Breast Cancer RESEARCH

LETTER

RAD51C deletion screening identifies a recurrent gross deletion in breast cancer and ovarian cancer families

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RAD51C is an integral part of the DNA double-strand repair through homologous recombination, and monoallelic mutations were found in ~1.3% of *BRCA1/2*-negative breast cancer (BC) and/or ovarian cancer (OC) families [1]. Several studies confirmed the occurrence of *RAD51C* mutations predominantly in BC and/or OC families, although with varying frequencies, clearly establishing *RAD51C* as a cancer-predisposing gene [2-4]. There is ongoing debate whether pathogenic *RAD51C* alterations increase the relative risk for BC in addition to that for OC, which was estimated to be 5.88 (95% confidence interval = 2.91 to 11.88; $P = 7.65 \times 10^{-7}$) [2].

Elucidating the role of *RAD51C* in BC pathogenesis is hampered by the low frequency of clearly truncating *RAD51C* mutations. Deleterious alterations, as deduced by mutation type, are virtually absent in BC-only families, and very few BC cases with a BC/OC family history have been experimentally proven to carry a truncating *RAD51C* mutation [1-4]. In this study, we screened for gross genomic alterations within the *RAD51C* gene in *BRCA1/2*-negative familial BC index cases, 500 of which showing a BC-only family history and 325 a BC/OC family history. Written informed consent was obtained from all patients and ethical approval was given by the Ethics Committee of the University of Cologne (07–185).

We identified a large heterozygous *RAD51C* deletion encompassing exons 5 to 9 in two independent families (Figure 1A,B,C). In the first (family #1), remarkably a BC-only family (Figure 1A), the mutation carrier was affected by early-onset and bilateral BC (age 33 years, age 39 years). The deletion was inherited from the mother who was affected by colon cancer (age 44 years). In the second (family #2), a BC/OC family (Figure 1B), the mutation was identified in dizygotic twins, one of which was affected by early-onset BC (age 42 years) and one by early-onset OC (age 43 years). The 36,637 base pair deletion (Figure 1D,E,F) appears to be rare because we identified no further case in another large cohort by junction fragment polymerase chain reaction (BC only: 1,011; BC/OC: 203). Strikingly, all three *RAD51C*-positive breast tumors were classified as intermediate to high grade (individual #1-IV-2: G3, G3; individual #2-III-6: G2 to G3), invasive ductal, and triple negative.

The early onset of BC in both families, the occurrence of bilateral BC and the triple-negative tumor phenotype resemble features closely associated with hereditary BC [5], and thus the presence of a clearly truncating mutation is supportive for a pathogenic role of *RAD51C*. Due to the low *RAD51C* mutation frequency, however, large collaborative studies are required to quantify the relative risk of *RAD51C* alterations for BC and potentially other cancer entities and, most importantly, to unravel genotype–phenotype correlations as well as genetic modifying factors that determine phenotypic variability with respect to cancer site and tumor subtype.

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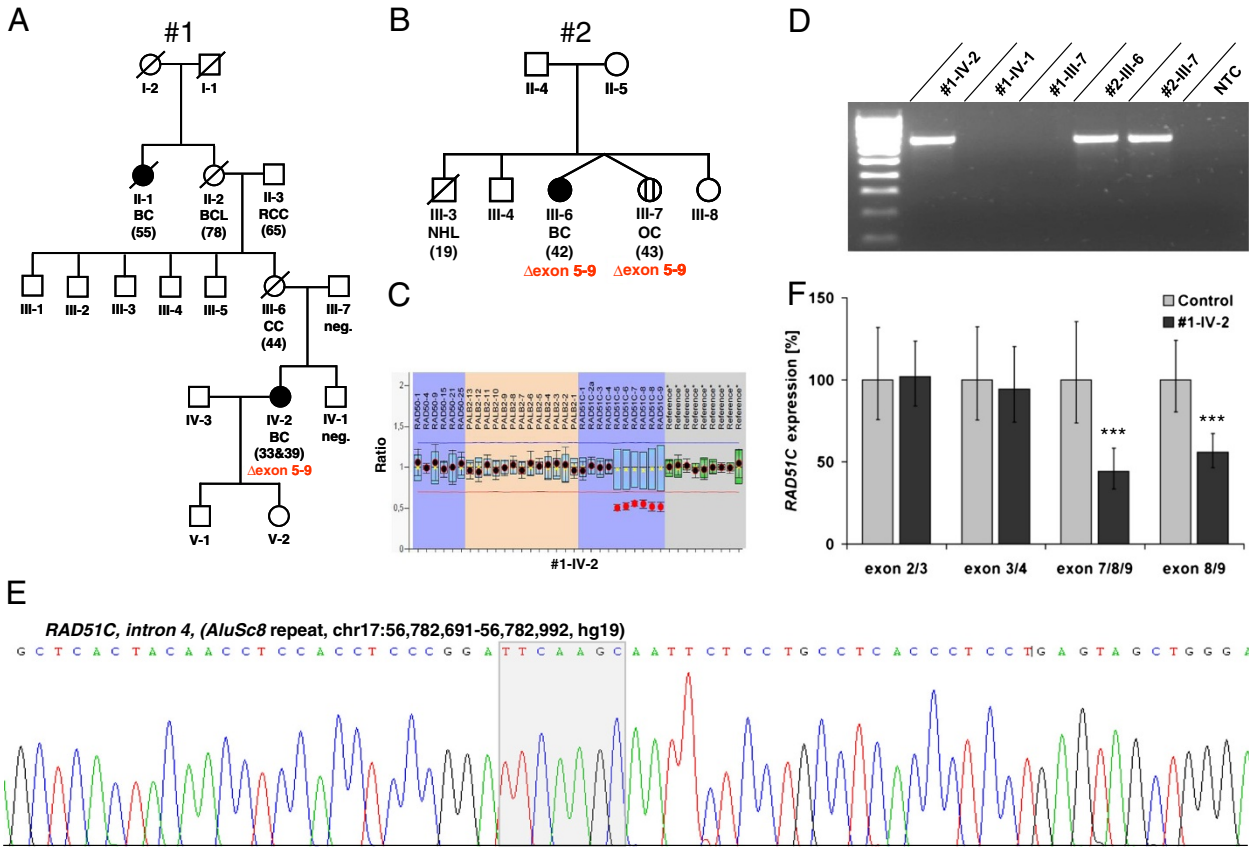

Figure 1 Identification and characterization of the *RAD51C* deletion. Identification of the *RAD51C* deletion: **(A), (B)** probands were recruited at the hereditary breast cancer (BC) and ovarian cancer (OC) centers in Cologne and Munich, Germany. Screening for genomic rearrangements was performed by employing a multiplex ligation-dependent probe amplification (MLPA) assay covering all nine coding exons of *RAD51C* as well as *PALB2* and partially *RAD50* (P260 MLPA probemix; MRC Holland, Amsterdam, the Netherlands). Deletions of exons 5 to 9 of the *RAD51C* gene were identified in two families (#1-IV-2; #2-III-6, #2-III-7) using blood-derived genomic DNA (gDNA). In both families, non-BC/OC entities were reported. BCL, B-cell lymphoma; RCC, renal cell carcinoma; CC, colon cancer; NHL, non-Hodgkin lymphoma. **(C)** MLPA data analysis was carried out using Coffalyser.Net software (MRC Holland). Characterization of the *RAD51C* deletion: **(D)** a deletion-specific junction fragment polymerase chain reaction (PCR) was performed using the primers 5′-TCTCTGTGTCCTCATATGATAGG-3′ and 5′-CTAGGATCACACTATTGCACTC-3′. A 681 base pair (bp) fragment was observed using gDNA derived from individuals #1-IV-2, #2-III-6 and #2-III-7, but was absent in individual #1-IV-1, indicating maternal inheritance in family #1. NTC, no template control. **(E)** Sequencing of the junction fragment in all cases revealed a recurrent 36,637 bp deletion, which is flanked by Alu repeats. The genomic breakpoint within intron 4 is located in a 7 bp region identical between both flanking Alu sequences (indicated). Hence, the deletion probably originates from an Alu repeat-mediated nonhomologous recombination event. **(F)** Blood-derived *RAD51C* transcripts from individual #1-IV-2 and a control were analyzed by real-time PCR as described previously [6]. Primer sequences are available on request. Amplicons spanning exons 2/3 and 3/4 were detected at similar levels, while those spanning exons 7/8/9 and 8/9 were less abundant in patient #1-IV-2 compared with the respective control (***P <0.001, t test). Detection levels in the control sample were set to 100%. Results given as mean ± standard deviation.

Abbreviations
BC: Breast cancer; OC: Ovarian cancer.

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

Authors' contributions
BW, SE and KR participated in the study design and helped to draft the manuscript. AM, RKS and EH wrote the manuscript. GS, JH, NW-L, HH, LG, AB and GN performed the molecular genetic studies. All authors read and approved the final manuscript.

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