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JNCI J Natl Cancer Inst (2019) 111(12): djz045

doi: 10.1093/jnci/djz045
First published online April 5, 2019
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Combined Tumor Sequencing and Case-Control Analyses of *RAD51C* in Breast Cancer

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Abstract

Background: Loss-of-function variants in *RAD51C* are associated with familial ovarian cancer, but its role in hereditary breast cancer remains unclear. The aim of this study was to couple breast tumor sequencing with case-control data to clarify the contribution of *RAD51C* to hereditary breast cancer.
Methods: *RAD51C* was sequenced in 3080 breast cancer index cases that were negative in *BRCA1/2* clinical tests and 4840 population-matched cancer-free controls. Pedigree and pathology data were analyzed. Nine breast cancers and one ovarian cancer from *RAD51C* variant carriers were sequenced to identify biallelic inactivation of *RAD51C*, copy number variation, mutational signatures, and the spectrum of somatic mutations in breast cancer driver genes. The promoter of *RAD51C* was analyzed for DNA methylation.
Results: A statistically significant excess of loss-of-function variants was identified in 3080 cases (0.4%) compared with 2 among 4840 controls (0.04%; odds ratio = 8.67, 95% confidence interval = 1.89 to 80.52, $P < .001$), with more than half of the carriers having no personal or family history of ovarian cancer. In addition, the association was highly statistically significant among cases with estrogen-negative ($P < .001$) or triple-negative cancer ($P < .001$), but not in estrogen-positive cases. Tumor sequencing from carriers confirmed bi-allelic inactivation in all the triple-negative cases and was associated with high homologous recombination deficiency scores and mutational signature 3 indicating homologous recombination repair deficiency.
Conclusions: This study provides evidence that germline loss-of-function variants of *RAD51C* are associated with hereditary breast cancer, particularly triple-negative type. *RAD51C*-null breast cancers possess similar genomic and clinical features to *BRCA1*-null cancers and may also be vulnerable to DNA double-strand break inducing chemotherapies and poly ADP-ribose polymerase inhibitors.

RAD51C is one of the five paralogs of RAD51 and is essential for DNA double-strand break repair by homologous recombination (HR). Bi-allelic loss-of-function (LoF) germline variants in *RAD51C* are responsible for Fanconi anemia-type complementation group O (1), whereas mono-allelic variants have been reported at a low frequency (1.3%) in families with a history of both breast and ovarian cancer (2), but rarely among families with a history of breast cancer only. Although studies have confirmed *RAD51C* as an ovarian cancer susceptibility gene, evidence for a role in breast cancer remains equivocal (3–9). Resolving the spectrum of cancers associated with pathogenic *RAD51C* germline variants is important for managing cancer

Received: December 11, 2018; **Revised:** February 7, 2019; **Accepted:** April 3, 2019
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risks in such families. However, given the rarity of *RAD51C* variants in most populations, current case-control studies remain substantially underpowered to establish a clear role for *RAD51C* in breast cancer predisposition in isolation. Data from genomic analysis of tumors from carriers of germline variants in candidate genes can provide powerful additional evidence for involvement of a gene in cancer predisposition. Characteristic somatic inactivation events and "mutational signatures" have recently been demonstrated for tumors from carriers of mutations in *ATM* and *PALB2* (10,11). In this study, sequencing data from breast cancer-affected cases in hereditary breast and ovarian cancer (HBOC) families and population-matched cancer-free controls was combined with tumor sequencing data to investigate the role of *RAD51C* in breast cancer.

Methods

Study Subjects and Sequencing

Case subjects were female index patients diagnosed with breast cancer from 3080 HBOC families that were negative for *BRCA1* and *BRCA2* pathogenic variants, and were ascertained from the Variants in Practice (ViP) Study from the combined Victorian and Tasmanian Familial Cancer Centres, Australia. Control subjects were 4840 women from the Lifepool study that were cancer free as of September 2017. The average age at diagnosis of the cases and the average age of controls were 45.8 years (range = 17–85 years) and 64.4 years (range = 40–97 years), respectively. This study was approved by the human research ethics committees at each participating ViP study recruitment center and the Peter MacCallum Cancer Centre (approval no. 09/29). All participants provided informed consent for genetic analysis of their germline and tumor DNA.

Germline DNA were sequenced for the coding region and exon-intron boundaries (≥10 bp) of *RAD51C* using a custom-designed HaloPlex Targeted Enrichment Assay panel (Agilent Technologies, Santa Clara, CA) as described previously (12–15). Tumor DNA was extracted from cancer cells in formalin-fixed, paraffin-embedded slides by needle microdissection and sequenced using an Agilent SureSelect XT Custom Panel that targeted all exons of *RAD51C* and an additional 487 genes (1.337 Mb total targeted region) including 27 breast cancer driver genes (16).

Statistical Analysis

To analyze data from the case and control study, the conditional maximum likelihood estimate was used to calculate odds ratios (ORs) with 95% confidence intervals (CIs), and the Fisher exact test was used to calculate *P* values [R 3.3.2 was used (17)]. The Mann–Whitney *U* test was performed for homologous recombination deficiency (HRD) score comparisons between groups of tumors in GraphPad Prism version 7.00 (California). A *P* value of less than .05 was considered statistically significant, and all tests were 2-sided.

Results

Frequency of Germline *RAD51C* Variants in HBOC Families and Controls

Breast cancer-affected index cases from 3080 HBOC families and 4840 controls (cancer free as of September 2017) from the Australian population were sequenced for all exons of *RAD51C*, at average sequencing depths of 147X and 170X, respectively. Overall, 98.7% of targeted bases in the cases and 99.4% in the controls were sequenced to a depth of more than 10-fold. LoF variants were identified in 11 cases (0.4%) and 2 controls (0.04%), suggesting a statistically significant enrichment in the familial cases (OR = 8.67, 95% CI = 1.89 to 80.52, $P < .001$) (Table 1). Seven of the 10 unique variants identified in this study were previously reported as pathogenic or likely pathogenic, associated with a hereditary cancer syndrome, in the ClinVar database.

The average age at the first breast cancer diagnosis in *RAD51C* carriers was 44.0 years (range = 26–60) and all were grade 2 or 3 invasive ductal carcinoma, with a proportion (7 of 11) lacking expression of estrogen (ER), progesterone (PR), and HER2 receptors (triple-negative [TN]) as summarized in Table 2.

Subgroup Analysis by Ovarian Cancer Family History and Hormone Receptor Status

To assess if these results could be explained by the known association with an increased risk of ovarian cancer, we considered the distribution of *RAD51C* variants according to personal and family cancer history of the case cohort. Of the cohort, 21% (638 of 3080 cases) had either a personal and/or a family history of one or more ovarian cancer diagnoses in any first- to third-degree relative. Five *RAD51C* carriers were identified among the 638 breast and ovarian cancer families (0.8%) compared with 6 among the 2442 breast cancer-only families (0.2%), indicating ovarian cancer family history explained at least part of the association of *RAD51C* with breast cancer (Table 3). Nevertheless, more than half (6 of 11) of the *RAD51C* carrier families did not have any history of ovarian cancer, which remained statistically significantly different to the control carrier frequency of 0.04% (OR = 5.96, 95% CI = 1.06 to 60.42, $P = .02$).

We examined the association between *RAD51C* and hereditary breast cancer by hormone receptor status. Among the cases where ER status was available (2699 of 3080), 1726 (63.9%) were ER-positive, 939 (34.8%) were ER-negative, and 34 were removed from the analysis because they were diagnosed with multiple primary breast cancers with both ER-positive and ER-negative tumors. In the ER-positive group, 0.2% were *RAD51C* variant carriers, which was not statistically significant (OR = 4.21, 95% CI = 0.48 to 50.44, $P = .12$) (Table 3). In contrast, 0.8% of the ER-negative group were *RAD51C* carriers, which was highly statistically significant (OR = 20.77, 95% CI = 4.14 to 200.58, $P < .001$), and this association was even stronger when considering only the subgroup of TN breast cancer cases, with 1.1% being variant carriers (OR = 27.33, 95% CI = 5.19 to 268.54, $P < .001$). However, the confidence intervals are broad and the estimates of the odds ratios need to be interpreted with caution.

A similar trend for an excess of missense variants was observed in the cases, particularly for TN cancers (Supplementary Tables 1 and 2, available online). The strength of the association varied with the variant classification tool used, but none of the comparisons would be considered statistically significant when accounting for multiple testing.

Bi-Allelic Inactivation Analysis in *RAD51C* Tumors

The status of the wild-type *RAD51C* allele was examined in tumor DNA from nine breast cancers and one ovarian cancer from nine families. Sequencing was performed using a custom gene panel that included all exons of *RAD51C* to an average depth of

Table 1. *RAD51C* variants identified in cases and controls study

CDS change*	Protein change†	Consequence	Case	Control	Exon (of 9)	Intron (of 8)	MAF in gnomAD‡	Clinical significance§
c.68_72dup	p.Val25CysfsTer3	Frameshift	1	0	1	N/A	0	N/A
c.146-4_146-2delTCA	N/A	Splice acceptor	1	0	N/A	1	0	LP
c.394dupA	p.Thr132AsnfsTer23	Frameshift	2	0	2	N/A	3.77×10^{-05}	N/A
c.397C>T	p.Gln133Ter	Stop gained	0	1	3	N/A	0	P
c.572-1G>T	N/A	Splice acceptor	1	0	N/A	3	4.22×10^{-06}	N/A
c.577C>T	p.Arg193Ter (rs200293302)	Stop gained	1	1	4	N/A	3.38×10^{-05}	P
c.705 + 1G>A	N/A	Splice donor	1	0	N/A	4	0	LP
c.706-2A>G	N/A	Splice acceptor	2	0	N/A	4	0	P/LP
c.904 + 5G>T	N/A	Splice donor	1	0	N/A	6	2.24×10^{-05}	LP
c.905-2_905-1delAG	N/A	Splice acceptor	1	0	N/A	6	0	P/LP
Total	N/A	N/A	11	2	N/A	N/A	N/A	N/A

*ENST00000337432 (NM 058216.1). CDS = coding sequence; LP = likely pathogenic; MAF = minor allele frequency; N/A = not applicable; P = pathogenic.
†ENSP00000336701(NP 478123.1).
‡Minor allele frequency in noncancer cohorts, GnomAD v2.1.
§Clinical significance reviewed by ClinVar (online database downloaded on July 18, 2018).

Table 2. Cancer diagnosis, pathology, and family history of *RAD51C* case carriers

		Breast cancer diagnosis					Other cancer diagnosis			Family history†		
Case	RAD51C variants*	Age, y	Histology	ER	PR	HER2	Age, y	Site	Histology	Total no. of relatives	Breast cancer	Ovarian cancer
3	p.Thr132AsnfsTer23	29, 48	IDC, G3‡	ER−	PR−	HER2−	37	Thyroid	N/A	23	1	0
4	c.905-2_905-1delAG	43	IDC, G3	ER−	PR−	HER2−	26	Cervix	N/A	34	3	0
5	c.572-1G>T	46	IDC, G3	ER−	PR−	HER2−	N/A	N/A	N/A	26	2	0
7	c.706-2A>G	50	IDC, G3	ER−	PR−	HER2−	N/A	N/A	N/A	33	5	1
8	c.706-2A>G	40	IDC, G3	ER−	PR−	HER2−	N/A	N/A	N/A	16	0	0
9	p.Arg193Ter	32	IDC, G3	ER−	PR−	HER2−	N/A	N/A	N/A	20	1	0
11	c.904 + 5G>T	55	IDC, G3	ER−	PR−	HER2−	N/A	N/A	N/A	13	1	1
1	p.Thr132AsnfsTer23	26	IDC, G2	ER−	PR−	HER2+	N/A	N/A	N/A	46	0	0
10	p.Val25CysfsTer3	53	IDC, G2	ER+	PR−	HER2+	52	Ovary	HGS	29	0	0
2	c.705 + 1G>A	50	IDC, G2	ER+	PR+	HER2−	N/A	N/A	N/A	61	2	1
6	c.146-4_146-2delTCA	60	IDC, G2	ER+	PR+	HER2−	45	Ovary	HGS	54	2	0

*ENST00000337432 (NM 058216.1), ENSP00000336701(NP 478123.1). ER = estrogen receptor; G = grade; HER2 = human epidermal growth factor receptor 2; HGS = high-grade serous ovarian carcinoma; IDC = invasive ductal carcinoma; N/A = not applicable; PR = progesterone receptor.
†Breast cancer and ovarian cancer affected cases in first-, second-, and third-degree relatives of the cases.
‡Histology and hormone receptor status data in the table are for the second breast cancer diagnosis. The first cancer was high-grade ER-negative breast cancer with no pathology report available.

Table 3. Subgroup analysis of the association between *RAD51C* and breast cancer by ovarian cancer family history and hormone receptor status

	Familial breast cancer cases			Cancer-free controls				
Personal/family history	RAD51C	Frequency, %	Total	RAD51C	Frequency, %	Total	OR (95% CI)*	P†
All subjects	11	0.4	3080	2	0.04	4840	8.67 (1.89 to 80.52)	<.001
Have OvCa history‡	5	0.8	638	2	0.04	4840	19.09 (3.12 to 200.42)	<.001
Excluding OvCa history§	6	0.2	2442	2‖	0.04	4840	5.96 (1.06 to 60.42)	.02
ER+ breast cancer	3	0.2	1726	2	0.04	4840	4.21(0.48 to 50.44)	.12
ER− breast cancer	8	0.8	939	2	0.04	4840	20.77 (4.14 to 200.58)	<.001
Triple-negative breast cancer	7	1.1	626	2	0.04	4840	27.33 (5.19 to 268.54)	<.001

*The ORs for each subgroup analysis were calculated using the reference group of cancer-free controls. CI = confidence interval; ER = estrogen; OR = odds ratio; OvCa = ovarian cancer.
†Fisher exact test, 2-sided.
‡Ovarian cancer diagnosis in the index case of one or more first- to third-degree relatives.
§No ovarian cancer diagnosis in the index case or any first- to third-degree relative.
‖Both of these control carriers reported having first- and/or second-degree relatives diagnosed with breast and/or ovarian cancer: One control carrier reported a sister diagnosed with breast cancer (age 45 years) and another sister diagnosed with ovarian cancer (age unknown). The other control carrier reported her mother was diagnosed with both breast cancer (age 30 years) and ovarian cancer (age 49) and that "multiple" second-degree relatives were diagnosed with breast or ovarian cancer (age unknown).

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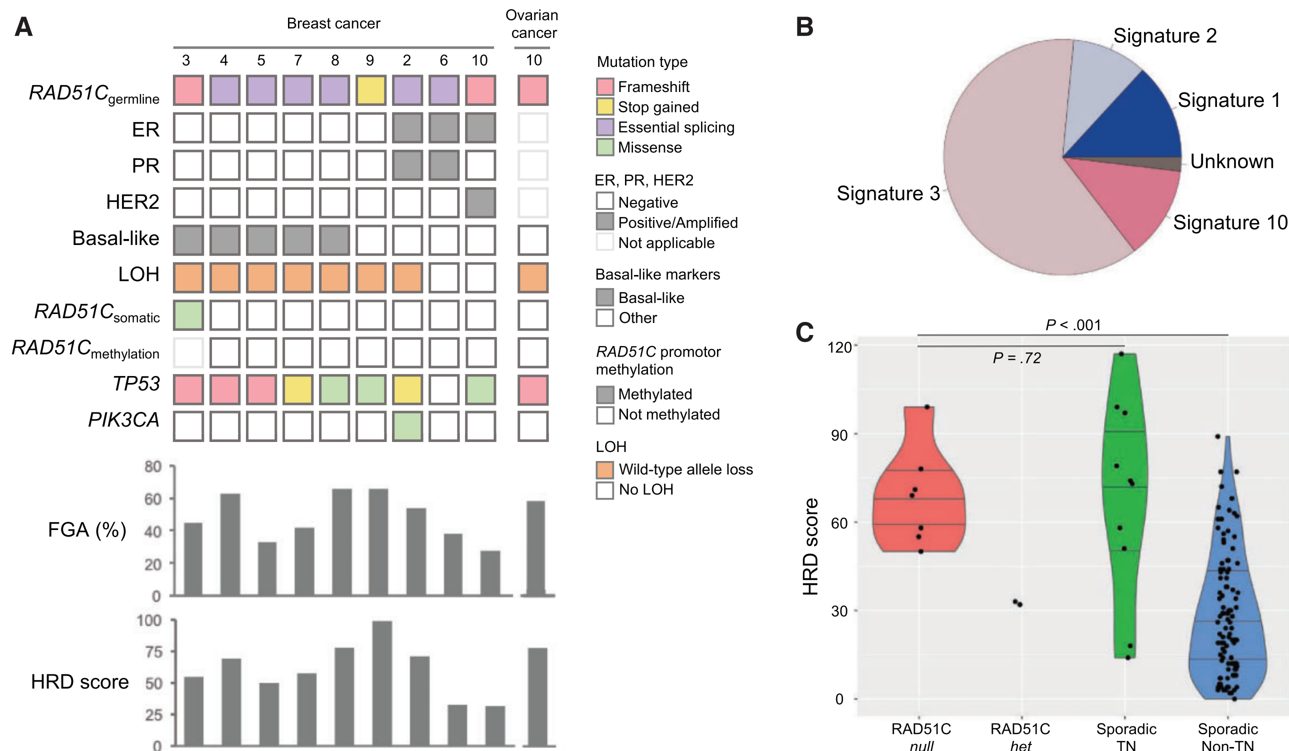

Figure 1. Genomic characterization of breast and ovarian cancers from carriers of *RAD51C* germline loss-of-function variants. **A)** Germline variants, bi-allelic inactivation events, somatic mutations, and genomic alterations of *RAD51C*-associated tumors. Germline and somatic mutation types are color-coded according to the legend. The phenobar (**top**) provides information about estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status, basal-like subtype, loss of heterozygosity (LOH) of the wild-type allele, and somatic mutations in *TP53* and *PIK3CA*. The fraction of the genome altered (FGA) and homologous recombination deficiency (HRD) score are shown for each case below. **B)** The weighted contribution of mutational signatures from breast cancers of *RAD51C* germline variant carriers. **C)** HRD scores of *RAD51C*-null (n = 7), *RAD51C*-het (n = 2), sporadic TN breast cancers (n = 10), and sporadic non-TN breast cancers (n = 105). *P* values were calculated using Mann–Whitney test, 2-tailed. ER = estrogen receptor; FGA = fraction of genome altered; HER2 = human epidermal growth factor receptor 2; HRD = homologous recombination deficiency; LOH = loss of heterozygosity; PR = progesterone receptor; TN = triple-negative.

396x. Seven of the nine breast tumors (77.8%) and the high-grade ovarian cancer showed loss of heterozygosity (LOH) across the *RAD51C* locus and all lost the wild-type allele (*RAD51C*-null). Six of the seven *RAD51C*-null breast cancers were TN cancers (Figure 1, A) and five of these were confirmed as basal-like type based on positive immunohistochemical staining for CK5 and EGFR. The two breast tumors that retained heterozygosity across the *RAD51C* locus were an ER-positive, PR-positive tumor and an ER-positive, HER2-positive tumor. Bisulfite sequencing excluded promoter hypermethylation-mediated silencing of *RAD51C* in these two tumors, and no somatic *RAD51C* point mutations were identified, confirming that they only had mono-allelic inactivation of *RAD51C* (*RAD51C*-het). As expected, promoter hypermethylation was not detected in any of the *RAD51C*-null breast cancers. A somatic missense mutation (p.Leu363Met) was identified in one case, but in silico predictions indicate that this is likely to be a benign passenger mutation.

Overall, 77.8% of the breast tumors from germline *RAD51C* variant carriers were established to have bi-allelic inactivation of *RAD51C*, and this were associated with TN breast cancers (100% of the TN breast tumors were *RAD51C*-null).

Somatic Mutations and Mutational Signature in *RAD51C* Tumors

Sequencing of the *RAD51C*-associated cancers identified *TP53* as the most common somatically mutated gene found in eight of nine breast tumors (88.9%), including all *RAD51C*-null tumors and one of the two *RAD51C*-het tumors. One *PIK3CA* mutation was detected in an ER-positive *RAD51C*-null tumor, and one *PTEN* and one *RB1* mutation was observed individually in one TN *RAD51C*-null tumor.

Somatic mutational signature 3 is a hallmark of tumors with *BRCA1* or *BRCA2* mutations (18) and is a robust indicator of HR DNA repair deficiency, particularly in tumors that harbor alterations in genes in the same complex of *BRCA1/BRCA2*-associated DNA repair (eg, *PALB2*, *RAD51C*, and *BARD1*) (19). To evaluate the common mutational processes underlying the *RAD51C* tumors, we used a pooled sample mutational signature method (10) to overcome the limitation of the small number of somatic variants per sample detected by targeted sequencing. The profile of mutational signatures was generated from 44 single-nucleotide substitution variants identified in nine *RAD51C* breast tumors and confirmed a predominant mutational signature 3 (Figure 1B) that was consistent with the *RAD51C*-associated HR deficiency.

Genomic Instability and HRD Score of *RAD51C* Tumors

Tumors demonstrating HR deficiency (including tumors associated with bi-allelic inactivation of *BRCA1*, *BRCA2*, or *PALB2* through the combination of a germline pathogenic variant and a somatic inactivation event) have been shown to be associated with an increased level of genomic instability (20) and elevated rates of large-scale chromosomal aberrations (21,22). To further

confirm that *RAD51C* bi-allelic inactivation drives breast cancer tumorigenesis through HR deficiency, we evaluated two HR deficiency measures using the genome-wide copy number data. First, we calculated the fraction of genome altered (FGA) (23,24) to measure the degree of broad-range genomic instability, previously reported as the genomic instability index (GII) (25); and second, we calculated an HRD score to measure the HR-deficiency-specific genomic aberrations (26,27), combined from three HRD score components: NtAI (number of telomeric allelic imbalances) (28), HRD-LOH (29), and large-scale state (LST) transitions (18) (Figure 1, A and C). *RAD51C*-null tumors all had a high level of genomic instability (FGA median = 53.6%, 33.3%–66.5%) and high HRD scores (median 70, 50–99) that exceeded the threshold for HR deficiency proposed for *BRCA1*-driven breast cancer (20,27). In contrast, neither of the two *RAD51C*-het tumors met the criteria for HR deficiency (HRD = 33 and 32, FGA = 37.8% and 27.9%, respectively) (Figure 1, A and C). Compared with a cohort of 115 sporadic breast cancers that were sequenced on the same platform, *RAD51C*-null breast tumors had HRD scores in the upper range observed for the 10 sporadic TN breast cancers and much higher than the 105 non-TN breast cancers (median 69 vs 24, $P < .001$, Mann–Whitney test, 2-sided) (Figure 1, C). The fraction of the genome that was altered showed good concordance with the HRD score, matching the previously reported positive association (21). The copy number profiles of the *RAD51C*-null tumors were similar to those described for *BRCA1*-driven tumors (30) and *BRCA*-like *PALB2*-driven breast cancers we previously reported (10). These tumors were also similar to sporadic TN breast cancers but distinct from non-TN breast cancers, for example, frequent gains of 3q, 5p, and 10p and loss of 5q (Supplementary Figure 1, available online). The two *RAD51C*-het tumors had few alterations and appeared similar to sporadic non-TN breast cancers (Supplementary Figures 1 and 2, available online).

Discussion

RAD51C was first reported as a predisposition gene to HBOC in 2010 (2), and subsequent studies have confirmed its role in ovarian cancer. The recent large-scale study by Song et al. (9) provided strong evidence that *RAD51C* is a moderate-risk ovarian cancer susceptibility gene and suggested that it should be included alongside *BRCA1* and *BRCA2* in clinical genetic tests for this indication. In contrast, the relevance of *RAD51C* to breast cancer predisposition remains unclear. A few studies have reported supportive evidence (3,7,8), whereas others suggested little or no increased risk for breast cancer (4,6,31). This uncertainty is predominantly due to the rarity of pathogenic variants in the population (we observed two carriers, 0.04%, in 4840 cancer-free Australia women), which severely limits the statistical power of case-control studies. To overcome these limitations, augmentation of case-control and family history data with information about the somatic landscape of the tumors occurring in carriers of rare pathogenic variants has recently been used to provide a detailed understanding of the role of *ATM* and *PALB2* in breast cancer predisposition (10,11). Using a similar approach, the data generated in this study have provided strong evidence to conclude that *RAD51C* is a genuine breast cancer predisposition gene that typically undergoes bi-allelic inactivation and is specifically highly associated with TN breast cancers. With detailed, validated family cancer histories available for our cohort, we were able to demonstrate that the association of *RAD51C* with breast cancer predisposition was not a consequence of enrichment of ovarian cancer families, as might be anticipated in a clinical cohort selected for *BRCA*-like features; 6 of the 11 carriers had no personal or family history of ovarian cancer, and there was a similar excess of *RAD51C* LoF variants compared with controls among the overall familial cases and the breast cancer-only families (8.67-fold vs 5.96-fold). The data shows that *RAD51C* conforms to Knudson's 2-hit model with loss of the wild-type allele in all the TN breast cancers, one ER-positive breast cancer and the high-grade serous ovarian cancer, consistent with previous observations (2,6). Together with the high level of genome alteration and HRD scores, the predominant mutational signature 3, and a pattern of copy number alterations consistent with *BRCA*-like tumors, we conclude that *RAD51C*-driven breast cancer tumorigenesis occurs via homologous recombination deficiency. Notably, the high proportion of TN breast cancers among carriers and the fact that all TN breast cancers have bi-allelic inactivation strongly links *RAD51C* LoF variants with TN breast cancer predisposition.

This connection is in agreement with the recent finding of germline variants of *RAD51C* associated with elevated risk in a TN breast cancer cohort (32) and the description of enrichment for epigenetic silencing of *RAD51C* in basal-like breast cancers in young individuals of African descent (19). Shimelis et al. (32) reported 23 *RAD51C* pathogenic variant carriers (0.4%) in 6093 Caucasian TN breast cancer patients, and a slightly attenuated frequency was observed after excluding patients with ovarian cancer family history (9 in 3313 patients, 0.3%). Although using slightly different criteria to define pathogenicity (Shimelis et al. included "pathogenic or likely pathogenic" variants with the classification not described in detail, whereas only LoF variants were included in the current study), the frequencies of carriers were similar between Shimelis et al. and our case cohort (0.4% overall, and 0.2% excluding any ovarian cancer family history). However, the overall odds ratio for TN breast cancer reported by Shimelis et al. in their case-only study is lower than reported in our study, reflecting the relatively high number of *RAC51C* carriers in their population-comparison group of 26 647 ExAC controls, which included 37 (0.1%) reported carriers of pathogenic variants in *RAD51C*. By comparison, we observe fewer LoF carriers in our control cohort (2 in 4840, 0.04%) compared with the gnomAD database excluding cancer cohorts (142 in 118 423, 0.1%, gnomAD V2.1 noncancer). It is possible that *RAD51C* variants were underrepresented in our controls, potentially reflecting the healthy, aged nature of our control group (average age 64.4 years). Alternatively, gnomAD or ExAC may overestimate the frequency of *RAD51C* variants because of the combination of different cohorts, not controlled for age, and lack of cancer diagnosis update. Notably, Song et al. (9) reported 2 carriers identified in 2769 cancer-free individuals aged 35 years or older, which is similar to our controls. However, additional large-scale studies in different populations will be required to determine the frequency of *RAD51C* LoF variants in the general population.

Deficiencies in the HR pathway are known to sensitize breast tumors to drugs such as platinum compounds that induce DNA damage through DNA double-strand breaks (33). The use of platinum-based chemotherapy regimens in early breast cancer has increased based on recent data suggesting an improved likelihood for pathological complete response in high-risk TN breast cancers regardless of *BRCA1* or *BRCA2* status (34). In advanced TN breast cancer only, the presence of a germline *BRCA1* or *BRCA2* variant was associated with improved response to platinum-based chemotherapy (35). With conflicting outcome data together with the additional toxicities associated with the

addition of platinum therapies to standard treatments, the ability to better identify the subgroups most likely to benefit is essential. Many TN tumors, particularly of the basal-like subtype, are known to have characteristic features of HR deficiency, and the HRD score has been shown to predict response to platinum-based chemotherapy in the neoadjuvant setting (27). Our results suggest that *RAD51C* germline variants result in many of the same tumor features previously associated with *BRCA* germline variants and have the potential to identify a further subgroup of women who may benefit from DNA double-strand break-inducing chemotherapies. Silencing of *RAD51C* expression in cancer cells has also been reported to be sufficient to induce sensitivity to the synthetic lethality effect of poly ADP-ribose polymerase (PARP) inhibitors (36). Recent clinical trials in ovarian cancer have confirmed that tumors harboring *RAD51C* variants were responsive to the PARP inhibitor Rucaparib (37,38), suggesting a potential therapeutic opportunity for other HR-deficient cancer types.

This study has two important limitations. First, the broad confidence intervals of the odds ratios indicate that the study is underpowered to give a stable estimate of the odds ratios of *RAD51C* LoF variants. The high odds ratios may be exaggerated as a result of the "extreme phenotype" cohort design of the study (familial and/or young onset cases vs aged cancer-free controls) and should not be considered as a relative risk, directly transferable to all carriers of *RAD51C* pathogenic variants in the population. Second, this study did not screen for large genomic rearrangements affecting *RAD51C*, although a previous study identified none in 141 non-BRCA1 and BRCA2 families, suggesting that this type of variant may be rare in *RAD51C* (39), in comparison to *BRCA1* and *BRCA2* (40).

Together, our findings indicate that although pathogenic germline variants in *RAD51C* are rare, they are associated with a statistically significantly increased risk, particularly of triple-negative breast cancers, and this information has the potential to greatly benefit patients not only in effective risk management but also in improved cancer treatment.

Funding

This work was supported by the National Breast Cancer Foundation, the Victorian Cancer Agency, and the National Health and Medical Research Council.

Notes

Affiliations of authors: Cancer Genetics Laboratory, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia (NL, MZ, DC, BWXL, SMR, IGC); Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Victoria, Australia (NL, DC, BWXL, SA, JL, SBF, KLG, PAJ, IGC); Parkville Familial Cancer Centre, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Victoria, Australia (SM, NG, PAJ); Bioinformatics Consulting Core, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia (MZ, JL); Lifepool, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia (LD); Department of Clinical Pathology, University of Melbourne, Melbourne, Victoria, Australia (DB, SBF, KLG, IGC); Molecular Genomics Core Facility, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia (JEAL); Familial Cancer Centre, Austin Health, Heidelberg, Victoria, Australia (TJ); Cabrini Family Cancer Clinic, Cabrini Hospital, Malvern, Victoria, Australia (YA); Cancer Genomics Program, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia (KLG).

The authors have no conflict of interest to declare.

PAJ and IGC contributed equally to this work.

The funders had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript for publication.

The authors thank Lyon Mascarenhas, Rebecca Driessen, the ViP study site principal investigators Geoffrey Lindeman, Marion Harris, and Ingrid Winship, and the staff at the Victorian and Tasmanian Familial Cancer Centres who enrolled participants and provided clinical data. We also thank all the participants of the ViP and Lifepool studies for donating their DNA samples and clinical information. We thank the following staff from Peter MacCallum Cancer Centre: Tim Semple, Gisela Mir Arnau from Molecular Genomics core facility for sequencing the tumor DNA; Kaushalya Amarasinghe, Niko Thio, and Richard Lupat from Bioinformatics core facility for helping with the bioinformatic analysis; Peter Mac Tissue Bank for preparing sections of tumor blocks; and Heather Thorne, Genna Glavich, and other staff from kConFab for assisting with obtaining the tumor blocks.

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