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AURKA F31I Polymorphism and Breast Cancer Risk in *BRCA1* and *BRCA2* Mutation Carriers: A CIMBA study

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Abstract

The AURKA oncogene is associated with abnormal chromosome segregation and aneuploidy and predisposition to cancer. Amplification of AURKA has been detected at higher frequency in tumors from BRCA1 and BRCA2 mutation carriers than in sporadic breast tumors, suggesting that overexpression of AURKA and inactivation of BRCA1 and BRCA2 co-operate during tumor development and progression. The F31I polymorphism in AURKA has been associated with breast cancer risk in the homozygous state in prior studies. We evaluated whether the AURKA F31I polymorphism modifies breast cancer risk in BRCA1 and BRCA2 mutation carriers from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). CIMBA was established to provide sufficient statistical power through increased numbers of mutation carriers to identify polymorphisms that act as modifiers of cancer risk and can refine breast cancer risk estimates in BRCA1 and BRCA2 mutation carriers. A total of 4935 BRCA1 and 2241 BRCA2 mutation carriers and 11 individuals carrying both BRCA1 and BRCA2 mutations were genotyped for F31I. Overall, homozygosity for the 31I allele was not significantly associated with breast cancer risk in BRCA1 and BRCA2 carriers combined (HR = 0.91; 95% CI 0.77-1.06). Similarly, no significant association was seen in BRCA1 (HR = 0.90; 95% CI 0.75-1.08) or BRCA2 carriers (HR = 0.93; 95% CI 0.67-1.29) or when assessing the modifying effects of either bilateral prophylactic oophorectomy or menopausal status of BRCA1 and BRCA2 carriers. In summary, the F31I polymorphism in AURKA is not associated with a modified risk of breast cancer in BRCA1 and BRCA2 carriers.

Keywords

BRCA1; *BRCA2*; *AURKA*; CIMBA; Breast cancer risk

Introduction

The *AURORA-A/AURKA/BTAK/STK15* gene encodes a serine/threonine kinase that regulates mitotic chromosome segregation. *AURKA* is amplified and overexpressed in breast and other tumors and is associated with centrosome amplification, failure of cytokinesis, and aneuploidy. Genetic mapping studies in mouse models suggest that *AURKA* is a genetic modifier of cancer risk (1). In addition, mouse models of *AURKA* exhibit infrequent mammary gland tumor formation, but display synergy in tumor formation when combined with overexpressed oncogenes or disrupted tumor suppressors, suggesting that *AURKA* is a low risk cancer susceptibility gene (2).

Further evidence for a role of *AURKA* in breast cancer comes from obervations that homozygosity for a F31I polymorphism in *AURKA* is associated with an increased risk for breast cancer. In a study of incident breast cancer cases (n = 941) and age-matched population controls (n=830), Egan et al. (3) found that the breast cancer risk for Ile/Ile homozygotes were at increased risk for breast cancer (OR = 1.54; 95% CI: 0.96-2.47), although this finding was not significant. Sun et al. (4) observed that the Ile encoding allele is the common allele in the Chinese population whereas the Phe encoding allele is more common in Caucasian populations (4). In addition, an association between Ile/Ile homozygotes and ER negative breast carcinomas (OR = 2.56; 95% CI: 1.24-5.26) was detected. Lo et al. reported a significant association between *AURKA* haplotypes and breast cancer risk (5). Ewart-Toland et al. also found an increase in cancer risk for the *Ile/Ile* homozygotes (OR = 1.35, 95% CI: 1.12–1.64; $p = 0.002$) in a meta-analysis of data from four case–control breast cancer populations (6). Furthermore, post-menopausal women homozygous for the F31I and I57V alleles of *AURKA* in a case-control study nested within the Nurses' Health Study prospective cohort had an increased risk of invasive breast cancer (OR 1.63, 95% CI 1.08–2.45) (7). In contrast, Dai et al. did not observe a significant association with breast cancer risk for Ile/Ile homozygotes (OR = 1.2; 95% CI, 0.9-1.6) in a population based case-control series of Han Chinese (8), and Fletcher et al. (9) found no association between Ile/Ile homozygotes and risk of bilateral breast cancer (OR = 0.63, 95% CI 0.34-1.13). Importantly, the F31I variant has been shown to alter the activity of the Aurora box-1 of the AURKA protein, resulting in disruption of p53 binding and a decreased rate of degradation of AURKA. The stabilized AURKA may lead to centrosome amplification and failure of cytokinesis, increased chromosomal instability and aneuploidy, and promotion of tumor formation (1).

Mutations in *BRCA1* and *BRCA2* are correlated with aberrant duplication of the centrosome leading to centrosome amplification, chromosome mis-segregation, and aneuploidy (10-12). Amplification of *AURKA* has also been detected at much higher frequency in tumors from *BRCA1* and *BRCA2* mutation carriers than in sporadic breast tumors, suggesting that over-expression of AURKA and inactivation of BRCA1 and BRCA2 co-operate during tumor development and/or progression. Based on these data, we hypothesized that the F31I polymorphism modifies the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers. To address this hypothesis *AURKA* F31I was genotyped on *BRCA1* and *BRCA2* deleterious mutation carriers from 16 clinic and population-based research studies and multi-center consortia participating in the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA) and the association of F31I with breast cancer risk was assessed.

Materials and Methods

Subjects

BRCA1 and *BRCA2* mutation carriers were identified through 16 clinic and population-based research studies and multi-center consortia participating in the CIMBA. This international consortium was established in 2005 by a group of investigators interested in identifying

modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers that could be used to refine cancer risk estimates. Recruitment of mutation carriers for this and other CIMBA studies was approved by institutional review boards or ethics committees at all sites. *BRCA1* and *BRCA2* mutation carriers were defined as carriers of frameshifting small deletions and insertions, nonsense mutations, splice site mutations verified *in vitro* and large genomic rearrangements that result in a premature stop codon in either *BRCA1* or *BRCA2*. These mutations were identified by a variety of screening techniques and were sequence verified. As the K3326X variant in exon 27 is not associated with high-risk of breast cancer this and other mutations causing stop codons in exon 27 were excluded. Missense mutations that have been classified as pathogenic by multifactorial likelihood approaches were included in the deleterious category (12-14), while carriers of all other missense and intronic mutations in *BRCA1* and *BRCA2* were excluded from the study. Phenotypic data for mutation carriers were provided by each contributing center. Data were collected on year of birth, mutation description, ethnicity, country of residence, age at last follow-up, ages at breast and ovarian cancer diagnosis, age at bilateral prophylactic mastectomy, age at bilateral prophylactic oophorectomy and status and age at menopause. These and other available epidemiological data obtained from risk factor questionnaires and/or medical records were uniformly coded and stored in a centralized CIMBA database.

Genotyping

The F31I polymorphism (rs2273535) of *AURKA* was genotyped by 13 groups by the 5′ nuclease assay (TaqMan) on an ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). PCR primers were F primer: 5′-CTGGCCACTATTTACAGGTAATGGA-3′ and R primer: 5′-TGGAGGTCCAAAACGTGTTCTC-3′. Probes were VIC-ACTCAGCAATTTCCTT and FAM-CTCAGCAAATTCCTT. The annealing temperature was 60°C. Lund investigators used an alternative R primer: CATCTTTTGCTTTCATGAATGCCAG and performed the 5′ nuclease assay on a RotorGene (Corbett Research, Australia). INHERIT investigators directly sequenced the polymorphism using the following primers: Forward:5′-GGGTGAGGAATTGGAGGGGAT-3′;Reverse: 5′-GGACACCAATTTATGCTGTGTCCT-3′. Genotyping for the HEBCS was done by Amplifluor(tm) fluorescent genotyping (KBiosciences, Cambridge, UK, KBioscience [http://www.kbioscience.co.uk]). Genotyping for the DKFZ and Polish studies was performed by fragment analysis. DNA fragments containing the polymorphism were amplified using forward primer 5′-AGTTGGAGGTCCAAAACGTG-3′ and Cy5-labeled reverse primer 5′-CGCTGGGAAGTATTTGAAGG-3′, digested with 2.5U XapI (Fermentas, St. Leon-Rot, Germany), separated on 3% agarose gel (Polish samples) or by capillary gel electrophoresis (German samples) on a CEQ 8000 DNA Analysis System (Beckmann, Krefeld, Germany) and sized relative to CEQ DNA Size Standard-400 in each well. Allele sizes were 114 bp for the T allele and 78 bp for the A allele.

Statistical methods

Hazard ratios were modeled using Cox proportional hazards regression analysis, with breast cancer as the outcome and age as the time variable (15). We corrected for possible ascertainment bias using a weighted cohort approach (16). Briefly, this involves assigning weights to the mutation-carrying subjects such that the reweighted incidence rates observed in the study sample are consistent with the age-dependent penetrances for breast cancer onset established in carriers of inactivating mutations in BRCA1 and BRCA2. Subjects were followed from birth until the earliest occurrence of breast cancer (3884), bilateral prophylactic mastectomy (232), ovarian cancer (643), age 80 (97) or age at last contact (2331). Subjects were censored at age 80 because population-based incidence rates for older mutation carriers are unreliable, and accurate sampling weights cannot be assigned. Carriers with both *BRCA1* and *BRCA2* mutations were included once in overall analyses and were also included in each

of the *BRCA1* and *BRCA2* gene specific analyses. The number of subjects in each family varied from one to 33, with 75% of families represented by a single individual. Since the exact relationships among the family members were not available we accounted for the non-independence of observations within families using a robust variance estimate (17). Primary analyses modeled *AURKA* as a recessive effect, comparing those with two copies of the minor allele to those with less than two copies. Secondary analyses examined associations using a two degree-of-freedom general model, simultaneously comparing subjects with one copy or with two copies of the minor allele to the subjects with zero copies.

Overall analyses were carried out for all subjects, regardless of whether they carried a mutation in *BRCA1* or *BRCA2* or both. All analyses accounted for birth cohort and country of residence by including them as stratification variables in the Cox regression. The overall analysis also accounted for study site and mutation status. Additional analyses were conducted to obtain risk estimates for individuals with different characteristics, as defined by gene status, menopausal status, oophorectomy status and study site. Gene-specific results accounted for study site along with birth cohort and country of residence by use of stratification variables. Site-specific results accounted for mutation status, birth cohort and country of residence. Menopausal status and oophorectomy status were modeled as time-dependent covariates and results accounted for group status and mutation status. In secondary analyses the influence of benign prophylactic oophorectomy and menopausal status on associations between the Ile/Ile genotype and breast cancer risk were also evaluated. As these covariates did not confound the observed associations the associations reported in Table 2 are not adjusted for these variables.

Among those who provided ethnicity information, 97% were Caucasian, 2% were Ashkenazi Jewish, and the remaining 1% were “other”. Those who did not provide ethnicity information were grouped in a separate “missing” category for analysis purposes. Ethnicity was initially included as an additional stratification variable but was subsequently excluded because of the absence of any effect on the results. We assessed the possible heterogeneity of risk ratios across study site using standard tests of interaction. A sensitivity analysis assessing the impact of possible survival bias was conducted by excluding cases ascertained more than three years post-diagnosis. All statistical tests were two-sided, and all analyses were carried out using the SAS (SAS Institute Inc., Cary, NC) and S-Plus (Insightful, Seattle, WA) software systems.

Results

A total of 4935 female *BRCA1*, 2241 female *BRCA2* deleterious mutation carriers and 11 individuals carrying both *BRCA1* and *BRCA2* mutations were included in this study. Of these 7187 mutation carriers, 3884 had a diagnosis of breast cancer at the end of follow up and 3303 were censored as unaffected at a mean age of 43.4 years. The distribution of *BRCA1* and *BRCA2* carriers by study site, gene and cancer status is shown in Table 1. To avoid overlap between studies we compared carriers by country of origin, year of birth, mutation and reported ages. Duplication of samples between MAYO and MAGIC and between GEMO and MAGIC was detected. In both instances the duplicated samples were excluded from the MAGIC dataset.

The distribution of the *AURKA* F31I genotypes is shown in Table 2. Of the 363 (5%) carriers homozygous for the Ile encoding allele, 188 were affected with breast cancer. The frequency of the recessive Ile/Ile encoding genotype in the 16 groups varied between 3% and 8%, which is similar to estimates from other populations (6). There was no difference in the frequency of the Ile/Ile recessive genotype across genotyping platforms (p=0.33). Similarly, the study sites with the highest Ile/Ile frequencies did not have ethnic mixtures significantly different to the other study sites. The F31I polymorphism did not deviate significantly from Hardy-Weinberg equilibrium (p=0.07) among all 7187 affected and unaffected carriers.

The estimated risk of breast cancer associated with the recessive genotype for F31I in *BRCA1* and *BRCA2* carriers using a weighted Cox proportional hazards model is shown in Table 2. While there was a suggestion of a protective effect (HR = 0.91; 95%CI 0.77-1.06) overall, the result was not statistically significant. Similarly, no association with risk was observed for individual participating centers, other than for two centers (Ontario and HEBCS) that contributed small numbers of carriers to the study (Table 2). A test for heterogeneity across study site was not significant (p=0.06). In an effort to account for the trend towards heterogeneity, we investigated the influence of the three sites that were significantly different from the other sites (MOD-SQUAD (p=0.02), GEMO (p=0.01), DKFZ (p=0.03)) on the overall effect. Exclusion of each site in turn did not substantially alter the overall hazard ratio or the significance of the association.

Because BRCA1 is phosphorylated by AURKA (18) we evaluated whether the Ile/Ile genotype was associated with risk of breast cancer in *BRCA1* or *BRCA2* carriers. No significant association with risk was detected for either *BRCA1* (HR = 0.90; 95%CI 0.75-1.08) or *BRCA2* carriers (HR = 0.93; 95%CI 0.67-1.29) (Table 2). As other studies have reported an association between the recessive Ile/Ile encoding genotype and postmenopausal status in non-carriers (3,7), we considered the influence of menopausal status of carriers on breast cancer risk. At the end of follow-up, 4201 carriers were pre-menopausal and 2986 were post-menopausal. No significant association with risk was detected (Table 2). Because prophylactic oophorectomy substantially reduces the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers (19), we also evaluated the influence of prophylactic oophorectomy status. A total of 707 individuals reported undergoing prophylactic oophorectomy, 4298 reported no history of oophorectomy, while 2182 (30%) provided no data at last follow up. Associations with breast cancer risk by category of prophylactic oophorectomy did not differ markedly from the overall results. Secondary analyses using a two degree-of-freedom general model also failed to detect a significant association for either a single copy (p=0.97) or two copies (p=0.24) of the F31I polymorphism compared to no copies.

In an effort to account for possible survival bias and the inclusion of prevalent cases in the collection of *BRCA1* and *BRCA2* carriers, we repeated our analysis after excluding cases diagnosed more than three years prior to the date of ascertainment. For this analysis we excluded records where an age at interview was not provided. Overall, the mean difference between age of diagnosis and age at interview for the 3422 cases with available data was 8.7 years. Of these 1,322 (38.6%) cases had been diagnosed less than three years prior to the date of ascertainment. When excluding prevalent cases no association between the Ile/Ile genotype and breast cancer risk was observed, and the risk estimates were similar to those obtained when using both prevalent and incident cases (Table 2).

Discussion

Overall, no evidence of a significant association between homozygosity for the F31I *AURKA* polymorphism and breast cancer risk in *BRCA1* and *BRCA2* mutation carriers in combination or alone was observed. These results were somewhat unexpected given the known functional relationship between AURKA and BRCA1 (18), the known influence of F31I on AURKA protein stability (1) and the significant associations with cancer risk reported in several studies of unselected breast cancer cases and controls. While the variant does not appear to modify predisposition to cancer in this combined group of mutation carriers, the possibility remains that the Ile/Ile genotype influences tumor progression or clinical outcome or modifies cancer risk in conjunction with other risk factors. The suggestion of a modestly protective effect of the Ile/Ile genotype in this study particularly when restricting the study to incident cases supports this possibility. Interestingly, a study of bilateral breast cancer cases also identified a non-significant protective effect for the Ile/Ile genotype (9). This common protective effect

among individuals at higher risk of breast cancer in the Caucasian population suggests that homozygosity for the F31I polymorphism may reduce cancer risk in high-risk groups while possibly increasing risk in the general population. Additional studies of other high-risk populations and the combined effects of other risk factors are needed to further evaluate these possibilities.

In this study we accounted for the effects of both bilateral prophylactic oophorectomy and menopausal status effects by treating these factors as time dependent variables in the analysis. As bilateral prophylactic oophorectomy is known to reduce breast cancer risk by approximately 50% in *BRCA1* and *BRCA2* mutation carriers (19), we chose to account for the remaining risk of cancer in women undergoing prophylactic oophorectomy by assessing it as an additional time-varying covariate rather than by censoring the follow-up of the women at the time they underwent this procedure. In addition, we performed a sensitivity analysis in order to assess the potential for survival bias in our analyses by restricting the study to women more likely to have incident cases of breast cancer. While no change in the significance of the results was observed following this approach, it is important to evaluate this possibility in any study, whether single site or multicenter, of individuals at significantly elevated risk of cancer.

This report represents the largest association study conducted to date in *BRCA1* and *BRCA2* carriers. It also is the first report from CIMBA, an international consortium established in order to provide sufficient statistical power to test candidate SNPs as modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers and to refine breast cancer risk prediction in this population. The operating principles of CIMBA are: 1) CIMBA is open to any group that can contribute genotype and phenotype information on at least 92 *BRCA1* and/or *BRCA2* mutation carriers. Groups with smaller collections of carriers are encouraged to participate through partnership with a larger group, 2) Phenotypic data obtained from risk factor questionnaires and/or medical records are uniformly coded and stored in a centralized CIMBA database. These data include year of birth, mutation description, ethnicity, country of residence, age at last follow-up, ages at breast and ovarian cancer diagnosis, age at bilateral prophylactic mastectomy, age at bilateral prophylactic oophorectomy and status and age at menopause, 3) Panels of single nucleotide polymorphisms (SNPs) for genotyping are selected every 6 months at a CIMBA group meeting, 4) Only SNPs that show significant associations, either in the published literature or in data available to a member group, at $p<0.01$ are considered, 5) Each investigator/group is free to participate or not in any round of genotyping, 6) Genotyping quality control standards must be followed (2% duplicates, call rates >95%, randomized arrangement of affected and unaffected carriers for genotyping). 7) Genotyping data from participating centers are pooled and analyzed as outlined in the CIMBA analysis plan. This study represents the first genetic modifier study conducted by CIMBA using these guidelines.

This study of 7,187 *BRCA1* and *BRCA2* carriers had 80% power to detect significant ($p<0.05$) protective recessive effects with hazard ratios of 0.82 or smaller for the F31I allele. We therefore conclude that the present study has a sufficient sample size to assess with reasonable confidence the involvement of the F31I allele in the modification of breast cancer risk among BRCA1 and BRCA2 mutation carries. It also demonstrates the importance of large consortia like CIMBA in evaluating the associations between genetic markers and cancer risk.

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Appendix

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Table 1

Characteristics of study subjects by site

Source*	Ascertainment	*BRCA1* Cases	*BRCA1* unaff.	Total *BRCA1*	*BRCA2* Cases	*BRCA2* unaff.	Total *BRCA2*	*B1/2*† Cases	*B1/2*† unaff.	Total *B1/2*†	Total Carriers
MAGIC	Clinic	303	428	731	137	160	297	3	0	3	1031
GEMO	Clinic	413	276	689	223	84	307	0	0	0	996
EMBRACE	Clinic	235	219	454	156	148	304	1	2	3	761
Poland	Clinic	307	427	734	0	0	0	0	0	0	734
kConFab	Clinic	203	201	404	169	143	312	0	0	0	716
GCHBOC	Clinic	286	113	399	173	52	225	3	0	3	627
MSKCC	Clinic	174	117	291	102	70	172	1	0	1	464
Ontario	Clinic and Population	125	52	177	100	41	141	0	0	0	318
LUMC	Clinic	99	120	219	12	20	32	0	0	0	251
Lund	Clinic	73	88	161	38	32	70	0	0	0	231
MOD-SQUAD	Clinic	82	67	149	28	15	43	0	0	0	192
HEBCS	Clinic	56	39	95	54	40	94	0	0	0	189
DKFZ	Clinic	82	41	123	30	21	51	0	0	0	174
MAYO	Clinic	53	23	76	26	20	46	0	0	0	122
INHERIT	Clinic	33	37	70	40	41	81	0	0	0	151
NCI	Clinic	47	116	163	17	50	67	0	0	0	230
Total		2571	2364	4935	1305	937	2242	8	2	10	7187

*Abbreviations: MAGIC: Modifiers and Genetics in Cancer; GEMO: Genetic Modifiers of cancer risk in BRCA1/2 mutation carriers study; GCHBOC: German Consortium for Hereditary Breast and Ovarian Cancer; EMBRACE: Epidemiological Study of *BRCA1* and BRCA2 Mutation Carriers; kConFab: Kathleen Cunningham Consortium for Research into Familial Breast Cancer; INHERIT BRCAs: Interdisciplinary Health Research International Team on Breast Cancer susceptibility. MSKCC: Memorial Sloan Kettering Cancer Center; MAYO: Mayo Clinic; LUMC: Leiden University Medical Center; MOD-SQUAD: Modifier Study of Quantitative Effects on Disease; HEBCS: Helsinki Breast Cancer Study; DKFZ: Deutsches Krebsforschungszentrum Heidelberg; NCI: National Cancer Institute. The term unaff. refers to individuals not affected with breast cancer.

†B1/2 refers to individuals with both *BRCA1* and *BRCA2* deleterious mutations.

Table 2

Association of *AURKA* F31I with breast cancer risk[1]

Group	0 or 1 copy Ile allele			2 copies Ile allele			HR (95% CI) All cases	HR (95% CI)* Incident cases
	Unaffected	Affected	Person Years	Unaffected	Affected	Person Years		
Overall	3128	3696	296122	175	188	15793	0.91 (0.77-1.06)	0.84 (0.65-1.08)
By mutation status								
BRCA1	2237	2460	200406	129	120	10754	0.90 (0.75-1.08)	0.90 (0.66-1.22)
BRCA2	893	1245	96110	46	68	5039	0.93 (0.67-1.29)	0.67 (0.44-1.03)
By menopausal status								
Pre-menopausal	1935	2049	242208	111	106	12834	0.84 (0.69-1.03)	0.83 (0.60-1.15)
Post-menopausal	1193	1647	53914	64	82	2959	0.96 (0.75-1.23)	0.77 (0.51-1.16)
By oophorectomy status								
No	1772	2318	201303	101	107	10474	0.85 (0.69-1.05)	0.82 (0.58-1.15)
Yes	510	160	3793	28	9	213	1.10 (0.56-2.18)	1.03 (0.39-2.78)
Missing	846	1218	91026	46	72	5106	0.97 (0.75-1.26)	0.86 (0.55-1.34)
By study site								
MAGIC	559	423	41554	29	20	2002	1.02 (0.63-1.67)	
GEMO	347	597	40913	13	39	2266	1.33 (0.97-1.82)	
EMBRACE	353	378	30757	16	14	1318	0.70 (0.37-1.32)	
Poland	399	285	30360	28	22	2197	0.98 (0.65-1.47)	
kConFab	322	362	29568	22	10	1251	0.64 (0.34-1.22)	
GCHBOC	157	432	24819	8	30	1698	0.94 (0.65-1.37)	
MSKCC	182	268	19371	5	9	591	0.79 (0.38-1.66)	
Ontario	79	217	13069	14	8	1012	0.33 (0.13-0.82)	
LUMC	129	106	10350	11	5	715	0.68 (0.32-1.44)	
Lund	113	102	11401	7	9	803	1.05 (0.55-1.99)	
MOD-SQUAD	78	104	7760	4	6	388	1.56 (1.04-2.36)	
HEBCS	75	108	8451	4	2	344	0.27 (0.05-1.96)	
DKFZ	61	110	6714	1	2	109	7.05 (0.66-75.2)	
MAYO	41	71	4998	2	8	442	1.41 (0.65-3.07)	
INHERIT	76	70	6668	2	3	225	1.29 (0.45-3.67)	
NCI	157	63	9371	9	1	433	0.28 (0.05-1.77)	

[1] Weighted Cox proportional hazards regression analysis, modeling *AURKA* F31I as a recessive genotypic effect. Results overall, by menopausal status and by oophorectomy status account for birth cohort, group status, country, and mutation status. Mutation-specific results account for birth cohort, group status and country. Group-specific results account for birth cohort, mutation status and country. Robust variance estimates were used to correct for possible non-independence of study subjects.

* Cox proportional hazards regression analysis restricted to cases for whom genetic diagnosis is less than three years after breast cancer diagnosis.