OXFORD

JNCI J Natl Cancer Inst (2016) 108(2): djv315

doi:10.1093/jnci/djv315
First published online November 19, 2015
Article

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BRCA2 Polymorphic Stop Codon K3326X and the Risk of Breast, Prostate, and Ovarian Cancers

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Received: March 30, 2015; **Revised:** August 13, 2015; **Accepted:** October 2, 2015

ARTICLE

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Abstract

Background: The K3326X variant in *BRCA2* (*BRCA2**c.9976A>T; p.Lys3326*; rs11571833) has been found to be associated with small increased risks of breast cancer. However, it is not clear to what extent linkage disequilibrium with fully pathogenic mutations might account for this association. There is scant information about the effect of K3326X in other hormone-related cancers.

Methods: Using weighted logistic regression, we analyzed data from the large iCOGS study including 76 637 cancer case patients and 83 796 control patients to estimate odds ratios (OR_w) and 95% confidence intervals (CIs) for K3326X variant carriers in relation to breast, ovarian, and prostate cancer risks, with weights defined as probability of not having a pathogenic *BRCA2* variant. Using Cox proportional hazards modeling, we also examined the associations of K3326X with breast and ovarian cancer risks among 7183 *BRCA1* variant carriers. All statistical tests were two-sided.

Results: The K3326X variant was associated with breast (OR_w = 1.28, 95% CI = 1.17 to 1.40, $P = 5.9\times10^{-6}$) and invasive ovarian cancer (OR_w = 1.26, 95% CI = 1.10 to 1.43, $P = 3.8\times10^{-3}$). These associations were stronger for serous ovarian cancer and for estrogen receptor–negative breast cancer (OR_w = 1.46, 95% CI = 1.2 to 1.70, $P = 3.4\times10^{-5}$ and OR_w = 1.50, 95% CI = 1.28 to 1.76, $P = 4.1\times10^{-5}$, respectively). For *BRCA1* mutation carriers, there was a statistically significant inverse association of the K3326X variant with risk of ovarian cancer (HR = 0.43, 95% CI = 0.22 to 0.84, P = .013) but no association with breast cancer. No association with prostate cancer was observed.

Conclusions: Our study provides evidence that the K3326X variant is associated with risk of developing breast and ovarian cancers independent of other pathogenic variants in BRCA2. Further studies are needed to determine the biological mechanism of action responsible for these associations.

Inheritance of a pathogenic variant in *BRCA2* is one of the strongest risk factors for breast and ovarian cancers (1–4). Estimates of the cumulative risk by age 70 years in *BRCA2* variant carriers are 45% for breast cancer and 11% for ovarian cancer (5,6). *BRCA2* variants have also been shown to increase risk of prostate cancer (7–9), with the lifetime risk of prostate cancer in *BRCA2* variant carriers estimated in the range of 19% to 34% (9). In some studies, carriers of *BRCA2* pathogenic variants also had increased risks of several other cancers, including pancreatic cancer (7,8,10,11), stomach cancer, and malignant melanoma (7).

*BRCA2**c.9976A>T; p.Lys3326*, hereafter referred to as K3326X, arises from a substitution of thymidine for adenine at nucleotide 9976 of the *BRCA2* coding sequence and results in loss of the final 93 amino acids of the BRCA2 protein. This premature stop codon was first described in 1996 by Mazoyer et al. (12), who found a minor allele frequency of about 1% in the control population and no increased prevalence of this sequence variant in patients with breast cancer; however, the study was small, with 462 control patients and 513 case patients. Since then, the K3326X variant has been identified in individuals with various types of cancers, either alone or in combination with known pathogenic variants in *BRCA2* (13–15). Genome-wide association studies have identified the association between the K3326X variant and risk of squamous-cell lung cancer (16) and breast cancer (17) at genome-wide statistical significance levels, with odds ratios (ORs) of 2.47 (95% CI = 2.03 to 3.00, $P = 4.7\times10^{-20}$) and 1.26 (95% CI = 1.14 to 1.39, $P = 4.9\times10^{-8}$), respectively. Recently, a large, pooled case-control study of cancers of the upper aero-digestive tract (UADT; including esophageal cancer) and the K3326X variant found that the K3326X variant was associated with UADT cancers (OR = 2.53, 95% CI = 1.89 to 3.38) (18) as well, with a particularly strong effect for esophageal cancer (OR = 3.30, $P = 3\times10^{-4}$). K3326X is in linkage disequilibrium (LD) with the pathogenic variants *BRCA2**c.6275_6276delTT (formerly reported as 6503-6504delTT) and *BRCA2**c.9257-16T>C (formerly reported as IVS 24-17T>C) (12,14). In a study of 1850 high-risk breast and ovarian cancer UK families fully sequenced for *BRCA2*, Higgs et al. (19) asserted that the association with increased breast cancer risk previously reported by Michailidou et al. (17) was because of LD with *BRCA2**c.6275_6276delTT and reported no associations with pancreatic, lung, or esophageal cancer risks, although the confidence intervals were wide and could not exclude the point estimates from the large-scale case-control studies cited above (16,18).

In this study, we therefore analyzed the association of the *BRCA2* K3326X sequence variant with respect to risk of breast cancer, adjusting for potential effects of LD with known *BRCA2* pathogenic variants, and for the first time examined the association between K3326X and ovarian and prostate cancer. We further assessed whether K3326X is an independent modifier of breast and ovarian cancer risk in *BRCA1* pathogenic variant carriers.

Methods

This study used data from the four consortia within the Collaborative Oncological Gene-Environment Study (COGS): the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA), the Breast Cancer Association Consortium (BCAC), the Ovarian Cancer Association Consortium (OCAC), and the PRostate cancer AssoCiation group To Investigate Cancer Associated aLterations in the genome (PRACTICAL). The COGS central focus was using data from high-throughput genotyping of large epidemiological studies, with state-of-the-art analysis and mathematical models to combine data on genetic and environmental/life style risk factors (20). All studies had approval from the relevant ethics committees, and all participants gave informed consent. Details on the numbers of participants in each consortium are shown in Table 1.

To validate the effect of the *BRCA2* K3326X variant on ovarian cancer risk, we sequenced the *BRCA2* gene in an independent sample of ovarian cancer case patients and control patients.

Statistical Analysis

Fisher's exact statistics were calculated to assess associations between the K3326X variant and known *BRCA2* pathogenic variants among study patients in CIMBA. Using the datasets from the BCAC, OCAC, and PRACTICAL consortia, we evaluated the association of the *BRCA2* K3326X variant with risks of breast,

invasive ovarian, and prostate cancer, respectively, through logistic regression models with adjustment for attained age, consortium study site, and principal components of population structure. To examine the hypothesis that K3326X is associated with breast and ovarian cancers independently from *BRCA2* pathogenic variants, we calculated and compared odds ratios using weighted logistic regression models (ie, OR_w), described below, and odds ratios using unweighted logistic regression models (ie, OR) using Wald Z-statistics. Because of the lack of *BRCA2* status in the BCAC and OCAC datasets, to account for possible LD with *BRCA2* pathogenic variants we developed a model using the CIMBA dataset to predict whether case patients and control patients were carriers of pathogenic *BRCA2* variants based on age at diagnosis (for case patients) and age at interview (for control patients) and carrier status at K3326X. We then used this model to predict the probability of not having a pathogenic *BRCA2* variant in the other datasets and used this as the weight in the logistic regression model. This weight allowed us to adjust for the contribution of each patient to the test statistics based on their probability of not having a pathogenic *BRCA2* variant. Details of the calculation of these weights are shown in the Supplementary Methods (Available online). Using a similar approach, we evaluated the association of the K3326X variant with breast cancer risk stratified by ER status, triple-negative status (ER-, PR-, and HER2-negative), and tumor morphology, specifically ductal and lobular. We also evaluated the association of the K3326X variant with ovarian cancer risk stratified by histological subtypes of ovarian cancer, specifically serous, mucinous, endometrioid, and clear cell. We used Z-statistics to determine whether or not the magnitude of the associations between the K3326X variant and breast cancer risk were statistically different across breast and ovarian cancer subtypes. Using the datasets from the CIMBA consortium and Cox proportional hazards model, we examined the associations of the K3326X variant with breast and ovarian cancer risks for BRCA1 pathogenic variant carriers with censoring as defined in other CIMBA consortium analyses of data from the iCOGS study (21). To account for the inclusion of multiple carriers from the same family, a robust variance approach clustering on family membership was used (22). We tested the proportional-hazards assumption on the basis of Schoenfeld residuals. Statistical analyses were performed in R (version 2.14.2) (23). All statistical tests were two sided, with cutoff for statistical significance being .05.

Table 1. Number of control patients and breast cancer, ovarian cancer, and prostate cancer case patients included in the analysis

Study	Included in the analysis
The Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) (51)	7183 *BRCA1* mutation carriers, of which 1658 were unaffected, 2497 were breast cancer case patients, 543 were ovarian cancer case patients, and 267 had both breast and ovarian cancer 5101 *BRCA2* mutation carriers, of which 2183 were unaffected, 2538 were breast cancer case patients, 240 were ovarian cancer case patients, and 140 had both breast and ovarian cancer
Breast Cancer Association **Consortium** (BCAC) **(52)**	41 081 breast cancer case patients and 38 693 female control patients
Ovarian Cancer Association Consortium (OCAC) (53)	14 542 invasive ovarian cancer case patients and 23 111 female control patients
PRostate cancer AssoCiation group To Investigate Cancer Associated aLterations in the genome (PRACTICAL) (54)	21 014 prostate cancer case patients and 21 992 male control patients

Homology-Directed Repair Assay

The HDR assay for BRCA2 has been described previously by Guidugli et al. (24). Full-length flag-BRCA2 wild-type and mutant expression constructs were cotransfected with an I-Sce1 expressing pcBASce plasmid into BRCA2 deficient V-C8 cells, stably expressing the DR-GFP reporter plasmid. HDR-dependent DNA double strand break was quantified by fluorescence-activated cell sorting (FACS) of GFP-positive cells after 72 hours. Equivalent expression of wild-type and mutant BRCA2 proteins was confirmed by western blot analysis of anti-Flag-M2 (Sigma F1804) antibody immunoprecipitates from V-C8 cell lysates.

Results

In summary, 7183 *BRCA1* mutation carriers and 5101 *BRCA2* mutation carriers from CIMBA were used in the weight calculation; 76 637 cancer case patients and 83 796 control patients from BCAC, OCAC, and PRACTICAL were used in the main analysis. Table 2 shows the frequencies of the two most frequent *BRCA2* pathogenic variants (c.6275_6276delTT and c.4889C>G) among K3326X variant carriers in CIMBA. The c.6275_6276delTT known pathogenic variant was identified in 233 of 306 K3326X variant carrier families carrying the K3326X variant and in 5/4795 K3326X variant noncarrier families ($P < .001$), and c.4889C>G was observed in nine

Table 2. Frequencies of the two most frequent *BRCA2* pathogenic variants that co-occurred with the K3326X common variant in the Consortium of Investigators of Modifiers of BRCA1/2

Human Genome Variation Society nomenclature	BRCA2 K3326X noncarriers	BRCA2 K3326X carriers	*P*
c.6275_6276delTT	5	233	<.001*
c.4889C>G	0	9	<.001*
Other BRCA2 known pathogenic variants†	4790	64	Reference

* *P* values using two-sided Fisher's exact test calculated to assess the association between the K3326X variant and known *BRCA2* pathogenic variants. Each family in the Consortium of Investigators of Modifiers of BRCA1/2 was counted only once in this analysis.
† Listed in Supplementary Table 1 (available online).

of 306 K3326X variant–positive families and zero of 4795 K3326X variant noncarrier families ($P < .001$). The list and frequency of all pathogenic variants in the *BRCA2* gene that co-occurred with the K3326X variant in CIMBA are shown in Supplementary Table 1 (available online). Within the BCAC dataset, among K3326X variant carriers, 1471 of 1490 individuals also harbored the *BRCA2**c.9257-16T>C variant, which was not considered pathogenic based on the low likelihood for splice site alteration (13). Within the OCAC dataset, among K3326X variant carriers, all K3326X variant carriers also carried the *BRCA2**c.9257-16T>C variant. Within the independently sequenced set of ovarian cancer case patients and control patients, 2240 ovarian cancer case patients and 1530 control patients were included. Twenty-seven K3326X carriers were found in the control patients, compared with 48 in the case patients (OR = 1.23, 95% CI = 0.70 to 2.00, $P = .45$), and three of these carriers (all case patients) also carried the BRCA2*c.6275_6276delTT pathogenic variant.

Association Between the K3326X Variant and Breast Cancer Risk

Previous principal component analyses of these data derived six components used as adjustments for population structure in logistic regression models (17). The analyses of K3326X and breast cancer are shown in Table 3. The K3326X variant was associated with all invasive breast cancer (OR_w = 1.28, 95% CI = 1.17 to 1.40, $P = 5.9\times10^{-6}$), and this association was stronger for triple-negative breast cancer (OR_w = 1.52, 95% CI = 1.18 to 1.92, $P = 4.77\times10^{-3}$) and estrogen receptor–negative breast cancer (OR_w = 1.50, 95% CI = 1.28 to 1.76, $P = 4.10\times10^{-5}$). Of note, the odds ratios adjusted for potential LD with known pathogenic *BRCA2* variants were only slightly attenuated (eg, from 1.31 to 1.28 for all invasive breast cancer). However, the weighted odds ratios were not statistically different between ER+ breast cancer data subsets and either ER- ($P = .06$) or triple-negative breast cancer data subsets ($P = .20$), which might be because of the small sample size of ER- (n = 158) and triple-negative breast cancer case patients (n = 53). There was no evidence that K3326X was associated with lobular breast cancer ($P = .70$), but the sample size of lobular breast cancer case patients among the K3326X variant carriers was relatively small (n = 67), and the association between K3326X and breast cancer was not statistically different between participants with ductal and lobular tumors ($P = .17$). No association with breast cancer risk for *BRCA1* pathogenic variant carriers was observed (HR = 1.00, 95% CI = 0.78 to 1.29, $P = 1.00$) (data not shown).

Association Between the K3326X Variant and Ovarian Cancer Risk

Previous principal component analyses derived five components used as adjustments for population structure in logistic regression models (25). Adjusted weighted and unweighted odds ratios by histologic subtype of ovarian cancer are presented in Table 4. The K3326X variant was present in 323 of 14 542 individuals (2.2%) with invasive ovarian cancer (OR = 1.29, 95% CI = 1.13 to 1.46, $P = 1.41\times10^{-3}$; OR_w = 1.26, 95% CI = 1.10 to 1.43, $P = 3.84\times10^{-3}$) compared with 1.8% of control patients. A statistically significant association of K3326X was observed with serous ovarian cancer (OR_w = 1.46, 95% CI = 1.26 to 1.70, $P = 3.44\times10^{-5}$). However, little evidence was seen for an association of K3326X with nonserous ovarian cancer, although the sample size of such cancer case patients among the K3326X variant carriers was small (n = 60). The weighted odds ratios were statistically different between serous ovarian cancer data subsets and nonserous ovarian cancer data subsets ($P = 9.0\times10^{-4}$). In contrast, among *BRCA1* carriers, a statistically significantly decreased risk for K3326X variant carriers vs noncarriers was observed (HR = 0.43, 95% CI = 0.22 to 0.84, $P = .013$) (data not shown).

Association Between the K3326X Variant and Prostate Cancer Risk

Previous principal component analyses derived six components used as adjustments for population structure in logistic regression models (26). The K3326X variant was present in 358 of 21 014 case patients (1.7%) compared with 364 of 21 992 control patients (1.7%) (OR = 0.92, 95% CI = 0.77 to 1.09, $P = .39$; OR_w = 0.90, 95% CI = 0.76 to 1.07, $P = .32$). We estimated that this study had 87.6% power to detect an odds ratio of 1.25 using a two-sided test, assuming that 1.7% of the population are K3326X carriers (data not shown).

Discussion

In this study, we confirmed that the *BRCA2* K3326X variant is associated with increased risk of breast cancer independent of additional *BRCA2* pathogenic variants and demonstrated an even stronger association with serous ovarian cancer. These

Table 3. Association between *BRCA2* K3326X variant and risk of breast cancer (BC) by tumor subtypes: Breast Cancer Association Consortium

Subtypes	No. of BC cases	Number of K3326X carriers in BC cases	OR (95% CI)*	*P* for OR	Weighted OR_w (95% CI)*,†	*P* for OR_w‡
Control women	38 693	637	1.00 (Ref)		1.00 (Ref)	
Invasive BC cases	41 081	852	1.31 (1.20 to 1.43)	6.05×10^{-7}	1.28 (1.17 to 1.40)	5.86×10^{-6}
ER-	6441	158	1.54 (1.31 to 1.79)	5.07×10^{-6}	1.50 (1.28 to 1.76)	4.10×10^{-5}
ER+	24 833	501	1.28 (1.15 to 1.42)	8.50×10^{-5}	1.25 (1.13 to 1.39)	2.29×10^{-4}
Triple-negative	2158	53	1.55 (1.22 to 1.96)	2.45×10^{-3}	1.52 (1.18 to 1.92)	4.77×10^{-3}
Ductal	21 490	466	1.33 (1.20 to 1.48)	3.38×10^{-6}	1.30 (1.18 to 1.44)	2.17×10^{-5}
Lobular	3752	67	1.07 (0.87 to 1.32)	.57	1.05 (0.85 to 1.29)	.70

* Adjusted for attained age (at interview for control patients and at diagnosis for case patients), principal components of European population structure, and study site. CI = confidence interval; ER = estrogen receptor; OR = odds ratio.

† Calculated using weighted logistic regression models. The weight applied for the i^{th} individual in the Breast Cancer Association Consortium dataset is (1 - Probability of K3326X carriage and harboring known pathogenic variants in *BRCA2*).

‡ *P* values calculated using a two-sided Wald test.

Table 4. Association between *BRCA2* K3326X variant and risk of ovarian cancer (OC) by histological subtype: Ovarian Cancer Association Consortium

Subtype	No. of OC cases*	No. of K3326X carriers in BC cases	OR (95% CI)†	*P* for OR	Weighted OR_w (95% CI)†,‡	*P* for OR_w§
Control women	23 111	411	1.00 (Ref)		1.00 (Ref)	
Invasive OC cases	14 514	322	1.29 (1.13 to 1.46)	$1.41x10^{-3}$	1.26 (1.10 to 1.43)	$3.84x10^{-3}$
Serous	8360	210	1.50 (1.29 to 1.74)	$9.21x10^{-6}$	1.46 (1.26 to 1.70)	$3.44x10^{-5}$
Nonserous	4031	60	0.83 (0.65 to 1.04)	.18	0.81 (0.63 to 1.02)	.14
Mucinous	943	16	0.93 (0.59 to 1.39)	.79	0.91 (0.58 to 1.37)	.72
Endometrioid	2066	31	0.82 (0.59 to 1.11)	.31	0.81 (0.58 to 1.10)	.27
Clear cell	1022	13	0.72 (0.44 to 1.12)	.26	0.71 (0.42 to 1.10)	.23

* In total, 2129 case patients did not have information on ovarian cancer histologic type. CI = confidence interval; OR = odds ratio; Ref = reference.
† Adjusted for attained age (at interview for control patients and at diagnosis for case patients), principal component of European population structure, and study site.
‡ Calculated using weighted logistic regression models. The weight applied for the k^{th} individual in the Ovarian Cancer Association Consortium dataset is (1 - Probability of K3326X carriage and harboring known pathogenic variants in *BRCA2*).
§ *P* values calculated using a two-sided Wald test.

results suggest a role for the K3326X variant in both breast and ovarian cancers etiology but not in prostate cancer etiology.

The prevalence of K3326X in control populations (1.7%) is consistent with the observed allele frequency of the K3326X variant in the European population reported by the International HapMap Consortium (27). More recent data (Exome Aggregation Consortium [ExAC], Cambridge, MA, http://exac.broadinstitute.org) found the variant in 609 of 33 749 (1.8%) non-Finnish European sequenced individuals and a carrier frequency of 2.4% in Finland. In our study, most K3326X carriers also carried the *BRCA2**c9257-16T>C variant, which is also consistent with previous studies (13,28). However, most individuals with *BRCA2* pathogenic variants do not carry the K3326X variant, with the exception of individuals with c.6275_6276delTT and c.4889C>G variants (Table 2).

The odds ratios obtained from unweighted logistic regression and weighted logistic regression were similar, suggesting that K3326X is associated with the risk of developing breast and ovarian cancers independently from *BRCA2* known pathogenic variants (Tables 3 and 4). The association of the K3326X variant with cancer risk was strongest in patients with triple-negative breast and serous ovarian tumors (OR_w = 1.52, 95% CI = 1.18 to 1.92; and OR_w = 1.46, 95% CI = 1.26 to 1.70, respectively). ER-negative and triple-negative status has been linked to *BRCA1* variants but not *BRCA2* variants (29–35). Because of these findings, *BRCA1* tumors were hypothesized to have a different hormone-independent mechanism than *BRCA2* tumors (33). More research should explore the potential role of the *BRCA2* K3326X variant in the etiology of these disease phenotypes. Interestingly, the finding for serous ovarian cancer is consistent with epidemiological and genetic data showing that serous tumors have a different etiology from other ovarian carcinomas (36–38). Notably, it is *BRCA1* pathogenic variant carriers that generally have a higher risk of ER- breast and serous ovarian tumors, while there is no clear differential association for *BRCA2* pathogenic variant carriers (33). Overall, our results, together with evidence of molecular commonalities between triple-negative breast and high-grade serous ovarian tumors (39), suggest a possible related etiology between these two tumor subtypes.

Our study also provides other interesting findings that warrant further investigation. First, the K3326X variant is associated with increased risks of breast and ovarian cancer in the general population; however, in *BRCA1* variant carriers the K3326X variant is inversely associated with risk of ovarian cancer and not with risk of breast cancer. Second, our study found no association between the K3326X variant and prostate cancer risk, in contrast to the increased risk of prostate cancer in carriers of *BRCA2* pathogenic variants (44–47). The difference between the risk associations of K3326X and known *BRCA2* pathogenic variants has been observed previously. Wang et al. (16) reported a more than two-fold increased squamous-cell lung carcinoma risk in K3326X variant carriers; however, to date, there has been no evidence of any altered risk of lung cancer in families carrying *BRCA2* pathogenic variants (8,40,41). Additional studies are required to analyze the discrepant risk associations of the K3326X variant and other known *BRCA2* pathogenic variants with prostate and lung cancer risks and whether the K3326X variant may have an association with cancer risk independent of other genes. In contrast to lung cancer, there have been some reports of carriers of *BRCA2* pathogenic variants having increased risk of some UADT cancers (40,41).

We can only speculate about how K3326X might affect key functions of the BRCA2 protein. Fanconi anemia is an autosomal recessive disease characterized by cancer susceptibility, cellular hypersensitivity to DNA cross-linking agents, and other conditions (42). *FANCD2* encodes the protein for Fanconi anemia group D2 and is monoubiquitinated in response to DNA damage (43). Interaction of monoubiquitinated *FANCD2* and *BRCA2* is essential for activation of the homologous recombination activity of *RAD51*, an important enzyme involved in DNA repair mechanisms, and for loading onto the damaged DNA (44–49). Cells that express *BRCA2* protein lacking the C terminal exon 27 coding region do not show colocalization of *FANCD2*, *BRCA2*, and *RAD51* on chromatin (44,49); mice with deletions of exon 27 and *FANCD2* knockout mice had increased susceptibility to various types of cancers (50). We hypothesize that the K3326X variant modifies breast and ovarian cancer risk by altering the C terminus of *BRCA2*, resulting in loss of the interaction between FANCD2 and BRCA2 and, thus, inactivating *RAD51*. Our preliminary functional analyses showed that the average scaled HDR fold change for K3326X is 2.92 on a scale of 1 to 5, with a value of 5 for wild-type and 1 for *BRCA2* pathogenic variant D2723H. Although this value is significantly higher than known *BRCA2* pathogenic mutations, it does fall slightly outside the range of neutral variants that have been observed to date (24). It is possible that this reduced efficiency of HDR capacity is sufficient to cause the small increased breast and ovarian cancers risks described in this study. Additional experiments are still being performed to demonstrate the role of the K3326X variant on the BRCA2 protein.

Our study is the largest study to date examining the association of the *BRCA2* K3326X variant and breast, ovarian, and

prostate cancer risks. The large sample size and the use of weighted logistic regression models allowed us to estimate the underlying association of K3326X with cancer risk independent of known *BRCA2* pathogenic variants that are in LD with K3326X. A limitation of our analysis was the lack of *BRCA2* status in the BCAC and OCAC datasets, resulting in our use of the CIMBA *BRCA2* dataset to calculate the probability of *BRCA2* in order to create weights for the logistic regression models. As shown in Tables 3 and 4, the effect of LD between K3326X and two *BRCA2* pathogenic variants (ie, c.6275_6276delTT and c.4889C>G) was quite small and did not materially change the results. Therefore, it is extremely unlikely that the association with breast cancer can be explained by LD with *BRCA2* pathogenic variants as hypothesized by Higgs et al. (19); we note that their conclusion is based on theoretical calculations and did not include a matched control dataset corresponding to the highly familial cases sequenced in the study.

In conclusion, our study provides evidence that the *BRCA2**c.9976A>T (K3326X) variant contributes to the risk of developing breast and ovarian cancers. It remains open whether the underlying mechanism for this association is identical to that for lung and UADT cancers; it is unlikely that this question can be resolved using genetic data alone. Additional functional studies will be needed to determine the biological mechanism of action of the K3326X variant in the diverse set of cancers associated with it.

Funding

This work was supported by the Government of Canada through Genome Canada and the Canadian Institutes of Health Research and the Ministère de l'Économie, de l'Innovation et des Exportations du Québec through Génome Québec.

Amanda Spurdle is supported by a National Health and Medical Research Council (NHMRC) Senior Research Fellowship, and aspects of this research were funded by Australian NHMRC Project grant ID #1010719. This work was also supported in part by National Institutes of Health (NIH) grants CA128978 and CA116167, an NIH specialized program of research excellence in breast cancer to the Mayo Clinic (P50 CA116201), and the Breast Cancer Research Foundation. The The Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) data management and analysis is funded through Cancer Research-UK grant C12292/A11174. Breast Cancer Association Consortium (BCAC) data management was funded by Cancer Research UK (C1287/A10118 and C1287/A12014) and by the European Community's Seventh Framework Programme under grant agreement 223175 (HEALTH-F2-2009–223175). BCAC meetings have been funded by the European Union COST programme (BM0606). Ovarian Cancer Association Consortium (OCAC) is supported by a grant from the Ovarian Cancer Research Fund thanks to donations by the family and friends of Kathryn Sladek Smith (PPD/RPCI.07). The scientific development and funding for this project were in part supported by the US National Cancer Institute Genetic Associations and Mechanisms in Oncology (GAME-ON) Post–Genome Wide Association Study (GWAS) Initiative (U19-CA148112). This study made use of data generated by the Wellcome Trust Case Control consortium. A full list of the investigators who contributed to the generation of the data is available at http://www.wtccc.org.uk/. Funding for the project was provided by the Wellcome Trust under award 076113. The results published here are in part based upon data generated by the Cancer Genome Atlas Pilot Project established by the National Cancer Institute and National Human Genome Research Institute.

Funding for the iCOGS infrastructure came from: the European Community's Seventh Framework Programme under grant agreement n° 223175 (HEALTH-F2-2009–223175) (COGS), Cancer Research UK (C1287/A10118, C1287/A 10710, C12292/A11174, C1281/A12014, C5047/A8384, C5047/A15007, C5047/A10692, C8197/A16565), the National Institutes of Health (CA128978) and Post-Cancer GWAS initiative (1U19 CA148537, 1U19 CA148065 and 1U19 CA148112 - the GAME-ON initiative), the Department of Defence (W81XWH-10-1-0341), the Canadian Institutes of Health Research (CIHR) for the CIHR Team in Familial Risks of Breast Cancer, Komen Foundation for the Cure, the Breast Cancer Research Foundation, and the Ovarian Cancer Research Fund.

Notes

This study would not have been possible without the contributions of the following: Per Hall (COGS); Douglas F. Easton, Paul Pharoah, Kyriaki Michailidou, Manjeet K. Bolla, Qin Wang (BCAC); Andrew Berchuck (OCAC); Rosalind A. Eeles, Douglas F. Easton, Ali Amin Al Olama, Zsofia Kote-Jarai, and Sara Benlloch (PRostate cancer AssoCiation group To Investigate Cancer Associated aLterations in the genome); Georgia Chenevix-Trench, Antonis Antoniou, Lesley McGuffog, Fergus Couch, and Ken Offit (CIMBA); Joe Dennis, Alison M. Dunning, Andrew Lee, and Ed Dicks; Craig Luccarini and the staff of the Centre for Genetic Epidemiology Laboratory; Javier Benitez, Anna Gonzalez-Neira, and the staff of the CNIO genotyping unit; Jacques Simard and Daniel C. Tessier; Francois Bacot, Daniel Vincent, Sylvie LaBoissière, Frederic Robidoux, and the staff of the McGill University and the Génome Québec Innovation Centre; Stig E. Bojesen, Sune F. Nielsen, Borge G. Nordestgaard, and the staff of the Copenhagen DNA laboratory; and Julie M. Cunningham, Sharon A. Windebank, Christopher A. Hilker, Jeffrey Meyer, and the staff of Mayo Clinic Genotyping Core Facility.

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