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RAD51 135G→C Modifies Breast Cancer Risk among *BRCA2* Mutation Carriers: Results from a Combined Analysis of 19 Studies

Antonis C. Antoniou, Olga M. Sinilnikova, Jacques Simard, Mélanie Léoné, Martine Dumont, Susan L. Neuhausen, Jeffery P. Struewing, Dominique Stoppa-Lyonnet, Laure Barjhoux, David J. Hughes, Isabelle Coupier, Muriel Belotti, Christine Lasset, Valérie Bonadona, Yves-Jean Bignon, Genetic Modifiers of Cancer Risk in BRCA1/2 Mutation Carriers Study (GEMO),* Timothy R. Rebbeck, Theresa Wagner, Henry T. Lynch, Susan M. Domchek, Katherine L. Nathanson, Judy E. Garber, Jeffrey Weitzel, Steven A. Narod, Gail Tomlinson, Olufunmilayo I. Olopade, Andrew Godwin, Claudine Isaacs, Anna Jakubowska, Jan Lubinski, Jacek Gronwald, Bohdan Górski, Tomasz Byrski, Tomasz Huzarski, Susan Peock, Margaret Cook, Caroline Baynes, Alexandra Murray, Mark Rogers, Peter A. Daly, Huw Dorkins, Epidemiological Study of *BRCA1* and *BRCA2* Mutation Carriers (EMBRACE),* Rita K. Schmutzler, Beatrix Versmold, Christoph Engel, Alfons Meindl, Norbert Arnold, Dieter Niederacher, Helmut Deissler, German Consortium for Hereditary Breast and Ovarian Cancer (GCHBOC), Amanda B. Spurdle, Xiaoqing Chen, Nicola Waddell, Nicole Cloonan, The Kathleen Cuningham Consortium for Research into Familial Breast Cancer (kConFab), Tomas Kirchhoff, Kenneth Offit, Eitan Friedman, Bella Kaufmann, Yael Laitman, Gilli Galore, Gad Rennert, Flavio Lejbkowicz, Leon Raskin, Irene L. Andrulis, Eduard Ilyushik, Hilmi Ozcelik, Peter Devilee, Maaike P. G. Vreeswijk, Mark H. Greene, Sheila A. Prindiville, Ana Osorio, Javier Benítez, Michal Zikan, Csilla I. Szabo, Outi Kilpivaara, Heli Nevanlinna, Ute Hamann, Francine Durocher, Adalgeir Arason, Fergus J. Couch, Douglas F. Easton, and Georgia Chenevix-Trench, on behalf of the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA)†

RAD51 is an important component of double-stranded DNA–repair mechanisms that interacts with both BRCA1 and BRCA2. A single-nucleotide polymorphism (SNP) in the 5′ untranslated region (UTR) of *RAD51,* 135G→C, has been suggested as a possible modifier of breast cancer risk in *BRCA1* and *BRCA2* mutation carriers. We pooled genotype data for 8,512 female mutation carriers from 19 studies for the *RAD51* 135G→C SNP. We found evidence of an increased breast cancer risk in CC homozygotes (hazard ratio [HR] 1.92 [95% confidence interval {CI} 1.25–2.94) but not in heterozygotes (HR 0.95 [95% CI 0.83–1.07]; $P = .002$, by heterogeneity test with 2 degrees of freedom [df]). When *BRCA1* and *BRCA2* mutation carriers were analyzed separately, the increased risk was statistically significant only among *BRCA2* mutation carriers, in whom we observed HRs of 1.17 (95% CI 0.91–1.51) among heterozygotes and 3.18 (95% CI 1.39–7.27) among rare homozygotes ($P = .0007$, by heterogeneity test with 2 df). In addition, we determined that the 135G→C variant affects *RAD51* splicing within the 5′ UTR. Thus, 135G→C may modify the risk of breast cancer in *BRCA2* mutation carriers by altering the expression of *RAD51. RAD51* is the first gene to be reliably identified as a modifier of risk among *BRCA1/2* mutation carriers.

Germline mutations in *BRCA1* (MIM 113705) and *BRCA2* (MIM 600185) confer high risks of breast and ovarian cancer. In a meta-analysis of mutation-positive families identified through population-based studies of breast and ovarian cancer cases, the cumulative risks of breast cancer by age 70 years were estimated to be 65% and 45% for *BRCA1* and *BRCA2* mutation carriers, respectively.[1] However, these and other population-based estimates of penetrance have, in general, been lower than estimates based on families with multiple affected individuals.[2–4] Moreover, the breast cancer risk has been found to vary by the age at diagnosis and the type of cancer in the index patient.[1,5] Such observations are consistent with the hypothesis that breast cancer risk in mutation carriers is modified by other genetic or environmental factors that cluster in families.

RAD51 is the homolog of bacterial *RecA,* which is required for recombinational repair of double-strand DNA breaks.[6,7] Both BRCA1 and BRCA2 interact with RAD51,[8,9] and the *Rad51*-knockout mouse phenotype resembles the *Brca1*- and *Brca2*-knockout phenotypes.[10] To examine the

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Address for correspondence and reprints: Dr. Antonis C. Antoniou, Cancer Research UK Genetic Epidemiology Unit, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 8RN, United Kingdom. E-mail: antonis@srl.cam.ac.uk

* Study members are listed in the Acknowledgments section.

† The authors' affiliations can be found at the end of the Acknowledgments.

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effect of *RAD51* SNPs on cancer risk in *BRCA1* and *BRCA2* mutation carriers, Wang et al.[11] searched for common sequence variants by resequencing the *RAD51* gene. No SNPs in the coding region were identified, but two SNPs (135G→C [*rs1801320*] and 172G→T [*rs1801321*]) were discovered in the 5′ UTR of *RAD51*. The latter SNP was found to have no effect on cancer risk, but carriers of the 135C allele were reported to have an increased risk of breast cancer among the subset of *BRCA2* carriers ($n = 216$; odds ratio [OR] 3.2 [95% CI 1.4–40]).[11] Two additional studies of the 135G→C SNP also found an association with cancer risk in *BRCA2* carriers. A study of Israeli Ashkenazi Jewish carriers from 141 *BRCA1* families and 64 *BRCA2* families found a significant association of the C allele with cancer risk (breast or ovarian) in *BRCA2* carriers (hazard ratio [HR] of 4.0 [95% CI 1.3–9.2]), largely because of its effect on breast cancer risk.[12] Kadouri et al.[13] also found an increased risk of breast cancer (HR 2.09 [95% CI 1.04–4.18]) for *BRCA2* carriers and reported that the median age at onset of breast cancer in *BRCA2* carriers with the *RAD51* C allele was 7 years younger than that in *RAD51* wild-type carriers.

In contrast, Jakubowska et al.[14] evaluated this *RAD51* SNP in a small study of 83 discordant pairs (affected with breast cancer and unaffected) of female carriers of the *BRCA1* founder mutation 5382insC and observed a significantly reduced risk of breast cancer among *RAD51* 135C allele carriers (OR 0.23 [95% CI 0.07–0.62]). In a recent report by the same group,[15] the effect was only marginally significant among the 5382insC carriers ($P = .046$) and became more significant when carriers of the two other *BRCA1* Polish founder mutations (4153delA and 300T→G) were included in the analysis (OR 0.58 [95% CI 0.38–0.91]; $P = .018$).

Studies of genetic modifiers of *BRCA1/2* have been hampered by small sample size, such that the power to detect even moderate effects on cancer risk has been limited. To address this problem, we established CIMBA to conduct collaborative analyses of genetic polymorphisms, involving many thousands of samples, as modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers.[16] In this report, we evaluate the association of the *RAD51* 135G→C polymorphism (*rs1801320*) with breast cancer risk in *BRCA1* and *BRCA2* female mutation carriers.

Material and Methods

Study Population

Eligible study subjects were women aged ⩾18 years who carry a pathogenic mutation in *BRCA1* or *BRCA2*. Information on study subjects was submitted from 19 participating studies from 13 countries. These women participated in clinical and research studies at the host institutions under institutional review board–approved protocols. Data collected included year of birth, mutation description, family membership, ethnicity, country of residence, age at last follow-up, ages at diagnosis of breast and ovarian cancer, and information on bilateral prophylactic mastectomy. Mutations were included in the analysis if they were pathogenic according to generally recognized criteria (from the Breast Cancer Information Core)—that is, (i) mutations generating a premature termination codon as a result of nonsense substitution, frameshift due to small deletion or insertion, aberrant splicing, or large genomic rearrangement (excluding truncating variants in exon 27 of *BRCA2*); (ii) mutations resulting in loss of expression due to deletion of promoter and transcription start site; (iii) large in-frame deletions spanning one or more exons caused by aberrant splicing or large genomic rearrangement; and (iv) missense mutations classified as pathogenic by use of the algorithms of Goldgar et al.[17] and Chenevix-Trench et al.[18]

The functional consequences of pathogenic *BRCA1/2* mutations may depend on their type.[19–21] To examine whether the effect of the *RAD51* 135G→C on breast cancer risk is different for carriers of different types of *BRCA1/2* mutations, we grouped the mutations into two categories. Class 1 corresponds to loss-of-function mutations expected to result in reduced transcript or protein level because of mRNA nonsense-mediated decay (NMD) and/or degradation or instability of truncated proteins,[20–22] translation reinitiation but no production of stable protein,[23] or the absence of expression because of deletion of transcription regulatory regions. Class 2 consists of mutations likely to generate potentially stable mutant protein that might have dominant negative action, partially preserved normal function, or loss of function. Class 2 mutations are missense substitutions and truncating mutations with premature stop codon occurring in the last exon.

Genotyping

Most centers genotyped the 135G→C SNP in the 5′ UTR of *RAD51* by the 5′ nuclease assay (TaqMan) on an ABI 7900HT Sequence Detection System (Applied Biosystems). PCR primers were forward primer 5′-GCTGGGAACTGCAACTCATCT-3′ and reverse primer 5′-GCAGCGCTCCTCTCTCCAGC-3′. Probes were VIC-5′-CAACGCCCGTGGCTTACGCT-3′ and FAM-5′-CCCCAACGCCC-CTGGCTTAC-3′. The annealing temperature was 60°C. The Interdisciplinary Health Research International Team on Breast Cancer Susceptibility (INHERIT BRCAs) and Iceland Landspitali-University Hospital (ILUH) samples and most of the samples from the Ontario Cancer Genetics Network (OCGN) were genotyped by direct sequencing. INHERIT BRCAs and OCGN used forward primer 5′-GTTTGGCGGGAATTCTGAAAGCCG-3′ and reverse primer 5′-GTTCTAAAGACTGAGGTCCACTTG-3′. ILUH used forward primer 5′-TGGGAACTGCAACTCATCTGG-3′ and reverse primer 5′-GCGCTCCTCTCTCCAGCAG-3′. GCHBOC used the iCycler technology (Bio-Rad Laboratories) with forward primer 5′-GGGCAAGCGAGTAGAGAAGTG-3′ and reverse primer 5′-CGCG-CTCCGACTTCAC-3′. Probes were VIC-5′-CAACGCCCCTGGCTT-3′ and FAM-5′-ACGCCCGTGGCTT-3′. The International Hereditary Cancer Centre (IHCC), Helsinki Breast Cancer Study (HEBCS), Deutsche Krebsforschungszentrum (DKFZ), and National Israeli Cancer Center Control (NICCC) used RFLP-PCR analysis, and Memorial Sloan-Kettering Cancer Center (MSKCC) used pyrosequencing (details available on request). All centers included at least 2% of samples in duplicate, no template controls on every plate, and a random mixture of samples of affected and unaffected mutation carriers on each plate. The minimum accepted call rate was 95%. Call rates for each study were in the range 96%–100% (mean 98.5%). There was one discordant result (0.1%) among the 1,008 duplicates genotyped. For each study, the genotype frequencies among unrelated carriers were consistent with the expected frequencies under the assumption of Hardy-Weinberg equilibrium.

All reported CC homozygotes were confirmed by sequencing

with use of forward primer 5′-GAGGGCAGTCTGTAAAACTC-3′ and reverse primer 5′-AACTGCCGCTGAGCACTGGA-3′. One putative homozygote was shown to be a GC heterozygote by sequencing; for another, there was no DNA available for sequencing, so it was excluded from the analysis.

A total of 8,606 mutation carriers with an observed *RAD51* 135G→C genotype were eligible for inclusion in the study. In some instances, more than one study had enrolled carriers from the same family. We therefore investigated for possible overlap between studies by comparing the year of birth, mutation, and the reported ages, to identify potential duplicate individuals. When a potential duplicate was identified, we contacted the relevant centers for further information about these individuals, without revealing their identity. When potential overlap was identified, centers were contacted to determine precisely the extent of true overlap in subjects and families appearing more than once in the data set. To avoid having families extending over several centers, we excluded the smallest version of the family. In total, 94 carriers with a submitted genotype were excluded: 85 appeared twice in the data set, and 9 were excluded because they were part of the family being studied at another center. The *RAD51* 135G→C genotypes between the two submissions of the 85 carriers who appeared twice were identical (one was a CC homozygote). A total of 8,512 unique *BRCA1* and *BRCA2* mutation carriers remained for analysis (table 1).

Statistical Analysis

Individuals were classified according to their age at diagnosis of breast cancer or their age at last follow-up. For this purpose, individuals were censored at the age at the first of the following events: breast cancer diagnosis (n = 4,443), ovarian cancer diagnosis (n = 798), bilateral prophylactic mastectomy (n = 176), or last observation (n = 3,095). For the purpose of our analysis, only carriers censored at breast cancer diagnosis were assumed to be affected. To investigate whether our results were sensitive to the inclusion of prevalent cancer cases, we also performed analyses after excluding patients with breast or ovarian cancer diagnosed >5 years before their age at last follow-up. The 5-year cutoff was selected to maintain a sample size that would still have reasonable power to investigate the effects under consideration, while excluding long-term survivors. Moreover, prognostic data suggest that 5-year survival after breast cancer is ~95%, so that, within this time frame, survival bias would be minimal.[24] The same cutoff has been used in studies investigating environmental modifiers of risk.[25] For this analysis, we excluded subjects for whom an age at interview was not provided. The IHCC study was also excluded because only a censoring age was provided. This left a total of 5,198 mutation carriers for the sensitivity analysis.

To examine whether our results are modified by consideration of information on bilateral prophylactic oophorectomy (BPO), we also performed analyses whereby carriers were censored at age at BPO.[26]

The analysis of associations in *BRCA1* and *BRCA2* mutation carriers is complicated by the fact that mutation carriers are not randomly sampled with respect to their disease phenotype. Many carriers are sampled through genetic clinics, and it is likely that affected individuals are oversampled. In such cases, standard methods of analysis, like Cox regression, do not give valid estimates of the HRs.[27] To correct for this potential bias, we analyzed the data within a retrospective likelihood framework, by modeling the likelihood of the observed *RAD51* 135G→C genotypes and disease phenotypes conditional on the disease phenotypes[28] (appendix A). In this model, the breast cancer incidence was assumed to depend on the underlying *RAD51* 135G→C genotype through a Cox proportional hazards model: $\lambda_i(t) = \lambda_0(t)\exp(\beta_i)$, where $\exp(\beta_i)$ is the HR for genotype i and $\lambda_0(t)$ is the breast cancer incidence rate in the baseline category. We estimated the log-HRs for genotypes GC and CC, using the GG homozygotes as the baseline category. The baseline age-specific incidence rates were chosen such that the overall breast cancer incidence rates, averaged over all genotypic categories, agreed with external estimates of *BRCA1* and *BRCA2* incidence rates. This process is described in detail elsewhere.[29] For this purpose, we used the calendar-specific, cohort-specific (for cohorts based on birth year: before 1920, 1920–1929, 1930–1939, 1940–1949, and 1950 and after), and age-specific incidence rates derived using combined data from the meta-analysis of the families of *BRCA1/2* carriers identified through population-based studies of breast and ovarian cancer[1] and data from three population-based studies of breast cancer[31–35] (A.C.A., unpublished data). These analyses were performed using the pedigree analysis software MENDEL.[30] We also fitted models in which the log-relative hazards were allowed to vary with age. Significance tests for the null hypothesis that the log-HRs are equal to 0 were also performed using a score test statistic based on the retrospective likelihood described above[36] (also see appendix A). Between-study heterogeneity was examined by comparing the log-likelihood of models with study-specific log-HRs (logL1) against the log-likelihood of models in which the same log-HR was assumed to apply to all studies (logL2). These likelihood-ratio tests are approximate because of the small numbers of carriers in each study and the fact that some parameter estimates converge to boundaries.

All analyses used mutation-, calendar-, and cohort-specific breast cancer incidence rates and were stratified by study, country of residence, and reported ethnicity. Analyses were performed for *BRCA1* and *BRCA2* mutation carriers combined and separately. Fourteen compound *BRCA1* and *BRCA2* mutation carriers were included in all analyses. In all instances, a robust variance approach was used to allow for the dependence between related carriers.[37,38]

RNA Extraction and RT-PCR

Total RNA was extracted from lymphoblastoid cell lines (in INHERIT BRCAs and GEMO studies) with the RNeasy mini kit (QIAGEN) according to the manufacturer's instruction, with a digestion step by use of DNase I (QIAGEN). A total of 5 μg of RNA was reverse transcribed with SuperScript II Rnase H-Reverse Transcriptase (Invitrogen Life Technologies) by use of random primers (Promega). To explore the alternative splicing within the 5′ UTR of *RAD51,* RT-PCR was performed in the presence of 1.5% dimethyl sulfoxide (DMSO) by use of a forward primer located in the 5′ UTR (5′-AGACCGAGCCCTAAGGAGAG-3′) and a reverse primer located at the exon 2–exon 3 junction (5′-CCACACTGCTCTAACCGTGA-3′) (fig. 1). This RT-PCR was also performed in human mammary epithelial cells (CC-2551 [Lonza]) and human breast cancer cell lines MCF-7 (HTB-22 [ATCC]), MDA-MB-157 (HTB-24 [ATCC]), ZR-75-1 (CRL-1500 [ATCC]), and T47D (HTB-133 [ATCC]). To test whether the *RAD51* 5′ UTR isoform 2 was associated with specific alternative splicing of coding exons, we performed RT-PCR by using a forward primer specific to isoform 2 (5′-GAAGTGGAGCTAATGGCAATG-3′) and a reverse primer in exon 7 (5′-CTGGTGGTCTGTGTTGAACG-3′). The amplified frag-

Table 1. Description of Studies and Number of Carriers Included in the Analysis

Study	Country of Residence	Ascertainment Basis	No. of Carriers (%) with *RAD51* 135G→C Genotype			Total	Genotyping Platform
			GG	GC	CC		
CNIO	Spain	Clinic	188 (82.8)	37 (16.3)	2 (.9)	227	TaqMan
DKFZ Heidelberg	Germany, Pakistan, Colombia	Clinic	151 (87.3)	21 (12.1)	1 (.6)	173	RFLP confirmed by sequencing
EMBRACE	United Kingdom, Ireland	Clinic	659 (87.2)	91 (12.0)	6 (.8)	756	TaqMan
GCHBOC	Germany	Clinic	630 (86.8)	92 (12.7)	4 (.5)	726	Bio-Rad iCycler
GEMO	France, United States, Greece	Clinic	1,098 (84.3)	197 (15.1)	8 (.6)	1,303	TaqMan
HEBCS	Finland	Clinic	160 (86.0)	25 (13.4)	1 (.5)	186	RFLP confirmed by sequencing
INHERIT BRCAs	Canada	Clinic	132 (86.8)	18 (11.8)	2 (1.3)	152	Sequencing
KConFab	Australia, New Zealand	Clinic	631 (88.0)	84 (11.7)	2 (.3)	717	TaqMan
Leiden University Medical Centre	Netherlands	Clinic	242 (93.4)	16 (6.2)	1 (.4)	259	TaqMan
MAGIC	United States, Austria, Canada	Clinic	888 (85.4)	142 (13.6)	10 (1.0)	1,040	TaqMan
MAYO Clinic	United States	Clinic	92 (82.1)	18 (16.1)	2 (1.8)	112	TaqMan
MSKCC	United States, Brazil, Canada, Philippines	Clinic	406 (90.0)	43 (9.5)	2 (.4)	451	Pyrosequencing
Mod-Squad	Czech Republic	Clinic/population	160 (83.3)	30 (15.6)	2 (1.0)	192	TaqMan
NCI	United States	Clinic/community	205 (86.9)	30 (12.7)	1 (.4)	236	TaqMan, sequencing
NICCC	Israel	Clinic/population	308 (93.6)	21 (6.4)	0 (0)	329	RFLP confirmed by sequencing
OCGN	Canada	Clinic/population	261 (86.4)	39 (12.9)	2 (.7)	302	TaqMan, sequencing
IHCC	Poland	Clinic	661 (77.1)	186 (21.7)	10 (1.2)	857	PCR-RFLP, sequencing
ILUH	Iceland	Clinic/population	101 (95.3)	5 (4.7)	0 (0)	106	Sequencing
Sheeba Medical Centre	Israel	Clinic	350 (90.2)	37 (9.5)	1 (.3)	388	TaqMan
All	...	...	7,323 (86.0)	1,132 (13.3)	57 (.7)	8,512	...

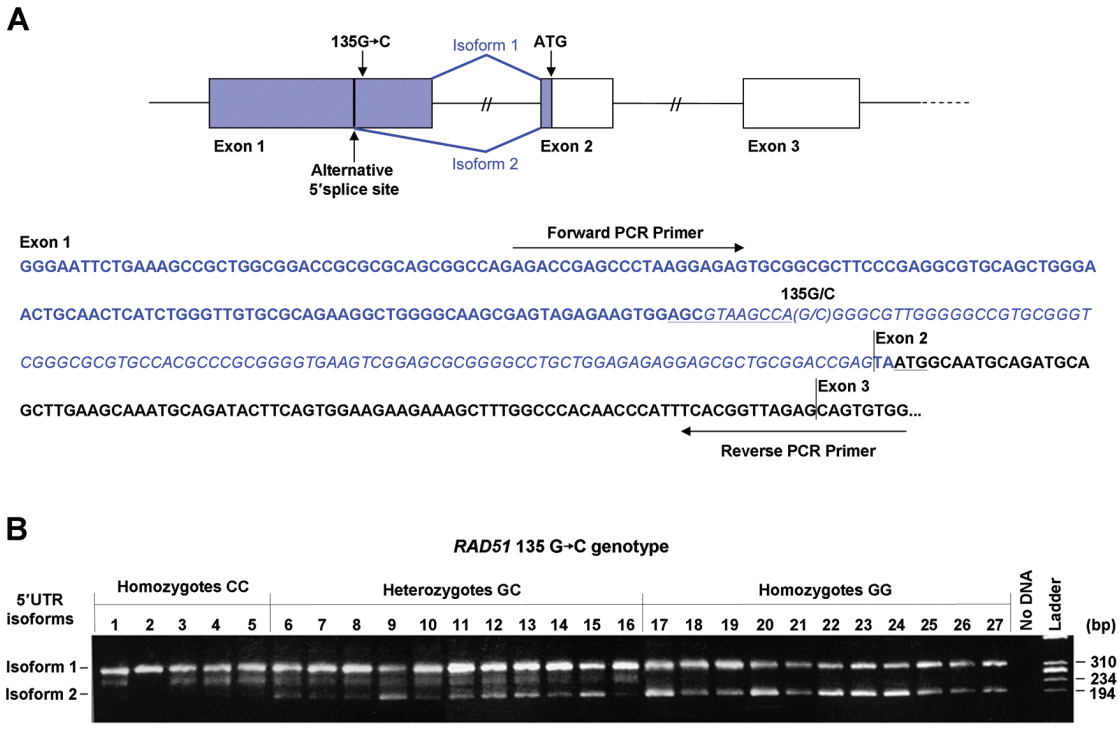

Figure 1. *RAD51* 135G→C variant and alternative splicing within the 5′ UTR. *A,* Schematic representation and sequence of 5′ *RAD51* exons. Exons are represented by hatched boxes (in 5′ UTR) and unblackened boxes (in coding region). Major splicing patterns are shown by blue connecting lines above (for isoform 1) and below (for isoform 2) the gene scheme. ATG is the translation initiation codon. The nucleotide sequence of the full-length 5′ UTR is in blue, the 5′ UTR sequence alternatively spliced as part of intron 1 is in italics, and the canonical motif of the alternative 5′ splice site within the 5′ UTR is underlined. *B,* Results of the RT-PCR performed with the primers shown in panel A in lymphoblastoid cell lines from carriers of three genotypes of the *RAD51* 135G→C variant. A predominant *RAD51* transcript with the longest 5′ UTR (isoform 1, with full-length 5′ UTR of length 257 nt [GenBank accession number NM_002875]) and a less abundant transcript, with the shortest 5′ UTR (isoform 2, with truncated 5′ UTR of length 153 nt [GenBank accession number AK131299]), as well as several minor *RAD51* transcript isoforms with intermediate 5′ UTR lengths characterized by sequencing, were detected.

ments were sequenced using BDT V1.1 (Applied Biosystems) on the ABI PRISM 3100 (Applied Biosystems). These experiments and the quantitative real-time PCR measurements were performed in the Cancer Genomics Laboratory, Centre Hospitalier Universitaire (CHU) de Quebec & Laval University, and in the Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Hospices Civils de Lyon–Centre Léon Bérard, Lyon.

Quantitative Real-Time PCR Experiments

Experiment 1.—First-strand cDNA synthesis was performed using 1–5 μg of total RNA with Superscript III Rnase H-Reverse Transcriptase (Invitrogen Life Technologies) and oligo-dT_{18}. The resulting products were purified with Qiaquick PCR purification kits (QIAGEN). cDNA corresponding to 40–300 ng of total RNA was used to perform fluorescent-based real-time PCR quantification by use of LightCycler FastStart DNA Master^{Plus} SYBR Green I (Roche) on the LightCycler Realtime PCR apparatus (Roche) as described by the manufacturer. The amplification of *RAD51* isoform 1 required the addition of 4% DMSO in the reaction mix for PCR. To ensure that a specific fluorescence signal was read, PCRs were brought for 3 s to a temperature a few degrees below the melting temperature of DNA fragments. The fluorescence signal was then registered at this temperature at the end of each cycle (for isoform 1, 84°C; for isoform 2, 78°C). A melting curve was performed at the end of each run to assess nonspecific signal. The primer pairs used for the specific amplification of *RAD51* isoforms were as follows: for *RAD51* isoform 1, forward primer 5′-AAGCGAGTAGAGAAGTGGAGCGTA-3′ and reverse primer 5′-ACTGCTCTAACCGTGAAATGGG-3′; for *RAD51* isoform 2, forward primer 5′-AGAGAAGTGGAGCTAATGGCAATG-3′ and reverse primer 5′-ACTGCTCTAACCGTGAAATGGG-3′. The specificity of isoform 1 primers was also verified by performing PCR with the purified isoform 2 amplicon as a template, and no amplified product was detected. The housekeeping gene glucose-6-phosphate dehydrogenase (*G6PD* [MIM 305900]) (forward primer 5′-GATGTCCCCTGTCCCACCAACTCTG-3′; reverse primer 5′-GCAGGGCATTGAGGTTGGGAG-3′) was used for normalization. Standard curves were established using serial dilutions of known cDNA amounts for each *RAD51* isoform and *G6PD,* and the expression was quantified as described elsewhere.[39] All experiments were done in duplicate.

Experiment 2.—A total of 2 μg of total RNA was reverse transcribed using the First-Strand cDNA Synthesis Kit and pd(N)6 random primers (Amersham Biosciences). Quantification of *RAD51*

Table 2. Patient Characteristics

Characteristic	All Patients	*BRCA1* Mutation Carriers[a]		*BRCA2* Mutation Carriers[a]	
		Unaffected	With Breast Cancer	Unaffected	With Breast Cancer
No. of carriers	8,512	2,902	2,876	1,174	1,574
Length of follow-up (person-years)	363,476	122,953	118,711	52,575	69,825
Mean ± SD age at censure (years)	42.9 ± 11.2	42.6 ± 12.5	41.4 ± 9.3	45.1 ± 13.1	44.5 ± 10.1
Year of birth:					
Before 1920	91 (1.1)	19 (.7)	29 (1.0)	17 (1.5)	26 (1.7)
1920–1929	334 (3.9)	74 (2.6)	115 (4.0)	43 (3.7)	102 (6.5)
1930–1939	829 (9.7)	214 (7.4)	291 (10.1)	105 (8.9)	221 (14.0)
1940–1949	1,704 (20.0)	422 (14.5)	701 (24.4)	193 (16.4)	389 (24.7)
1950–1959	2,590 (30.4)	807 (27.8)	985 (34.3)	299 (25.5)	504 (32.0)
1960 or later	2,964 (34.8)	1,366 (47.1)	755 (26.3)	517 (44.0)	332 (21.1)
Ethnicity:					
White	6,935 (81.5)	2,344 (80.8)	2,351 (81.8)	916 (78.0)	1,334 (84.8)
Ashkenazi Jewish	1,329 (15.6)	473 (16.3)	425 (14.8)	229 (19.5)	206 (13.1)
African American	54 (.6)	17 (.6)	21 (.7)	9 (.8)	7 (.4)
Asian	57 (.7)	16 (.6)	26 (.9)	2 (.2)	13 (.8)
Hispanic	79 (.9)	29 (1.0)	28 (1.0)	12 (1.0)	10 (.6)
Other	58 (.7)	23 (.8)	25 (.9)	6 (.5)	4 (.3)
Parity:					
Nulliparous	693 (8.1)	285 (9.8)	190 (6.6)	123 (10.5)	95 (6.0)
1 child	652 (7.7)	190 (6.6)	249 (8.7)	89 (7.6)	124 (7.9)
2 children	1,564 (18.4)	486 (16.8)	544 (18.9)	222 (18.9)	314 (20.0)
3 children	885 (10.4)	257 (8.9)	303 (10.5)	141 (12.0)	185 (11.8)
⩾4 children	517 (6.1)	168 (5.8)	154 (5.4)	86 (7.3)	110 (7.0)
Data missing	4,201 (49.4)	1,516 (52.2)	1,436 (49.9)	513 (43.7)	746 (47.4)
Oophorectomy:					
None	4,148 (48.7)	1,275 (43.9)	1,457 (50.6)	621 (52.9)	803 (51.0)
BPO	452 (5.3)	243 (8.4)	62 (2.2)	105 (8.9)	42 (2.7)
Data missing	3,912 (46.0)	1,384 (47.7)	1,357 (47.2)	448 (38.2)	729 (46.3)

NOTE.—Data are no. (%) of carriers, unless otherwise indicated.

[a] Includes 14 compound *BRCA1* and *BRCA2* mutation carriers.

isoform 1 and isoform 2 expression by real-time PCR was performed using LightCycler FastStart DNA Master^{PLUS} SYBR Green I (Roche) on the LightCycler 2.0 instrument (Roche) as described by the manufacturer. Primer pairs used for specific amplification of *RAD51* isoforms were as follows: for *RAD51* isoform 1, forward primer 5′-GGCCTGCTGGAGAGAGGA-3′ and reverse primer 5′-CCACACTGCTCTAACCGTGA-3′; for *RAD51* isoform 2, forward primer 5′-GAAGTGGAGCTAATGGCAATG-3′ and reverse primer 5′-CCACACTGCTCTAACCGTGA-3′. A melting curve was performed at the end of each run to assess nonspecific signal. The levels of the reference housekeeping genes glyceraldehyde-3-phosphatase dehydrogenase (*GAPDH* [MIM 138400]) (forward primer 5′-AGCCACATCGCTCAGACAC-3′ and reverse primer 5′-GCCCAATACGACCAAATCC-3′) and β-actin (*ACTB* [MIM 102630]) (forward primer 5′-ATTGGCAATGAGCGGTTC-3′ and reverse primer 5′-GGATGCCACAGGACTCCAT-3′) were quantified using TaqMan probes UPL#60 and UPL#11, respectively, and LightCycler Taqman Master (Roche) as described by the manufacturer. All experiments were done in duplicate and were normalized to the geometric mean of the level of these reference genes. Relative standard curves determining the PCR efficiencies of the *RAD51* isoforms, *GAPDH,* and *ACTB* were established using cDNA serial dilutions. Efficiency-corrected and calibrator-normalized calculations were performed using the LightCycler Relative Quantification Software (Roche). The nonparametric Kruskal-Wallis test was used to test for differences in the distribution of expression levels between genotypes implemented in STATA (version 8.2 for Unix [Statacorp]).

Results

A total of 8,512 *BRCA1* and *BRCA2* mutation carriers were used in the analysis (tables 1 and 2). The overall *RAD51* genotype frequencies (GC 13.3%; CC 0.7%) were similar to those reported in population-based studies in the United Kingdom and Australia,[40,41] but there was some variation in the frequencies between the CIMBA centers. Carriers of the *RAD51* 135C allele were least common in the Icelandic (ILUH) study and were most frequent among Polish *BRCA1* mutation carriers. The *RAD51* genotype frequencies were similar across the larger studies of GEMO, Modifiers and Genetics in Cancer (MAGIC), GCHBOC, EMBRACE, and kConFab.

The genotype frequencies by mutation and disease status are shown in table 3, along with the estimated HRs. There was evidence of association between the *RAD51* 135G→C genotype and breast cancer risk among *BRCA1* and *BRCA2* mutation carriers combined ($P = .002$, by 2-df test). The estimated breast cancer HR was 0.95 (95% CI 0.83–1.07; $P = .292$) in GC individuals and 1.92 (95% CI 1.25–2.94; $P = .0008$) in CC individuals. The estimated HR for the CC homozygotes was higher among *BRCA2* mutation carriers (HR 3.18 [95% CI 1.39–7.27]; $P = .0004$) than among *BRCA1* mutation carriers (HR 1.59

Table 3. *RAD51* 135G→C Genotype Distribution and Estimated HRs in the Total Sample of *BRCA1* and *BRCA2* Mutation Carriers

Gene and Genotype or Test	No. (%) of Carriers		HR (95% CI)	*P*[a]
	Unaffected	With Breast Cancer		
BRCA1/2:				
GG	3,485 (85.6)	3,838 (86.4)	1.00	…
GC	565 (13.9)	567 (12.7)	.95 (.83–1.07)	.292
CC	19 (.5)	38 (.9)	1.92 (1.25–2.94)	.0008
2-df Test	…	…	…	.002
Trend test	…	…	…	.801
BRCA1:				
GG	2,456 (84.6)	2,475 (86.0)	1.00	…
GC	429 (14.8)	376 (13.1)	.86 (.77–1.02)	.095
CC	17 (.6)	25 (.9)	1.59 (.96–2.63)	.067
2-df Test	…	…	…	.046
Trend test	…	…	…	.386
BRCA2:				
GG	1,036 (88.2)	1,370 (87.0)	1.00	…
GC	136 (11.6)	191 (12.1)	1.17 (.91–1.51)	.123
CC	2 (.2)	13 (.8)	3.18 (1.39–7.27)	.0004
2-df Test	…	…	…	.0007
Trend test	…	…	…	.007

NOTE.—Compound *BRCA1* and *BRCA2* carriers were included in all analyses.

[a] *P* value by the score test.

[95% CI 0.96–2.63]), although the difference in HR was not statistically significant. There were no statistically significant differences between the estimated HRs for patients aged <40 years and those aged ⩾40 years, for either gene (results not shown). Likelihood-ratio tests suggest some evidence of heterogeneity in the HR estimates among centers for *BRCA2* ($\chi^2 = 41.6$, df = 24) but not for *BRCA1* ($\chi^2 = 32.7$, df = 32).

To investigate whether our results were sensitive to the inclusion of patients with cancer diagnosed a long time before their recruitment into the various studies (i.e., "prevalent" cancer cases) who may introduce a survival bias, we repeated our analysis after excluding patients with breast or ovarian cancer diagnosed >5 years before their last follow-up. The estimated HRs for CC individuals were very similar to those obtained with the complete data set (*BRCA1/2* combined HR 2.36 [95% CI 1.31–4.26]; *BRCA1* HR 1.98 [95% CI 0.88–4.46]; *BRCA2* HR 3.37 [95% CI 1.33–8.55]).

It is well established that BPO significantly reduces the risk of breast cancer among *BRCA1* and *BRCA2* mutation carriers.[26,42] We therefore examined whether our results are modified by considering the information on BPO, by censoring carriers at BPO in two ways: mutation carriers with missing information on BPO either were included in the analysis under the assumption that they had not undergone BPO or were excluded from the analysis. When all carriers were used in the analysis, the results were very similar to those of the primary analysis (data not shown). When subjects with missing information on BPO were excluded, the HR among *BRCA2* mutation carriers with the GC genotype was estimated to be 1.35 (95% CI 0.97–1.89) and, among *BRCA2* carriers with the CC genotype, was estimated to be 5.67 (95% CI 1.64–19.63). Thus, the estimated HRs were comparable to those in the overall analysis, although the effect in the combined sample of *BRCA1* and *BRCA2* mutation carriers was no longer statistically significant.

Although there are limited experimental data on the functional consequence of *BRCA1* and *BRCA2* mutations, current evidence suggests that most of the premature stop-codon mutations result in loss of function caused by reduced transcript and protein levels due to NMD and/or instability of the truncated proteins (class 1 mutations).[20–23] On the other hand, missense or in-frame deletion/insertion mutations, as well as truncating mutations that are not subject to NMD, might generate stable mutant protein that has consequences other than loss of function (class 2 mutations). It is possible that the *BRCA1/2* mutations in these two categories may interact in different ways with the potential cancer risk modifier effect of *RAD51* 135G→C. Of the *BRCA1* mutation carriers, 64% carried class 1 mutations, 33% carried class 2 mutations, and 3% carried mutations of unpredictable consequence at transcript or protein level. The majority (95%) of the *BRCA2* mutation carriers carried class 1 mutations. There was some indication that the *RAD51* 135G→C genotype association was greater in carriers of class 1 *BRCA1* mutations, with an estimated HR among CC homozygotes of 1.97 (95% CI 1.05–3.70; $P = .041$), and had no effect in carriers of class 2 mutations (HR 1.11 [95% CI 0.42–2.93]) (table 4). However, the difference in the HRs by mutation type was not statisically significant. The corresponding HR

Table 4. *RAD51* 135G→C Genotype Distribution and Estimated HRs by *BRCA1* and *BRCA2* Mutation Class

Mutation Class and Genotype or Test	No. (%) of Carriers		HR (95% CI)	*P*[a]
	Unaffected	With Breast Cancer		
BRCA1 class 1:				
GG	1,639 (85.8)	1,562 (87.5)	1.00	…
GC	262 (13.7)	208 (11.6)	.83 (.68–1.00)	.058
CC	9 (.5)	16 (.9)	1.97 (1.05–3.70)	.041
2-df Test	…	…	…	.021
Trend test	…	…	…	.284
BRCA1 class 2:				
GG	753 (81.9)	825 (83.9)	1.00	…
GC	158 (17.2)	150 (15.3)	.93 (.74–1.17)	.567
CC	8 (.9)	8 (.8)	1.11 (.42–2.93)	.780
2-df Test	…	…	…	.817
Trend test	…	…	…	.525
BRCA2 class 1:				
GG	995 (87.8)	1,289 (87.0)	1.00	…
GC	136 (12.0)	179 (12.1)	1.14 (.88–1.48)	.123
CC	2 (.2)	13 (.9)	3.24 (1.41–7.45)	.0004
2-df Test	…	…	…	.0007
Trend test	…	…	…	.007

NOTE.—See main text for definition of mutation classes.

[a] *P* value by the score test.

estimates among the GC heterozygotes did not differ significantly from unity (0.83 [95% CI 0.68–1.00] and 0.93 [95% CI 1.05–3.70] for carriers of class 1 and class 2 BRCA1 mutations, respectively).

To evaluate whether the observed association between the *RAD51* 135G→C genotype and breast cancer risk was an indirect effect due to another (untested) variant in the *RAD51* region, we used the HapMap data for CEPH individuals from Utah data to identify SNPs in strong linkage disequilibrium with 135G→C (International HapMap Project). Fifty SNPs had an $r^2 > 0.50$ with 135G→C, in a region of 285 kb. Of these, 44 were perfectly correlated with 135G→C ($r^2 = 1.0$) and would not be distinguishable by genetic epidemiological studies in European populations. Only 2 of the 50 SNPs, however, were located within *RAD51* ($r^2 = 1.0$ and 0.51). SNP *rs2304579* ($r^2 = 1.0$) is in the *RAD51* intron 2 (IVS2+110A→G), and SNP *rs4144242* ($r^2 = 0.51$) is in intron 5 (IVS5-4016G→A). By use of software tools that assess splice sites and splice-enhancer motifs, these deep intronic SNPs were not predicted to be of functional significance (SpliceSiteFinder, Splice Site Prediction, and ESE Finder). Three of the other proxy SNPs for *RAD51* 135G→C are located within the neighboring cancer susceptibility candidate 5 gene (*CASC5* [MIM 609173]). SNP *rs10518696* is in an intron, and SNPs *rs8034726* and *rs16970854* are in the promoter region. *CASC5* (also known as "*AF15Q14*" and "*D40*") is a component of the hMis12 kinetochore complex essential for chromosome segregation[43] and is fused to the *MLL* gene as a result of translocations in some cases of acute lymphoblastic and myeloblastic leukemias.[44–46] However, there is no known interaction between *CASC5* and *BRCA1* or *BRCA2,* so we consider *RAD51* to be the more plausible modifier of risk.

The molecular mechanism by which the *RAD51* 135G→C SNP results in increased breast cancer risk in *BRCA2* mutation carriers is unclear. Alternative splicing of the human *RAD51* gene, involving coding and noncoding exons, has been reported in genomic databases (including GenBank). Some mRNAs had 5′ UTRs shorter than the 5′ UTR of the full-length *RAD51* transcript (GenBank accession number NM_002875), with 135G→C located 1 nt next to the canonical motif of the alternative 5′ splice site within the 5′ UTR (fig. 1*A*).

To investigate whether 135G→C is involved in the regulation of the pattern of the 5′ UTR alternatively spliced *RAD51* transcripts, we examined their relative abundance in lymphoblastoid cell lines established from 5 CC homozygotes, 20 GC heterozygotes, and 20 GG homozygotes by performing RT-PCR, encompassing the 5′ UTR, exon 1, and exon 2, including the beginning of the coding region (fig. 1*A* and 1*B*). Several *RAD51* transcripts with varying length for the 5′ UTR were detected. The two predominant *RAD51* transcripts had the longest and the shortest 5′ UTRs: isoform 1 (full-length 5′ UTR of 257 nt [GenBank accession number NM_002875]) and isoform 2 (truncated 5′ UTR of 153 nt [GenBank accession number AK131299]), generated by an alternative splicing within the 5′ UTR, which removes the 104-nt 5′ UTR sequence recognized as part of intron 1. These RT-PCR experiments suggested that the level of the isoform 2 transcript varied between cell lines with different 135G→C genotypes and was found to be particularly low in CC homozygotes (fig. 1*B*). To investigate this observation further, we used quantitative real-time PCR to compare the expression of the *RAD51* isoforms 1 and 2 across three 135G→C genotypes. The quantitative real-time PCR quantification was performed in two laboratories with overlapping sets of samples, by use of different housekeeping genes for normalizing the *RAD51* expression data. The results between the two experiments were very similar and are shown in detail in figure 2. There was no statistically significant difference in the expression levels for isoform 1 among the three genotypes (experiment 1, $P = .92$; experiment 2, $P = .44$), but there were significant differences among the genotypes in the expression of isoform 2 (experiment 1, $P = .008$; experiment 2, $P = .001$). Pairwise genotype comparisons of the mean expression levels of isoform 2 revealed 3–7-fold differences between GG and CC homozygotes (experiment 1, $P = .007$; experiment 2, $P = .002$) and 2–4-fold differences between GC heterozygotes and CC homozygotes (experiment 1, $P = .012$; experiment 2, $P = .002$). There were no statistically significant differences between the expression levels of isoform 2 between GG homozygotes and GC heterozygotes (experiment 1, $P = .137$; experiment 2, $P = .087$).

To test whether the *RAD51* 5′ UTR isoform 2 is associated with specific alternative splicing of coding exons and may, therefore, give rise to a variant *RAD51* protein, we performed RT-PCR, using the forward primer specific to isoform 2 and the reverse primer in exon 7 (see the "Material and Methods" section), generating an amplicon covering the *RAD51* exons reported to be alternatively spliced. However, no isoform 2 transcripts with alternative splicing of coding exons were detected.

To find out whether alternative *RAD51* splicing that produces isoforms 1 and 2 transcripts is present in mammary gland and is not limited to lymphoblastoid cell lines, we studied human mammary epithelial cells and four human breast cancer cell lines: MCF-7, MDA-MB-157, ZR-75-1, and T47D. Both isoforms 1 and 2 were detected in the breast epithelium and cancer samples tested (data not shown).

Discussion

In this study, we combined data from 19 studies to investigate the effect of the *RAD51* 135G→C SNP on breast cancer risk among *BRCA1* and *BRCA2* female mutation carriers and found evidence that this polymorphism is associated with breast cancer risk among *BRCA2* mutation carriers ($P = .007$, by trend test; $P = .0007$, by 2-df test). We found no evidence of a higher risk in GC heterozygotes, and the sample size was sufficient to rule out any

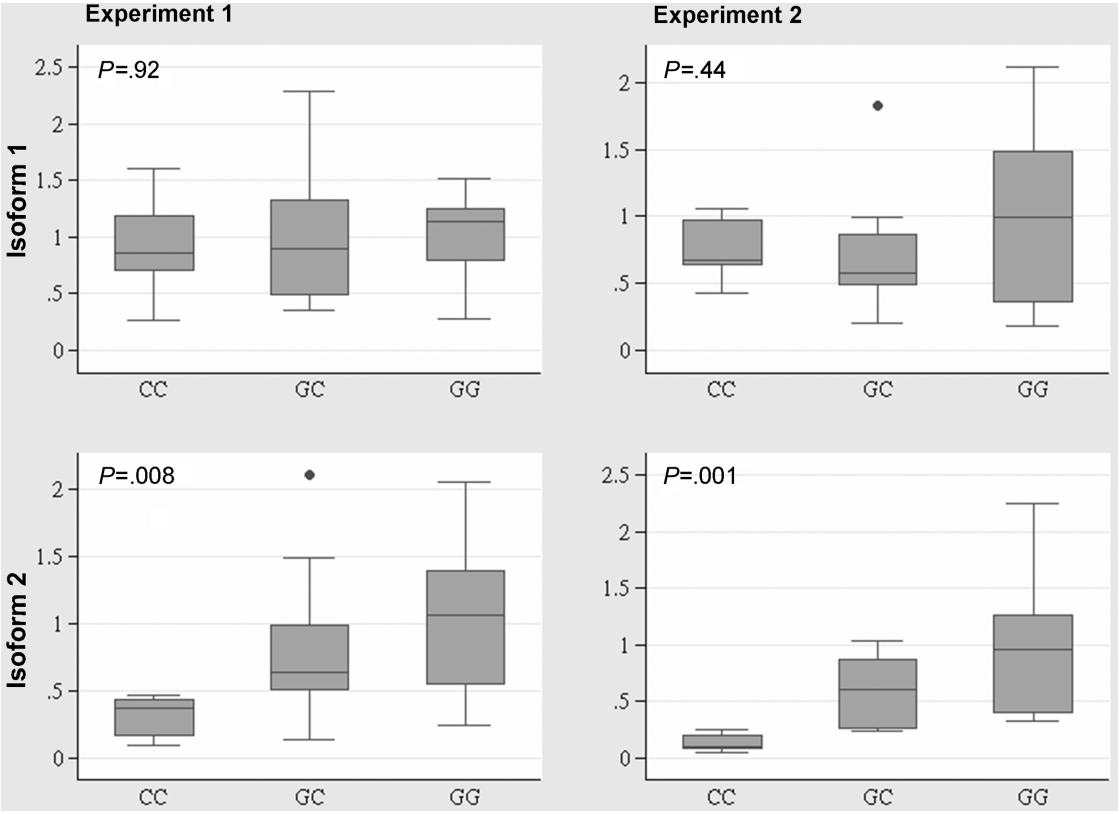

Figure 2. Relative levels of the *RAD51* isoforms 1 and 2 transcripts by genotype, measured by quantitative RT-PCR in lymphoblastoid cell lines established from individuals with different *RAD51* 135G→C genotypes. The relative expression level of each *RAD51* isoform across the three 135G/C variant genotypes was normalized by the geometric mean of the expression level of the reference housekeeping genes (in experiment 1, *G6PD*; in experiment 2, *GAPDH* and *ACTB*) and are given in arbitrary units relative to the mean level for the GG genotype. Two replicates were performed for each experiment. The nonparametric Kruskal-Wallis tests were performed to investigate differences in the distributions of the isoform levels across the genotypes.

substantial risk in this group (HR 1.17 [95% CI 0.91–1.51]). However, we found strong evidence of an increased risk in CC homozygotes (HR 3.18 [95% CI 1.39–7.27]). The association with breast cancer risk was weaker or nonexistent in *BRCA1* carriers, but larger studies will be needed to determine whether there is any association in this group.

Since CC homozygotes are rare, previous studies would have been too small to detect this effect. Only a study of this size, made possible through the CIMBA collaboration, allows such effects to be detected reliably. The association with risk in *BRCA2* carriers was essentially unaltered by restriction to incident cases and by adjustment for prophylactic oophorectomy.

There was some evidence of heterogeneity in the HRs between the studies for *BRCA2* mutation carriers. However, this may be partly because of the very small number of rare homozygotes in each study, which results in imprecise study-specific estimates. Studies with larger numbers of *BRCA2* mutation carriers are required to clarify this further.

Three previously published studies found evidence that carriers of the 135C allele among *BRCA2* mutation carriers were at a significantly increased risk of breast cancer,[11–13] with the evidence largely coming from the heterozygotes. Although the data from one of these studies are included in the current combined CIMBA analysis[11] and although partial overlap may exist with the other two studies, which we cannot establish with certainty, we did not find significant evidence of an increased risk among the heterozygotes.

Two additional studies investigated the effect of the *RAD51* 135G→C polymorphism among Polish *BRCA1* mutation carriers and found a significant protective effect among carriers of the 135C allele.[14,15] These data were included in the CIMBA analysis (as a subset of the IHCC study), but the combined analysis does not support this finding. This discrepancy may reflect differences in the eligibility criteria for participation in each study, differences in the study size, or differences in the statistical methods used. In the present analysis, the IHCC study–specific HR estimates were not significantly different from 1 for either the heterozygotes or the rare homozygotes. Interestingly, 93% of the carriers in the IHCC group carry

BRCA1 class 2 mutations, for which no effect was detected in our analysis.

The effect of the *RAD51* 135G→C SNP in unselected series of breast cancer cases has been investigated in a small number of studies. Kuschel et al.[40] found no significant association with breast cancer among heterozygote carriers, whereas the OR estimate for rare homozygotes was 2.5 (95% CI 0.6–10.9). However, preliminary analyses of a larger sample from the same population found an OR of 0.97 (95% CI 0.45–2.07) among the CC homozygotes (K. Pooley, personal communication). Another population-based study from Australia also found no evidence of association with breast cancer risk.[41] These observations raise the possibility that the association between *RAD51* 135G→C and breast cancer risk is specific to *BRCA2* carriers.

BRCA2 mediates the homologous recombination activity of RAD51 through binding to this DNA recombinase and is essential for the repair of DNA double-strand breaks.[6,47] The effect on breast cancer risk observed in our study suggests an interaction between the functional outcomes of the *RAD51* 135C allele and *BRCA2*-inactivating mutations. Because of the strong linkage disequilibrium in this region, genetic studies cannot distinguish whether the association is being driven by *RAD51* 135G→C itself or by other polymorphisms in the region (including *CASC5*), but *RAD51* 135G→C is the most plausible causative variant. Prior characterization of *RAD51* suggested that it contains a 747-nt basic promoter region that includes the 135G→C polymorphic site.[48] Furthermore, expression constructs containing the 135C allele had higher activity than that of the wild type.

Our study suggests an effect of the *RAD51* 135G→C variant on the *RAD51* alternative splicing within the 5′ UTR. The level of the *RAD51* alternative isoform 2 transcript is significantly decreased in the lymphoblastoid cell lines of 135CC homozygotes, compared with other genotypes. Interestingly, the part of the 5′ UTR sequence that is lacking in isoform 2 has a particularly high GC content (77%) and is predicted to create highly stable stem-loop secondary structures (RNA and DNA Folding Applications).[49] Such structures are known to negatively regulate the translation potential, by inhibiting binding or scanning of the translation machinery.[50,51] Therefore, isoform 2 would be expected to have high translation efficiency. These findings suggest that the *RAD51* 135C allele may cause an overall lower abundance of RAD51 protein, thereby providing an insight into the molecular mechanism through which this *RAD51* variant may affect cancer risk. Further studies will be needed to characterize the consequences of this SNP at the protein level, particularly in breast tissue.

The identification of *RAD51* as a genetic modifier may have implications for the clinical management of *BRCA2* mutation carriers. On the basis of the estimated HR in CC homozygotes, the estimated absolute risk of breast cancer in *BRCA2* carriers by age 70 years in the most recent birth cohort would be 90% in CC homozygotes, compared with 51% in GG homozygotes. This difference may be sufficiently large to alter management for those rare patients who are *RAD51* CC homozygotes.

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Author affiliations.—Cancer Research UK, Genetic Epidemiology Unit, Department of Public Health and Primary Care (A.C.A.; S.P.; M.C.; D.F.E.), and Cancer Research UK, Human Cancer Genetics Group, Department of Oncology (C.B.), University of Cambridge, Cambridge, United Kingdom; Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Hospices Civils de Lyon–Centre Léon Bérard (O.M.S.; M.L.; L.B.), Laboratoire de Génétique Moléculaire, Signalisation et Cancer UMR5201 CNRS, Université Claude Bernard (O.M.S.; L.B.), Université de Lyon, Université Lyon 1, CNRS UMR5558, Laboratoire de Biometrie et Biologie Evolutive (C.L.; V.B.), and Centre Léon Bérard (C.L.; V.B.), Lyon, France; Cancer Genomics Laboratory, Oncology and Molecular Endocrinology Research Center, CHU de Quebec and Laval University, Quebec (J.S.; M.D.; F.D.); Department of Epidemiology, University of California–Irvine, Irvine (S.L.N.); Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics (J.P.S.; M.H.G.; S.A.P.), and Laboratory of Population Genetics (J.P.S.) and Cancer Genetics Branch (S.A.P.), Center for Cancer Research, US NCI, Bethesda; INSERM U509, Service de Génétique Oncologique, Institut Curie, Paris (D.S-.L.; I.C.; M.B.); Department of Clinical Medicine, Trinity College (D.J.H.), Department of Medical Oncology, St. James's Hospital and Trinity College (P.A.D.), and Cancer Genetics Program, St. James's Hospital (P.D.), Dublin; INSERM UMR484, Centre Jean Perrin, Clermont–Ferrand, France (Y.-J.B.); Center for Clinical Epidemiology and Biostatistics (T.R.R.), Abramson Cancer Center (T.R.R.; S.M.D.; K.L.N.), and Department of Medicine (S.M.D.; K.L.N.), The University of Pennsylvania School of Medicine, and Fox Chase Cancer Center (A.G.), Philadelphia; University of Vienna, Vienna (T.W.); Creighton University, Omaha (H.T.L.); Dana Farber Cancer Institute, Boston (J.E.G.); City of Hope National Medical Center, Duarte, CA (J.W.); Women's College Hospital (S.A.N.), Fred A. Litwin Center for Cancer Genetics, Samuel Lunenfeld Research Institute, Mount Sinai Hospital (I.L.A.; E.I.; H.O.), and OCGN, Cancer Care Ontario (I.L.A.), Toronto; Department of Pediatrics, University of Texas, Southwestern, Dallas (G.T.); Department of Medicine, University of Chicago, Chicago (O.I.O.); Lombardi Cancer Center, Georgetown University, Washington, DC (C.I.); IHCC, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland (A.J.; J.L.; J.G.; B.G.; T.B.; T.H.); Medical Genetics Services for Wales, University Hospital of Wales, Cardiff (A. Murray; M.R.); North West Thames Regional Genetics Service, Northwick Park Hospital, Harrow, United Kingdom (H. Dorkins); Division of Molecular Gyneco-Oncology, Department of Obstetrics Gynaecology, University of Cologne, Cologne, Germany (R.K.S.; B.V.); Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany (C.E.); Department of Gynecology and Obstetrics, Technical University, Munich (A. Meindl); Department of Gynecology and Obstetrics, University of Schleswig-Holstein, Kiel, Germany (N.A.); Molecular Genetics Laboratory, Department of Gynecology and Obstetrics, University of Düsseldorf, Düsseldorf (D.N.); Department of Gynecology and Obstetrics, University of Ulm, Ulm, Germany (H. Deissler); Queensland Institute of Medical Research (A.B.S.; X.C.; N.W.; G.C.-T.) and Institute of Molecular Biosciences, University of Queensland (N.C.), Brisbane, Australia; Peter MacCallum Cancer Centre, Melbourne (kConFab); Clinical Genetics Service, MSKCC, New York (T.K.; K.O.); The Susanne Levy Gertner Oncogenetics Unit and the Oncology Division, Sheba Medical Center, Tel-Hashomer, Israel (E.F.; B.K.; Y.L.; G.G.); CHS NICCC at Carmel Medical Center and B. Rappaport Faculty of Medicine, Technion, Haifa, Israel (G.R.; F.L.; L.R.); Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands (P.D.; M.P.G.V.); Human Cancer Genetics Programme, CNIO, Madrid (A.O.; J.B.); Department of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University Prague, Prague (M.Z.); Department of Laboratory Medicine and Experimental Pathology (C.I.S.) and Department of Laboratory Medicine and Pathology (F.J.C.), Mayo Clinic College of Medicine, Rochester, MN; Helsinki University Central Hospital, Department of Obstetrics and Gynecology, Helsinki (O.K.; H.N.); Deutsches Krebsforschungszentrum Heidelberg, Heidelberg, Germany (U.H.); and Department of Pathology, Landspitali-University Hospital, Reykjavik (A.A.).

Appendix A

Retrospective Likelihood Approach and Score Test

Analytical Framework

We assume that the study includes n carriers who receive a diagnosis of breast cancer at ages $t_1, t_2, \ldots, t_n$ and $m - n$ unaffected carriers censored at ages $t_{n+1}, \ldots, t_m$ and that we observe genotype vectors $\mathbf{z}_1, \mathbf{z}_2, \ldots, \mathbf{z}_m$ (or, in general, other covariates) for these individuals. We assume that the breast cancer incidence rate for individual i depends on the underlying genotype through a Cox proportional hazards model: $\lambda_i(t) = \lambda_0(t) \exp(\boldsymbol{\beta}^t \mathbf{z}_i)$. Our aim is to estimate and perform significance tests on the log-risk ratios β. Note that the baseline incidence rate $\lambda_0(t)$ is not known but can be inferred from the overall incidence rates $\mu(t)$ obtained, for example, from external sources.

Retrospective Likelihood

The conditional likelihood of the genotypes (assumed to be random effects) given the disease phenotypes can be written as

$$L = \prod_{i=1}^{m} \frac{p_{g_i} \exp\left[-\Lambda_0(t_i) \exp(\boldsymbol{\beta}^t \mathbf{z}_{g_i}) + \boldsymbol{\beta}^t \mathbf{z}_{g_i} O_i\right]}{\sum_k p_k \exp\left[-\Lambda_0(t_i) \exp(\boldsymbol{\beta}^t \mathbf{z}_k) + \boldsymbol{\beta}^t \mathbf{z}_k O_i\right]},$$

where the summation in the denominator is over all genotypes, p_k is the frequency of genotype k, and O_i is 0 (zero) if individual i is unaffected and 1 if affected. $\Lambda_0(t)$ is the baseline cumulative incidence rate ($= \sum \lambda_0(u)$) and is unknown but can be obtained by constraining the overall incidence to agree with the assumed incidence rates for *BRCA1* and *BRCA2* mutation carriers as described elsewhere.[29] This is a true likelihood and can be maximized jointly over $\mathbf{p}$ and $\boldsymbol{\beta}$ by use of the software MENDEL.[30] Stratified data can be dealt with by considering the stratified conditional likelihood

$$L = \prod_{j=1}^{S} \prod_{i=1}^{m_j} \frac{p_{g_{ji}} \exp\left[-\Lambda_{j0}(t_{ji}) \exp\left(\boldsymbol{\beta}^t \mathbf{z}_{g_{ji}}\right) + \boldsymbol{\beta}^t \mathbf{z}_{g_{ji}} O_{ji}\right]}{\sum_{k_j} p_{k_j} \exp\left[-\Lambda_{j0}(t_{ji}) \exp\left(\boldsymbol{\beta}^t \mathbf{z}_{k_j}\right) + \boldsymbol{\beta}^t \mathbf{z}_{k_j} O_{ji}\right]} ,$$

where p_{k_j} is the frequency of the kth genotype within stratum j ($j = 1, \ldots, S$) and the subscript ji indicates the observed data for individual i in the jth stratum. This likelihood can be maximized jointly over $\boldsymbol{\beta}$ and the stratum specific genotype frequencies.

Score Test

By use of the likelihood, it is also possible to derive the score test statistic for testing the null hypothesis H_0: $\boldsymbol{\beta} = 0$. Let $\mathbf{U}$ represent the score vector. Following the notation in the previous section, it can be shown that, under the null hypothesis, the kth element of $\mathbf{U}$ has the form

$$U_k = \sum_{i=1}^{m} (z_{ki} - \bar{z}_k) \left[O_i - \Lambda_0(t_i)\right] ,$$

where z_{ki} is value of the covariate for the ith individual and $\bar{z}_k$ is the mean of the kth covariate over all individuals. Under the null hypothesis, the statistic $\mathbf{U}^t \mathbf{V}^{-1} \mathbf{U}$ has a χ^2 distribution with $\nu - 1$ df, where V is the information matrix under the null hypothesis and ν is the dimension of the covariate vector $\mathbf{z}$. A stratified version of the test can also be derived. This test is fully efficient when the effect size is small and $\lambda_0(t)$ agrees with the true incidence rates but is valid even if these assumptions are not met. The score test is similar in concept to the log-rank test used in standard survival analysis.[32] The difference is that, in the log-rank test, the expected number of events is computed using observed data, whereas, in this test, the expected number is based on the assumed carrier incidence rates.

Web Resources

Accession numbers and URLs for data presented herein are as follows:

Breast Cancer Information Core, http://research.nhgri.nih.gov/projects/bic/

ESE Finder, http://rulai.cshl.edu/tools/ESE/

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *RAD51* transcripts [accession numbers NM_002875 and AK131299])

International HapMap Project, http://www.hapmap.org/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *BRCA1, BRCA2, G6PD, GAPDH, ACTB,* and *CASC5*)

RNA and DNA Folding Applications, http://www.bioinfo.rpi.edu/applications/mfold/

SpliceSiteFinder, http://violin.genet.sickkids.on.ca/~ali/splicesitefinder.html

Splice Site Prediction, http://www.fruitfly.org/seq_tools/splice.html

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