrance in \textit{BRCA1} and \textit{BRCA2} carriers.

Conclusions
The present study was the first to investigate the role of genetic variation in IGF signaling and breast cancer risk in women carrying deleterious mutations in \textit{BRCA1} and \textit{BRCA2}. We identified significant associations for variants in \textit{IGF1R} and \textit{IRS1} for \textit{BRCA1} carriers and for variants in \textit{IGFBP2} for \textit{BRCA2} carriers. Given the known interaction of \textit{BRCA1} and IGF signaling, specifically the regulation of IRS1 and IGF1R by \textit{BRCA1}, further replication and identification of causal mechanisms are needed to validate and better understand these associations.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
SLN designed and conceived of this work, was responsible for the genotyping and collection of all data, interpreted the results, and drafted and wrote the manuscript. SB was responsible for analysis of the data, interpreted the results, prepared tables and figures, and drafted and edited the manuscript. YCD was responsible for performing and overseeing the genotyping and quality control of the genotyping, and edited the manuscript. CFS, GP, HTL, KLN, TRR, JEG, FC, JW, SAN, PAG, MBD, AG, CI, OIO, GT, WSR, NT, and JLB all provided the \textit{BRCA1} and \textit{BRCA2} mutation carriers, including samples and data, and reviewed and edited the manuscript. DLG was responsible for developing the statistical analysis and overseeing the programming and analysis of SB, interpreted the results, and drafted and wrote the manuscript. All authors read and approved the final manuscript.

Additional files
The following Additional files are available online:

Additional file 1
Word file containing a table that lists the LD blocks for the SNPs within each gene and the minor allele frequencies (MAF) for each SNP
See http://www.biomedcentral.com/content/supplementary/bcr2414-S1.docx

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