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EPIDEMIOLOGY

RAD51C germline mutations found in Spanish site-specific breast cancer and breast-ovarian cancer families

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Abstract *BRCA1* and *BRCA2* are the most well-known breast and ovarian cancer susceptibility genes. Additional genes involved in DNA repair have been identified as predisposing to breast cancer. Recently, *RAD51C*, a new Fanconi Anemia gene, essential for homologous recombination repair, has been reported to be a rare hereditary breast and ovarian cancer susceptibility gene. Indeed, several pathogenic mutations have been identified in *BRCA1/BRCA2*-negative hereditary breast and ovarian cancer families. Here, we present the results of the screening of *RAD51C* mutations in a large series of 516 *BRCA1/BRCA2*-negative Spanish patients from breast and/or ovarian cancer families, and the evaluation of these results in the context of all *RAD51C* carriers. *RAD51C* mutation screening was performed by DNA analysis for all index cases. All the genetic variants identified were analyzed in silico for splicing and protein predictions. cDNA analysis was performed for three selected variants. All previous *RAD51C* mutation studies on breast and/or ovarian cancer were reviewed. We identified three inactivating *RAD51C* mutations. Two mutations were found in breast and ovarian cancer families and one mutation in a site-specific breast cancer family. Based on the mean age of ovarian cancer diagnosis in *RAD51C* carriers, we would recommend prophylactic bilateral salpingo-ophorectomy in premenopausal *RAD51C* mutation carriers. Our results support that *RAD51C* is a rare breast and ovarian cancer susceptibility gene and may contribute to a small fraction of families including breast and ovarian cancer cases and families with only breast cancer. Thus, *RAD51C testing* should be offered to hereditary breast and/or ovarian cancer families without selecting for specific cancer origin.

Keywords Hereditary breast and/or ovarian cancer · *BRCA1/2* negative · *RAD51C* · Germline mutation

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Introduction

Breast cancer is the most common cancer and the leading cause of cancer-related death among women worldwide. Familial cases of breast cancer comprise approximately 5–10 % of all breast cancer. The most important susceptibility genes are *BRCA1* and *BRCA2* accounting for 20–25 % of familial predisposition to breast cancer [1]. Mutations in these genes confer high risk of early onset breast cancer and have been also associated with an increased risk of ovarian cancer [2, 3]. Breast cancer risk is also associated with mutations in *TP53* and *PTEN* genes within the well-defined cancer syndromes Li-Fraumeni and Cowden disease, respectively [4, 5].

BRCA1 and BRCA2 proteins are involved in the maintenance of genomic stability by facilitating DNA repair. Therefore, it has been hypothesized that genes coding for proteins that interact with BRCA1 and BRCA2 or act in the same DNA repair pathway would be good candidate for breast cancer susceptibility. After intensive research examining many candidates, *CHEK2* [6, 7], *ATM* [8, 9], and the Fanconi Anemia genes *BACHI (BRIP1)/FANCJ* [10] and *PALB2/FANCN* [11] have been shown to confer moderate breast cancer risk.

RAD51C is a member of the RAD51 protein family. The RAD51 protein is a key player in the homologous recombination repair pathway, and its activity appears to be strictly regulated by a number of cofactors including five RAD51 paralogs, namely RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 [12]. These proteins form a complex (BCDX2 complex) [13], which is responsible for RAD51 recruitment or stabilization at DNA damage sites. It has been shown that the role of *BRCA1* and *BRCA2* in homologous recombination is regulated by RAD51 function [14]. Meindl et al. identified inactivating mutations in *RAD51C* in six of the 1100 *BRCA1/BRCA2*-negative subjects from pedigrees with gynecological cancers [15]. Interestingly, mutations were identified in a subcohort of 480 pedigrees with breast and ovarian cancer but not among families with breast cancer only. At the same time, a biallelic mutation in *RAD51C* was reported in a family with multiple severe abnormalities characteristic of Fanconi Anemia [16]. After that, several studies have been performed in different populations. Some of these studies identified deleterious mutations among breast and/or ovarian cancer families [17–21], while quite a few studies have not identified any pathogenic mutation [22–28].

However, only few breast cancer only carrier families have been detected, which keeps open a debate whether pathogenic *RAD51C* alterations increase the risk of breast cancer in addition to ovarian cancer. The frequencies of *RAD51C* mutations in these studies were about 1 %, indicating the necessity of large population-based studies for investigating the role of *RAD51C* in cancer susceptibility.

Table 1 Clinical characteristics of the 516 index cases of the hereditary breast/ovarian cancer families

Clinical diagnosis and patient information	
Mean age in years at first diagnosis (breast/ovarian)	43.3/49.1
Index cases with breast and ovarian cancer (Bilateral breast cancer)	20(2)
Index cases with breast cancer (Bilateral breast cancer)	458 (62)
Index cases with ovarian cancer	38
Family information	
Family history with breast cancer only	410
Family history with ovarian cancer only	17
Family history with both breast and ovarian cancer	89

In Spain, two previous studies detected a 1.3 % mutation prevalence in a series of 300 breast and/or ovarian cancer families and one mutation in 485 breast cancer only families [29, 30].

The aim of the present study was to investigate the frequency of *RAD51C* germline mutations in a cohort of 516 *BRCA1/BRCA2*-negative breast and/or ovarian cancer families from other geographical areas of Spain.

Materials and methods

Patients

Index cases from 516 *BRCA1/BRCA2* mutation-negative Spanish breast and/or ovarian cancer families (243 families from Galicia, North West of Spain, and 272 families from Catalonia, North East of Spain) were screened for mutations within the entire coding sequence and splicing sites of *RAD51C* gene. Clinical characteristics of the families are presented in Table 1. None of the index cases had relatives diagnosed with Fanconi anemia. All index cases were previously screened for mutations in *BRCA1* and *BRCA2* and were found to be negative for mutations in either gene. Large deletions and duplications in *BRCA1* and *BRCA2* were also included in the mutational analysis by MLPA. The ethics committees approvals were obtained as well as informed consent for all participants in the study.

Mutation analysis of *RAD51C* gene

DNA samples were obtained from peripheral blood leukocytes using standard methods. The entire coding sequence of *RAD51C* along with flanking intron boundaries was amplified using primers previously described [15]. The PCR products were purified and bi-directionally sequenced with the amplification primers on an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

In silico analysis

All *RAD51C* genetic variants were analyzed for their potential effect on splicing and in the protein. Splicing predictions were performed with Splicing Sequences Finder (SSF) (http://www.umd.be/searchSpliceSite.html), MaxEntScan (http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html), NNSplice (http://www.fruitfly.org/seq_tools/splice.html), and Human Splicing Finder (HSF) (http://www.umd.be/HSF/) algorithms through the Alamut-Mutation Interpretation Software v.1.54 (http://www.interactivebiosoftware.com/alamut.html). Default thresholds were used for all the analyses.

Potential consequences of missense substitutions were obtained using the prediction softwares PolyPhen-2 (Polymorphism Phenotyping-2, see http://genetics.bwh.harvard.edu/pph2/), SIFT (Sorting Intolerant From Tolerant, see http://sift.jcvi.org/), and Align-GVGD (Grantham score difference, see http://agvgd.iarc.fr/) tools. Native alignments of each algorithm have been used. We consider an optimal prediction when the three algorithms produce the same prediction.

CEU-population data from 1000 Genomes Project database (http://www.1000genomes.org/) were used to obtain allelic frequency of the identified variants in our samples.

cDNA analysis

cDNA analysis was performed for variants c.404 + 63_404 + 71dup, c.405-58A > G, and c.705 + 79A > G. RNA was extracted from total blood leukocytes of carrier patients, and ten non-carrier controls using either TRIzol (Invitrogen, Paisley, UK) or Qiagen RNeasy Minikit (Qiagen, N·V., The Netherlands) according to the manufacturer's protocols. Isolated RNA was further processed on columns from RNeasy Kit and RNase-Free DNase set to cleanup RNA and remove any DNA traces, according to the protocols provided by the manufacturer (Qiagen Sciences, Maryland, USA). The integrity of RNA purified was checked in agarose gel 1.5 % stained with ethidium bromide. After quantification with a NanoDrop spectrophotometer (NanoDrop Technologies, Inc. Wilmington, DE, USA), total RNA was reverse transcribed using High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, Life Technologies, Foster City, USA) with random and Oligo(dT) primers or Superscript II (Invitrogen, Paisley, UK) according to the manufacturers' protocols.

For the variant c.404 + 63_404 + 71dup, cDNA forward primer was GTTCCGCTTTGAAATGCAGC (located in exon 1), and reverse primer was GTCGAACCTTTGAGTG TTCTGA (between exons 4 and 5). For c.405-58A > G, the forward primer was GCACTGGAACTTCTTGAGCA (in exon 2), and the reverse primer was CTAATGCAGGAA-CAAGCAAGG (in exon 6). For c.705 + 79A > G, we used the forward primer GTGGCAGGTGAAGCAGTTTT (exon 3) and the reverse primer GCCGTATTGTAGCAGCATGT (in exon 7).

The amplifications of c.404 + 63_404 + 71dup and c.705 + 79A > G were performed with 37 cycles and with an annealing temperature of 57 °C. The amplification of c.405-58A > G was performed with a touchdown PCR with 3 cycles at 68 °C of annealing temperature, 3 cycles at 66 °C, and 30 cycles at annealing temperature of 64 °C.

The amplified fragments for variants c.404 + 63_404 + 71dup and c.705 + 79A > G were visualized in agarose gel 1.5 % and using an Agilent 2100 Expert Bioanalyzer DNA 500/DNA 1000 (Agilent Technologies Inc. Santa Clara, CA USA) in the case of the variant c.405-58A > G. All these amplicons were sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Life Technologies, Foster City, USA). The electropherograms were manually examined with the NCBI RNA NM_058216.1 sequence as reference.

Nomenclature and databases

Sequences used for *RAD51C* nomenclature were obtained from the NCBI RefSeq database (NG_023199.1 for genomic, NM_058216.1 for mRNA and NP_478123.1 for protein) (http://www.ncbi.nlm.nih.gov). Standardized nomenclature was reported considering the A of the ATG initiation codon of the coding DNA reference sequence as nucleotide position +1.

Results

We completely sequenced all the nine coding exons and exon/intron boundaries of the *RAD51C* gene in 516 index cases of *BRCA1/BRCA2*-negative high risk breast and/or ovarian cancer families.

We identified twelve different *RAD51C* sequence variants: three missense (c.376G > A (p.Ala126Thr), c.656T > C (p.Leu219Ser), c.859A > G (p.Thr287Ala)), two nonsense (c.577C > T (p.Arg193Stop), c.709C > T (p.Arg237Stop)), six intronic (c.404 + 63_404 + 71dup9, c.405-58A > G, c.572-17G > T, c.705 + 79A > G, c.904 + 34T > C, and c.905-115A > C), and one variant in the 5′UTR region (c.-26C > T) (Table 2). From these, two were novel changes (c.709C > T (p.Arg237Stop), c.905-115A > C).

Among all the variants, three are deleterious mutations: c.577C > T (p.Arg193Stop), c.709C > T (p.Arg237Stop), and c.656T > C (p.Leu219Ser). This missense variant has been previously reported in a breast and ovarian cancer family and was characterized as deleterious by functional assay [30].

The mutation c.577C > T (p.Arg193Stop), in exon 4, was identified in a breast cancer family. However, this mutation has been previously detected in a breast and

Table 2 Summary of germline *RAD51C* variants

Location	Variant[a]	Proteic change	rs	Genotypes HBC/HBOC families[b]				Allelic frequency (%)[c]								CONTROL POPULATION	
				BC ($x = 410$)	BC/OC ($x = 89$)	OC ($x = 17$)	TOTAL ($x = 516$)	BC ($N = 820$)		BC/OC ($N = 178$)		OC ($N = 34$)		TOTAL ($N = 1,032$)		1000 genomes[d]	
								A(A %)	B(B %)	A(A %)	B(B %)	A(A %)	B(B %)	A(A %)	B(B %)	A %	B %
Pathogenic mutations																	
EXON 4	c.577C > T	p.Arg193Stop	rs200293302	1	–	–	1	819(99.9)	1(0.1)	178(100)	0	34(100)	0	1,031(99.9)	1(0.1)	ND	ND
EXON 4	c.656T > C[e]	p.Leu219Ser	rs201529791	–	1	–	1	820(100)	0	177(99.4)	1(0.6)	34(100)	0	1,031(99.9)	1(0.1)	ND	ND
EXON 5	c.709C > T	p.Arg237Stop	–	–	1	–	1	820(100)	0	177(99.4)	1(0.6)	34(100)	0	1,031(99.9)	1(0.1)	–	–
Non-synonymous																	
EXON 2	c.376G > A	p.Ala126Thr	rs61758784	6	1	–	7	814(99.3)	6(0.7)	177(99.4)	1(0.6)	34(100)	0	1,025(99.3)	7(0.7)	99	1
EXON 6	c.859A > G	p.Thr287Ala	rs28363317	13	3	2	18	807(98.4)	13(1.6)	175(98.7)	3(1.7)	32(94.1)	2(5.9)	1,014(98.3)	18(1.7)	99	1
Non-coding																	
5′UTR	c.-26C > T	–	rs12946397	131(13)	23(1)	6(1)	160(15)	676(82.4)	144(17.6)	154(86.5)	24(13.5)	27(79.4)	7(20.6)	857(83.1)	175(16.9)	82	18
IVS 2	c.404 + 63_404 + 71dup9	–	rs142735413	–	2	–	2	820(100)	0	176(89.9)	2(1.1)	34(100)	0	1,030(99.8)	2(0.2)	ND	ND
IVS 2	c.405-58A > G	–	–	1	–	–	1	819(99.9)	1(0.1)	178(100)	0	34(100)	0	1,031(99.9)	1(0.1)	–	–
IVS 3	c.572-17G > T	–	rs193023469	4	1	–	5	816(99.5)	4(0.5)	177(99.4)	1(0.6)	34(100)	0	1,027(99.5)	5(0.5)	99	1
IVS 4	c.705 + 79A > G	–	–	1	–	–	1	819(99.9)	1(0.1)	178(100)	0	34(100)	0	1,031(99.9)	1(0.1)	–	–
IVS 6	c.904 + 34T > C	–	rs28363318	249(38)	50(11)	12(5)	311(54)	533(65)	287(35)	117(65.7)	61(34.3)	17(50)	17(50)	667(64.6)	365(35.4)	77	23
IVS 6	c.905-115A > C	–	–	1	–	–	1	819(99.9)	1(0.1)	178(100)	0	34(100)	0	1,031(99.9)	1(0.1)	–	–

[a] In bold variants not previously reported

[b] Breast cancer only family (BC), ovarian cancer only family (OC), breast and ovarian cancer family (BC/OC). *x* is the number of cases studied in each group

[c] Allelic frequency is the percentage of n/N, where *n* is the number of times of minor allele, and N is the total of alleles

[d] MAF for CEU population in 1000 genomes (http://browser.1000genomes.org/index.html); ND: SNPs without information about population frequencies in 1000 genomes

[e] Missense variant classified as deleterious by Osorio et al. (2012)

ovarian cancer family [31]. Our index case was diagnosed at 34 years of age. The patient's mother had been diagnosed with breast cancer at the age of 48. The affected mother and the 25-year-old asymptomatic daughter of the index case carried the variant (Fig. 1a).

The nonsense mutation c.709C > T (p.Arg237Stop) in exon 5 of *RAD51C* was detected in a woman diagnosed with follicular thyroid cancer at the age of 50 and with ovarian cancer at 54 years of age. The patient's mother had been diagnosed with ovarian cancer at 60 years of age and her grandmother with a colon cancer at 63 years of age. The proband has three third-degree affected relatives, two of them with breast cancer and one with ovarian cancer (Fig. 1b). Unfortunately, we could not confirm the origin of the mutation in the maternal branch.

We identified the c.656T > C (p.Leu219Ser) missense mutation in a woman diagnosed with ovarian cancer at 44 years. In her family, two maternal aunts had been diagnosed with breast cancer at 40 and 86 years of age, respectively. Two other family members were diagnosed with prostate cancer at the age of 70 and a glioblastoma at 53 years of age. A healthy sister of the proband was negative for the variant. The proband's mother died at age 88 without cancer (Fig. 1c).

In addition, two *RAD51C* non-synonymous variants were identified. Both variants are reported in 1000 genomes with a minor allele frequency of 1 %. The variant c.859A > G (p.Thr287Ala) was predicted to be damaging by one of the three programs used to analyze the variant effect on the protein. For the variant, p.Ala126Thr bioinformatic analyses consider the amino acid change not deleterious (Table 3).

We also identified seven non-coding variants, one of them located in the 5′UTR of the gene. The intronic variants c.404 + 63_404 + 71dup, c.405-58A > G, and c.705 + 79A > G were predicted to alter the splicing by the creation of new cryptic sites (Table 3). However, cDNA analyses for these three variants do not show any difference between cases and controls (Online resource 1).

Five of the sequence variants detected (c.-26C > T, c.376G > A (p.Ala126Thr), c.572-17G > T, c.859A > G (p.Thr287Ala), and c.904 + 34T > C) have been identified before in Spanish patients and described as non-pathogenic by previous reports [29, 30].

Discussion

We have identified three germline pathogenic mutations in the *RAD51C* gene in a new Spanish cohort of 516 breast and/or ovarian cancer families.

The prevalence of *RAD51C* mutations in our cohort is 0.6 % (3/516 families). Previous studies of breast and/or ovarian cancer families have reported a prevalence ranging from 0 to 2.9 % [15, 17, 18, 20–36], although most of these studies, including our, do not include the analysis of large genomic rearrangements, which could increase these estimates (reviewed in Table 4). It has been suggested that these differences in prevalence might be explained by stochastic variation due to the low frequency of *RAD51C* mutations, as well as by differences in the ascertainment of collected families [31]. Nevertheless, these differences could be originated by specific genetic features of the population studied such as the presence of founder mutations.

In our cohort, two of the *RAD51C* carriers belong to the group of 89 breast and ovarian cancer families, leading to a prevalence of *RAD51C* mutations in this group of 2.25 %. We found 16 additional reports in the literature investigating germline *RAD51C* mutations in breast and ovarian families. In 15 of them, the complete coding and splice site regions of the gene were screened but did not include large genomic rearrangement analysis, and one analyzed exclusively large genomic alterations [34]. In the 3,296 families with both breast and ovarian cancers analyzed, 27 unequivocal deleterious *RAD51C* mutations were identified (0.8 %) (Table 4).

Two prior studies analyzed *RAD51C* in Spanish breast and/or ovarian cancer families [29, 30]. Romero et al. found one pathogenic *RAD51C* mutation among 492 families (0.2 %). Interestingly, the carrier of this mutation (c.774delT) was of Swedish origin. This mutation was recently reported by Vuorela et al. in an ovarian case from a study of Swedish and Finnish familial breast and/or ovarian cancer cases and unselected ovarian cancer cases [18]. Later, Osorio et al. extended this series to 785 patients. The authors performed functional assays to evaluate the effect of missense variants and demonstrated that three of them were pathogenic [30]. Another variant was previously classified as pathogenic by Somyajit [37]. In the subgroup of breast and ovarian cancer families, the frequency of missense *RAD51C* mutations was 1 % (3 of 300) [30]. One of these missense mutations is the variant c.656T > C (p.Leu219Ser), which was also detected in our cohort. This missense variant was characterized as deleterious based on three approaches: i) evolutionary conservation of the original amino acid among RAD51 and its five paralogs; ii) prediction of in silico programs; and iii) functional complementation assay [30]. Overall, these results confirm the association of deleterious *RAD51C* mutations in families with both breast and ovarian cancer.

Some previous reports identified *RAD51C* mutations in ovarian cancer families as well as in unselected ovarian cancer cases [17, 20, 21, 31, 35, 36]. In a cohort of Finnish familial breast and/or ovarian cancer patients, *RAD51C* mutations were associated with an increased risk of familial breast and ovarian cancer (OR 13.59, 95 % CI

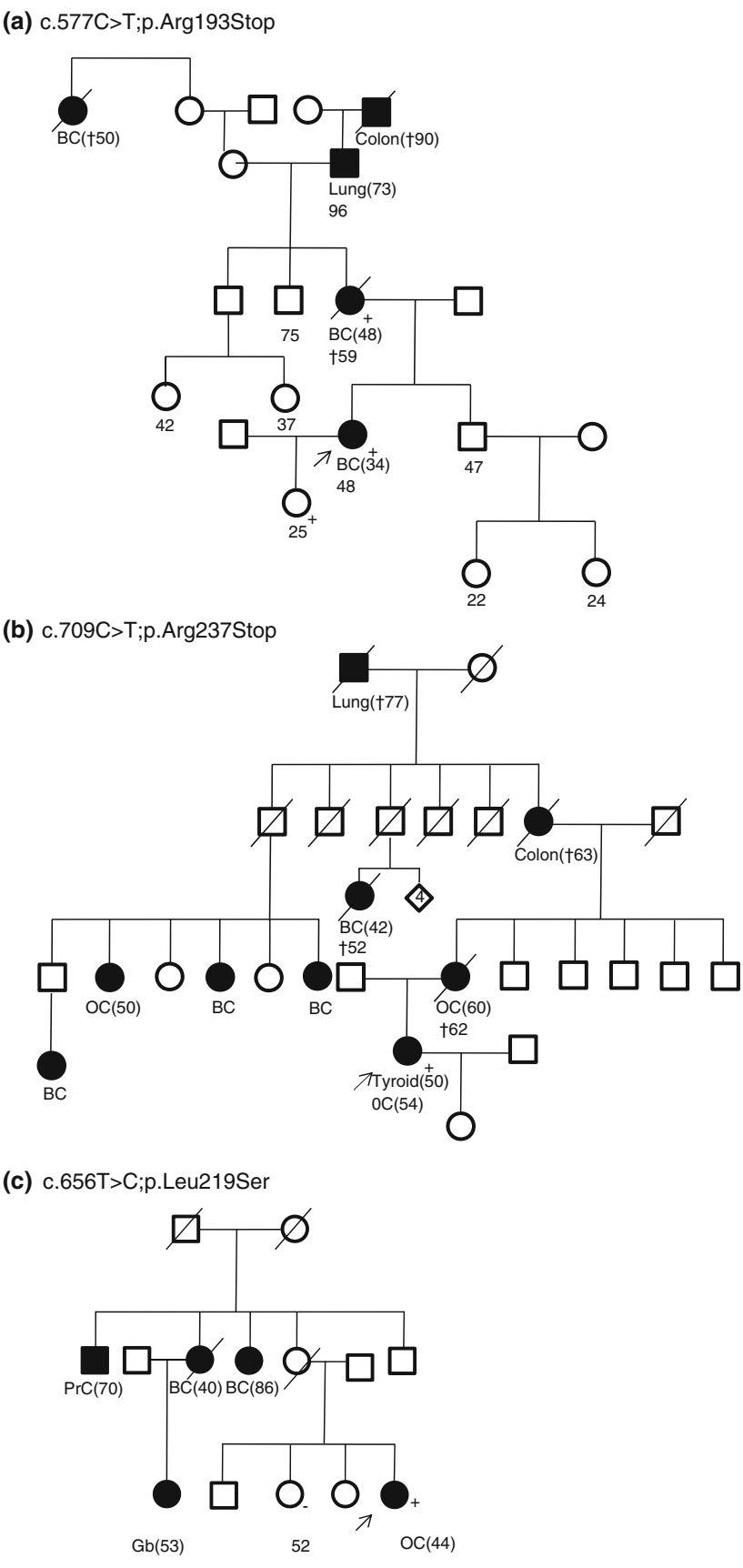

Fig. 1 Pedigrees of the breast/ovarian cancer families carrying the mutations: **a** c.577C > T; p.Arg193Stop. **b** c.709C > T; p.Arg237Stop. **c** c.656T > C; p.Leu219Ser. Cancer diagnoses are indicated in those affected patients; in *brackets* the age at diagnosis or exitus. The *arrow* indicates the index case analyzed. BC breast cancer, OC ovarian cancer, *PrC* prostate cancer, *Gb* glioblastoma

Table 3 Results of bioinformatic analysis for *RAD51C* variants

Variant	Protein change	Prediction aminoacidic change			Splice signal detection		
		Polyphen	SIFT	A-GVGD	Location, SS, distance	5′ or 3′ score modification (% variation)	Proximal cryptic/de novo (% variation)
Pathogenic mutations							
c.577C > T	p.Arg193Stop	–	–	–	Exon 4, 3′, 6	NNSPLICE: 0.96→0.95(−1.2 %)	
c.656T > C	p.Leu219Ser	Possibly damaging	Deleterious	Class C0	Exon 4, 3′, 84	GeneSplicer: 2.27→2.02(−11.0 %)	
c.709C > T	p.Arg237Stop	–	–	–	Exon 5, 5′, 137		c.706-1 SSF:-→70.49 c.706-1 MaxEnt:1.12→1.31(+17.1 %)
Non-synonimous							
c.376G > A	p.Ala126Thr	Benign	Tolerated	Class C0	Exon 2, 5′, 29		c.373 MaxEnt:- →0.92 c.373 HSF: 69.19→70.35(+1.7 %)
c.859A > G	p.Thr287Ala	Probably damaging	Tolerated	Class C0	Exon 6, 3′, 22	GeneSplicer: 7.51→7.18(−4.4 %)	
Non-coding							
c.-26C > T	–	–	–	–	5′UTR, 5′, 26		c.-20 NNSPLICE: 0.67→0.68(+1.4 %) c.-20 GeneSplicer: 0.96→0.51(−47.2 %)
c.404 + 63_404 + 71dup	–	–	–	–	Intron 2, 5′, 63		c.404 + 71 MaxEnt:-→3.48 c.404 + 71 HSF: -→69.81
c.405-58A > G	–	–	–	–	Intron 2, 3′,58	GeneSplicer: 2.19→2.19(−0.1 %)	c.405-57 SSF: -→0.07 c.405-57 HSF: -→81.89
c.572-17G > T	–	–	–	–	Intron 3, 3′, 17	MaxEnt: 7.43→7.86(+5.9 %) NNSPLICE: 0.96→0.98(+1.9 %) GeneSplicer: -→2.23	
c.705 + 79A > G	–	–	–	–	Intron 4, 5′, 79		c.705 + 74 MaxEnt: -→3.84 c.705 + 74 HSF: 61.77→73.93(+19.7 %) c.705 + 79 MaxEnt: -→3.14 c.705 + 79 HSF: 61.03→71.61(+17.3 %)
c.904 + 34T > C	–	–	–	–	Intron 6, 5′, 34		
c.905-115A > C	–	–	–	–	Intron 6, 3′, 115		

In bold variants where cDNA have been studied

Table 4 Overview of the literature on role of *RAD51C* in breast and/or ovarian cancer families

Study	Country	Patients analyzed						RAD51C mutations (%)					
		Total	BC	BC/OC	OC	uOC	Controls[a]	Total (%)	BC (%)	BC/OC (%)	OC (%)	uOC (%)	Controls[a] (%)
Meindl et al. (2010) [15]	Germany	1,100	620	480	0	0	0	6 (0.5)	0	6 (1.25)	0	0	0
Zheng et al. (2010) [22]	USA	92	0	92	0	0	0	0	0	0	0	0	0
Akbari et al. (2010) [23]	Jewish, French-Canadian, other ethnicities	454	N/S	N/S	N/S	0	0	0	N/S	N/S	N/S	0	0
Silvestri et al. (2011) [32]	Italy	97 (MBC)	72	25	0	0	0	0	0	0	0	0	0
Wong et al. (2011) [24]	Australia	70	67	3	0	0	0	0	0	0	0	0	0
Pang t al (2011) [25]	China	273	N/S	N/S	N/S	0	475	0	N/S	N/S	N/S	0	0
Pelttari et al. (2011) [17]	Finland	2,747	130 + 491[b] + 884[b,c] + 686[b,c]	139	8	409[b]	2,086	8 (0.3)	0 + 0+0 + 0	2 (1.4)	2 (25)	4[b](1.0)	2 (0.1)
Vuorela et al. (2011) [18]	Finland and Sweeden	1,936	112 + 993[b]	35	0	232 + 332[b]	871[b]	2 (0.1)	0	1 (2.8)	0	1 (0.4) +0[b]	0[b]
Clague et al. (2011) [26]	USA	286	113	34	119	0	0	0	0	0	0	0	0
Walsh et al. (2011) [19]	USA	360	0	0	0	281	0	2 (0.6)	N/S	N/S	N/S	0	0
Thompson et al. (2012) [20]	Australia	1,655	1,053	314	21	267	427	3 (0.2)	0	1 (0.3)	1 (4.8)	1 (0.4)	0
De Leeneer et al. (2012) [27]	Belgium, The Netherlands and Canada	351	0	239	112	0	0	0	0	0	0	0	0
Osorio et al. (2012) [30]	Spain	785 [492]	485 [391]	300[101]	0 [0]	0 [0]	550 [0]	5 (0.6)	1 (0.2) [0]	4 (1.3)	0 [0]	0 [0]	0[0]
[Romero et al. (2011) [29]][d]								[1(0.2)]		[1[e](1.0)]			
Lu et al. (2012) [28]	USA	192	157	35	0	0	0	0	0	0	0	0	0
Loveday et al. (2012) [21]	UK	1,404	0	1,102	30	272	1,156	12 (0.9)	0	8 (0.7)	1 (3.3)	3 (1.1)	1 (0.09)
Coulet et al. (2012) [31]	France and European origin	117	0	82	35	0	0	3 (2.6)	0	2 (2.4)	1 (2.9)	0	0
Kushnir et al. (2012) [33]	Jewish	206	190	2	14	0	200	0	0	0	0	0	0
Schnurbein et al. (2013)[f] [34]	Germany	825	500	325	0	0	0	2 (0.3)	1 (0.2)	1 (0.3)	0	0	0
Pennington et al. (2014) [35]	USA	311	0	0	0	311	0	2 (1.0)	0	0	0	2 (1.0)	0
Cunningham et al. (2014) [36]	UK	899	0	0	0	899	0	26 (2.9)	0	0	0	26 (2.9)	0
This study	Spain	516	410	89	17	0	0	3 (0.6)	1 (0.24)	2 (2.2)	0	0	0
TOTAL		14,676	3,929	3,296	326	3,146	5,290	73 (0.5)	3 (0.07)	27 (0.8)	5 (1.5)	38 (1.2)	3 (0.06)

[a] Healthy controls are not included in the total

[b] In this subset of population, only mutations detected previously in the previous analysis of samples has been studied

[c] BC unselected for family history

[d] Osorio et al. include samples of Romero et al

[e] Mutation of family of Swedish origin

[f] Only study large genomic rearrangements

N/S Not Specified; MBC Male Breast Cancer; uOC unselected ovarian cancer

1.89–97.6) but especially with familial ovarian cancer in the absence of breast cancer (OR 213, 95 % CI 25.6–1,769) and also with unselected ovarian cancer (OR 6.31, 95 % CI 1.15–34.6). The mutation rate was significantly higher among the familial cases than in the unselected ovarian cancer cases (OR 33.8, 95 % CI 5.15–221). Based on these results, authors suggest *RAD51C* as the first moderate-to-high risk susceptibility gene for ovarian cancer [17].

Besides that one *RAD51C* mutation was detected in a cohort of 232 ovarian cancer patients unselected for family history from Sweden and Finland [18]. Moreover, Loveday et al. found one mutation in 30 ovarian cancer only families and three mutations in 272 ovarian cancer cases unselected for family history [21]. These authors estimated a relative risk of ovarian cancer for *RAD51C* mutation carriers of 5.88 (95 % CI 2.91–11.88, $P = 7.65 \times 10^{-7}$) which constitutes a > 9 % cumulative risk by age 80 [21]. Coulet et al. identified one mutation in a cohort of 35 ovarian cancer patients, eight of them with at least one relative with ovarian cancer [31]. Recently, two new studies analyzed *RAD51C* mutations in 311 and 899 unselected ovarian cancer and reported a frequency of 1 and 2.9 %, respectively [35, 36]. Despite that the reported frequency of *RAD51C* mutations in families with only ovarian cancer is 1.5 and 1.2 % in unselected ovarian cancers, in our study no *RAD51C* pathogenic variants were identified among the 17 families with only ovarian cancer history (Table 4).

Although the association of *RAD51C* with ovarian cancer seems clear, its association with breast cancer is more controversial. A previous study found no evidence of association (RR = 0.91; 95 % CI = 0.45–1.86, $P = 0.8$) [21]. However, in our study, one out of the 410 families with breast cancer only was carrier of the c.577C > T (p.Arg193Stop) *RAD51C* mutation (0.24 %). This mutation has been previously reported in a French breast and ovarian cancer family [31]. To our knowledge, another two *RAD51C* mutations have been described to date in breast cancer only families (Table 4): one missense variant characterized as pathogenic by a functional assays (p.Gln143Arg) [30] and a large deletion from exon 5 to 9 [34]. Thus, three *RAD51C* pathogenic mutations have been described in 3929 breast cancer only families (0.07 %).

In addition to these mutations, several missense variants were detected in breast cancer families [15, 20]. Seven of these variants (p.Arg2Gly, p.Ala30Glu, p.Asp159Asn, p.Gly162Glu, p.Asp215Gly, p.Arg249Cys, and p.Leu262-Val) were predicted as possibly pathogenic by in silico programs [15, 20, 25, 29], increasing the potential frequency of *RAD51C* mutations in families with breast cancer to 0.25 %, which would give further support to the involvement of *RAD51C* in breast cancer.

The age at diagnosis in *RAD51C* mutation carriers could guide the age interval in which prophylactic surgeries could be recommended. The mean age at diagnosis of ovarian cancer among *RAD51C* mutation carriers is 57.3 years (range 42–83) (Online resource 2), which is similar for carriers of *BRCA1* and *BRCA2* mutations (52 and 55 years, respectively), and lower than the mean age of 62 years described for ovarian cancer in the general population [30]. We then would recommend prophylactic bilateral salpingo-ophorectomy in *RAD51C* mutation carriers as in *BRCA2* carriers. Regardless the family history, the mean age at diagnosis of breast cancer in *RAD51C* carriers is younger than that of the general population but older than that for *BRCA1* and *BRCA2* carriers (Online resource 2). All these data point out a role of *RAD51C* gene in breast cancer development and suggest that testing for mutations in this gene might have clinical utility for breast cancer patients [35, 36].

Finally, recent studies have shown that, as with *BRCA1* and *BRCA2* genes, mutations in *RAD51C* gene are associated with homologous recombination deficient phenotypes in epithelial ovarian cancer and may predict a better response to specific targeted therapies [36]. Pennington et al. demonstrated that germline and somatic mutations in homologous repair genes, including *RAD51C*, predict higher rates of platinum sensitivity and better overall survival in primary ovarian carcinoma. They hypothesized that individuals with mutations in homologous recombination genes other than *BRCA1* and *BRCA2* genes will also have increased response rates to PARP inhibitors [35]. These data suggest that massive parallel sequencing of a panel of homologous repair genes, including *RAD51C,* might be considered in ovarian cancer patients to tailor their systemic therapy.

In summary, we have identified three *RAD51C* pathogenic variants c.577C > T (p.Arg193Stop), c.656T > C (p.Leu219Ser), and c.709C > T (p.Arg237Stop) in our cohort of 516 Spanish breast and/or ovarian cancer families (0.6 %). Two *RAD51C* carriers had a family history of breast and ovarian cancer, whereas the mutation c.577C > T (p.Arg193Stop) was identified in a breast cancer only family. Our results suggest that *RAD51C* testing should be offered to hereditary breast and/or ovarian cancer families in addition to *BRCA1* and *BRCA2* testing. Data derived from larger cohorts may help to delineate the cancer penetrance in *RAD51C* mutation carriers and provide evidence for tailoring medical management of mutation carriers.

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