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EPIDEMIOLOGY

Germline pathogenic variants in BRCA1, BRCA2, PALB2 and RAD51C in breast cancer women from Argentina

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Abstract

Purpose Each year, 17,000 new breast cancer cases are diagnosed in Argentina, and 5400 women die of breast cancer. The contribution of cancer-related mutations to the incidence of breast cancer in Argentina has not yet been explored.

Methods We sequenced the entire coding regions of *BRCA1*, *BRCA2*, *PALB2* and *RAD51C* in 112 unselected Argentinian breast cancer patients.

Results A pathogenic genetic variant was found in 12 of 112 (10.7%) patients; two in *BRCA1* (1.8%), five in *BRCA2* (4.5%), four in *PALB2* (3.6%) and one in *RAD51C* (0.9%). Three of four (75%) *PALB2* mutation carriers carried the same variant (c.1653T > A).

Conclusions A founder mutation in *PALB2* accounts for up to 4% of breast cancer patients in Argentina. *BRCA1*, *BRCA2*, *PALB2* and *RAD51C* should be included in the genetic testing panel of breast cancer patients in Argentina.

Keywords Breast cancer · Argentina · PALB2 · Founder mutation · Genetics · Hereditary

Introduction

Argentina has the second highest rate of breast cancer incidence in all Latin American countries (38.4 per 100,000/year) and an annual mortality of 5400 people [1]. 56% of the Argentinean women have some indigenous ancestry while the remaining 44% are mostly descendants of Europeans (mostly Spanish and Italians) [2]. This unique demographic suggests that Argentina may have unique founder populations due to their historical immigration patterns and relative reproductive isolation.

Mutations in *BRCA1* and *BRCA2* can account for the majority of hereditary breast cancers. Other genes such as *PALB2* or *RAD51C* may account for cases that cannot be explained by *BRCA1* and *BRCA2* [3]. Being able to determine the mutation carrier frequency and to identify frequent gene mutations may lead to the development of national guidelines for genetic testing. These data could help the implementation of genetic counselling and testing, as well as for the development of prevention and early-detection strategies. We have examined the prevalence of *BRCA1*, *BRCA2*, *PALB2*, and *RAD51C* in a population of 112 unselected Argentinian women with breast cancer.

Methods

Study subjects

Breast cancer patients were recruited from the Centro Nacional de Genética Médica, ANLIS; a medical genetics

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institute in Buenos Aires, Argentina. All participants provided consent for the study. Our protocol was approved by the Bioethics Committee for Human Research at the Centro Nacional de Genética Médica, as well as by the Research Ethics Board at Women's College Hospital. Research participants were deemed eligible for this study if they presented with either a primary invasive breast cancer or a ductal carcinoma in situ, and if at least one of their grandparents was born in Argentina. A total of 112 unselected breast cancer patients were enrolled between the years of 2009 and 2012. Participants provided blood, completed a risk factor questionnaire, and provided a family history of cancer. We reviewed patient medical records to collect information regarding age of diagnosis, other primary tumors, tumor stage, grade, histology, lymph node involvement, and ER/HER2 status. We also recorded age at menarche, age of first pregnancy, parity, birth order and age at menopause.

Laboratory

Participants' DNA was extracted from their blood samples using the 'salting out method' at the Departamento de Genética Experimental del Centro Nacional de Genética Médica, ANLIS, in Buenos Aires. Once extracted, the DNA samples were sent to the molecular genetics laboratory at Women's College Hospital in Toronto.

All 22 coding exons of *BRCA1* (NM_007294.3), 26 coding exons of *BRCA2* (NM_000059.3), 13 coding exons (exons 1–13) of *PALB2* (NM_024675.3), and 9 coding exons (exons 1–9) of *RAD51C* (NM_058216) plus 20 base pairs from the exon boundaries were amplified in 81, 134, 54, and 19 amplicons, respectively, using Wafergen SmartChip technology (Wafergene Inc, CA). Illumina panel next-generation sequencing adaptors and sample unique DNA barcodes were incorporated into the amplified amplicons with a second PCR. The prepared DNA library was pooled and paired-end sequenced at 2 × 250 cycles using an Illumina MiSeq sequencer.

The sequence reads for each DNA sample aligned with the reference sequences from the four genes using a Burrows-Wheeler Aligner. Next, the Picard package was used to convert the files from sequence alignment/map format (SAM) to a compressed, binary alignment/map (BAM) format. The BAM files were subsequently sorted and indexed. As for the unmapped reads, reads with low mapping quality, and reads aligned to more than one region; they were filtered out from the BAM file using the genome analysis toolkit (GATK) package. The HaplotypeCaller module of the GATK was then used to identify SNPs, insertions and deletions in these reads. Regions with at least 20-fold depth of coverage were used for calling variants, and a nucleotide that differed from the reference sequence in at least 25% of the reads aligned to a given position was called as a variant. Once these variants were identified, we used the SNP & Variation Suite (GoldenHelix Inc., Bozeman, MT) to annotate them, and confirmed all truncating and missense mutations using Sanger sequencing. Sequencing reactions were performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Fisher Scientific) according to the manufacturer's protocol on the Applied Biosystem Prism 3500XL Genetic Analyzer (Fisher Scientific), and were analyzed for variant detection using the Mutation Surveyor software (SoftGenetics LLC, State College, Pa).

We completed sequencing of all coding exons of *BRCA1, BRCA2, PALB2*, and *RAD51C* for 112 Argentinian patients. The mean depth of coverage was 2962× (range 1050× to 3989×). On average, 99.5% (range 95.3% to 100%) of the coding exons of the four genes were covered at 20x depth of coverage and higher, which is used for variant calling.

Results

A total of 112 unselected Argentinian women with breast cancer were studied. The mean age at diagnosis was 46.9 years (range 26–84) (Table 1). 76 of 112 patients (67.9%) were premenopausal. Of those with known ER/HER2 status, 68 of 95 patients (71.6%) presented with ER-positive breast cancers, and 46 patients (48.4%) were

Table 1 Characteristics of all Argentinian breast cancer patients

	N	Proportion
Mean age at Dx	46.9	
Age range	26–84	
Age at breast cancer diagnosis		
19–29	6	0.05
30–39	22	0.20
40–49	47	0.42
50+	37	0.33
Bilateral cases	26	0.23
Histology		
ER+	68	0.61
ER−	7	0.06
Unknown	17	0.15
HER2/neu+	46	0.41
HER2/neu−	29	0.26
Unknown	17	0.15
Triple negative	20	0.33
Heredity		
European	50	0.45
Indigenous	4	0.04
European/indigenous	40	0.36
Other	12	0.15

HER2-positive. 20 patients (21.1%) were recorded as having triple-negative breast cancers (Table 1).

Pathogenic mutations were found in 12 of 112 (10.7%) patients. Of these, two (1.8%) had a pathogenic mutation in *BRCA1*, five (4.5%) in *BRCA2*, four (3.6%) in *PALB2*, and one (0.9%) in *RAD51C* (Table 2). The *PALB2* variant, c.1653T > A, was the only recurrent variant in our study and was found in three of the 12 (25%) mutation carriers. This recurrent variant constituted three of the four *PALB2* mutations (75%) in our study. We did not find any recurrent *BRCA1/BRCA2*-genetic variant.

The mean age at diagnosis of the 12 mutation carriers was 44.7 years (range 31–56). The mean age at diagnosis of the 100 non-carriers was 46.9 years (range 26–84; $p = 0.405$). The mean age at diagnosis was 42.6 for *BRCA2*, 43.0 for *RAD51C*, 45.3 for *PALB2*, and 49.5 for *BRCA1*. The prevalence of mutations amongst carriers of ages 20–29 was 0% (0/6), 30–39 was 9.1% (2/22), 40–49 was 14.9% (7/47), and 50 or older was 8.1% (3/37).

A mutation was discovered in six of 68 ER-positive patients (8.8%), in seven of 46 HER2-positive patients (15.2%) and in four of 20 (20%) triple-negative patients. All 12 carriers had European ancestry, and six of the 12 carriers (50%) had Amerindian ancestry as well.

42 of 112 patients (37.5%) had a first-degree relative with breast cancer, and 62 of 112 patients (55.4%) had a first or second-degree relative with breast cancer. Five of 112 patients (4.5%) had a first-degree relative with ovarian cancer, and 17 of 112 patients (15.2%) had a first or second-degree relative with ovarian cancer. Mutations occurred in 16.1% of patients (10/62) who reported having one or more first or second-degree relatives with breast cancer, and in 4.0% of patients (2/50) who reported having no first or second-degree relatives with breast cancer ($p = 0.040$).

Three of the four *PALB2* carriers had a first or second degree relative with breast cancer (Fig. 1). The single *RAD51C* carrier had no relative with breast cancer, but she had one relative with ovarian cancer. Other cancers observed in first and second degree relatives of these twelve carrier families included pancreas cancer (1 case), colon cancer (3 cases), prostate cancer (3 cases), gastric cancer (4 cases), ovarian cancer (2 cases), lymphoma (2 cases), lung cancer (3 cases), liver cancer (1 case), melanoma (1 case) and sarcoma (2 cases).

Discussion

This is the first report of genetic screening in unselected breast cancer patients from Argentina. We identified pathogenic genetic mutations in *BRCA1*, *BRCA2*, *PALB2*, or *RAD51C* in 12 of 112 (10.7%) women with breast cancer. The frequency of *BRCA* pathogenic variants was 6.3% (7/112), which is comparable to similar, reported populations of unselected South American women with breast cancer (1.2% to 7.1%) [3–11]. All *BRCA1* and *BRCA2* variants had been described in other populations [12, 13].

Interestingly, we detected a relatively high frequency of *PALB2* mutations (4/112, 3.6%) among Argentinian breast cancer patients. The prevalence exceeds those reported in

Table 2 Mutation characteristics and family histories of all Argentinian breast cancer patients

Sample ID	AgeDgn	Gene 1	Exon 1	HGVS Coding 1	Ancestry	ER	HER2	PR	Family History of Cancer
F249P1	40	BRCA2	10	c.1138delA	Italian/Spanish	−	+	+	Breast: Mother, 2 aunts, Pancreatic: 2 uncles pancreatic cancer
F126P1	39	BRCA2	11	c.5351_5352insA	Indigenous/Spanish	−	−	−	Breast: Mother, 1 aunt Gastric: 1 uncle
F3P1	31	BRCA2	11	c.5681_5682insA	German/Paraguayan	+	+	+	Breast: Sister
F252P1	47	BRCA2	17	c.7857G > A	Italian/Irish	+	+	+	Breast: 2 aunts Prostate: 1 uncle
F202P1	56	BRCA2	23	c.8987T > A	Indigenous/Spanish	−	−	−	Breast: Mother, grandmother Gastric: Grandfather
F119P1	41	PALB2	5	c.2283_2284ins GCACACCCCAACTTGCT	Italian	+	+	+	Prostate: Uncle
F95P1	45	PALB2	4	c.1653T > A	Spanish/Indigenous	+	+	+	Breast: Mother, grandmother
F140P1	53	PALB2	4	c.1653T > A	Spanish/Arabic	−	+	−	Breast: Mother, 2 sisters Colon: Daughter
F247P1	42	PALB2	4	c.1653T > A	Spanish/Indigenous	−	−	−	Breast: Mother, sister
F134P1	55	BRCA1	10	c.4035delA	Ukrainian/Russian	−	−	−	Breast: Sister
F69P1	44	BRCA1	4	c.211A > G	Spanish/Indigenous	+	−	+	Breast: Mother, sister, aunt
F109P1	43	RAD51C	7	c.905-2_905-1delAG	Italian/Indigenous	+	+	+	Ovarian: Aunt Gastric: Grandfather

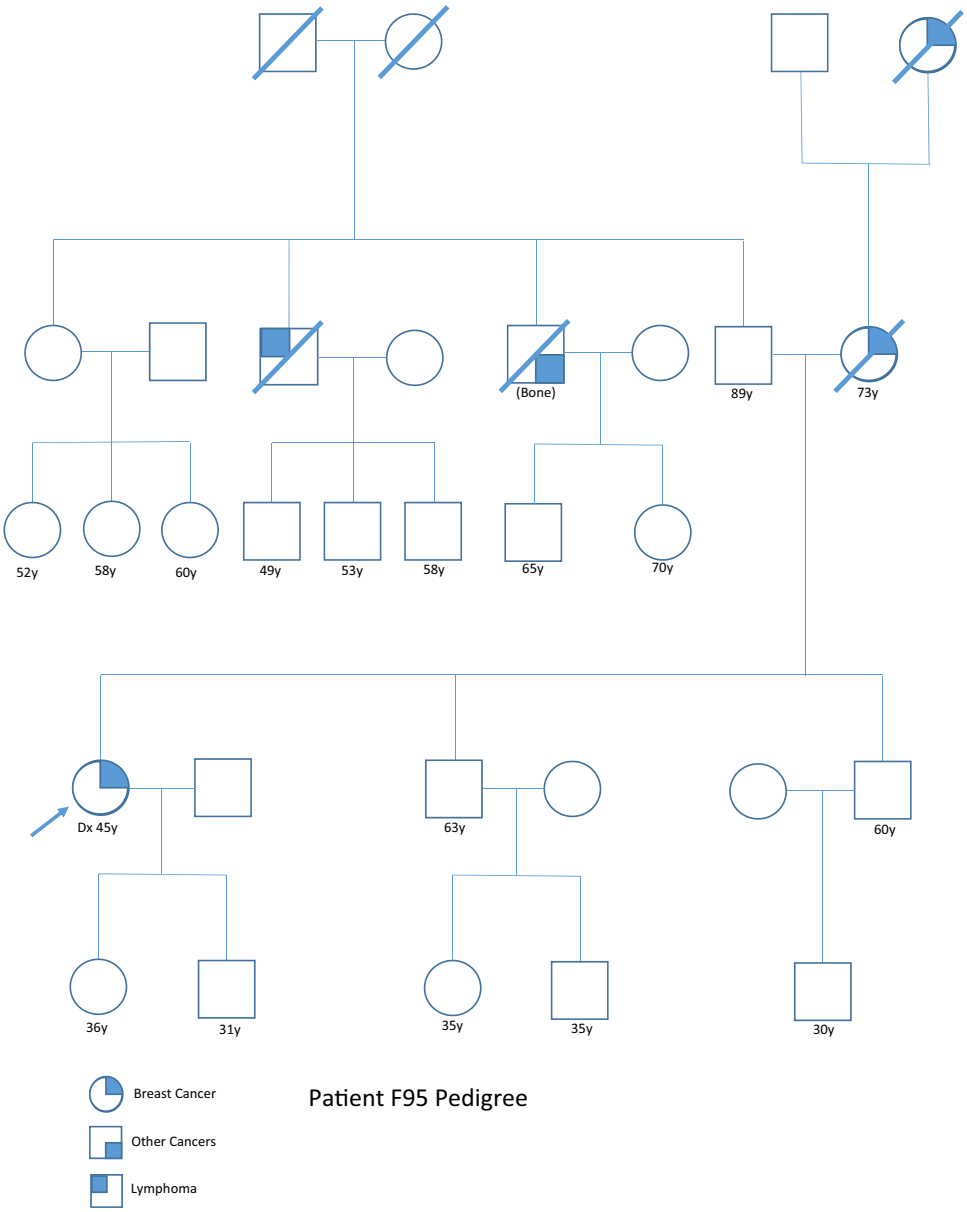


Fig. 1 Pedigrees of the four Argentinian patients (F95, F119, F140 and F247) with *PALB2* mutations. Patient familial history of cancer is presented in the form of a pedigree. Breast cancer is defined by shading in the upper-right corner, lymphoma is defined by shading in the upper-left corner, and other cancers are defined by shading in the lower-right corner of an individual

Finnish, French Canadian, Australian and African-American populations (0.4–1.1%) [14–18], but our sample was relatively small. Interestingly, similar high proportion was observed in our previous study in Jamaican, in which *PALB2* mutations were found in 2.8% of study participants [19]. Argentina exhibits the highest reported rate of *PALB2* mutation for an unselected population of women with breast cancer. *PALB2* mutation carriers have been reported as having a lifetime breast cancer risk of between 33 and 58% [20]. In our study four of 12 patients with a mutation (33.3%) had *PALB2* mutations, compared to only two with a *BRCA1* mutation. Three of the four patients with *PALB2* mutations presented with the: c.1653T > A variant. Two of these presented with bilateral breast cancer (Table 2). This *PALB2* mutation was also reported in breast/ovarian cancer patient with Spanish ancestry and a familial breast cancer case from the United States. This suggests that c.1653T > A is probably a Spanish *PALB2* founder mutation. To our knowledge, there are four other *PALB2* founder mutations, one in the French Canadian population from Quebec (c.2323 C > T) [21], one in Greece (c.2257C > T) [22], and two in Poland (c.509_510delGA and 172_175delTTGT) [23–25].

RAD51C was originally regarded as a predictive marker for breast and ovarian cancer [26–28]. After further investigation, the general dogma was changed to consider RAD51C as a predictor of ovarian cancer alone, effectively disregarding its role in predicting breast cancer [29, 30]. As more research has been conducted on RAD51C, it is once again being considered as a predictor for breast and ovarian cancer, particularly in patients whom present with a triple negative receptor status [31, 32].

Fig. 1 (continued)

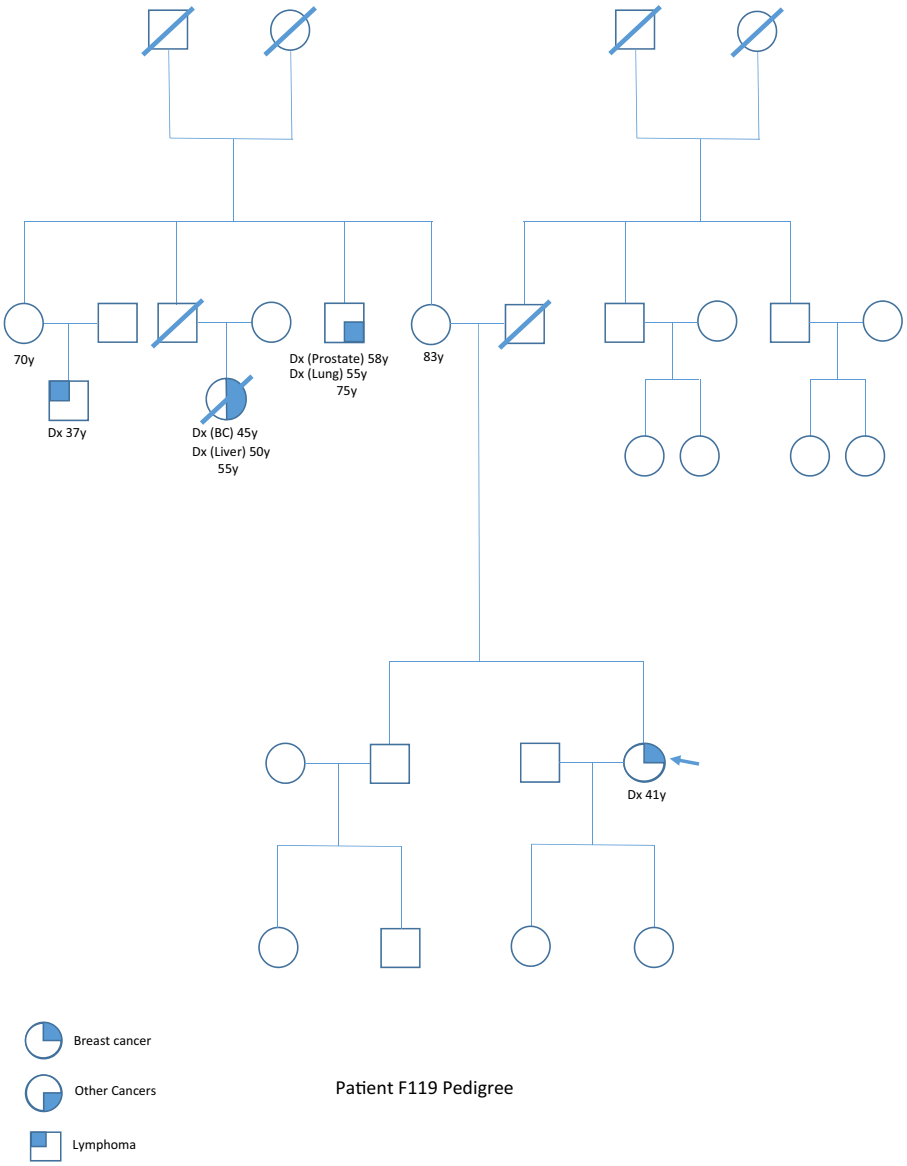
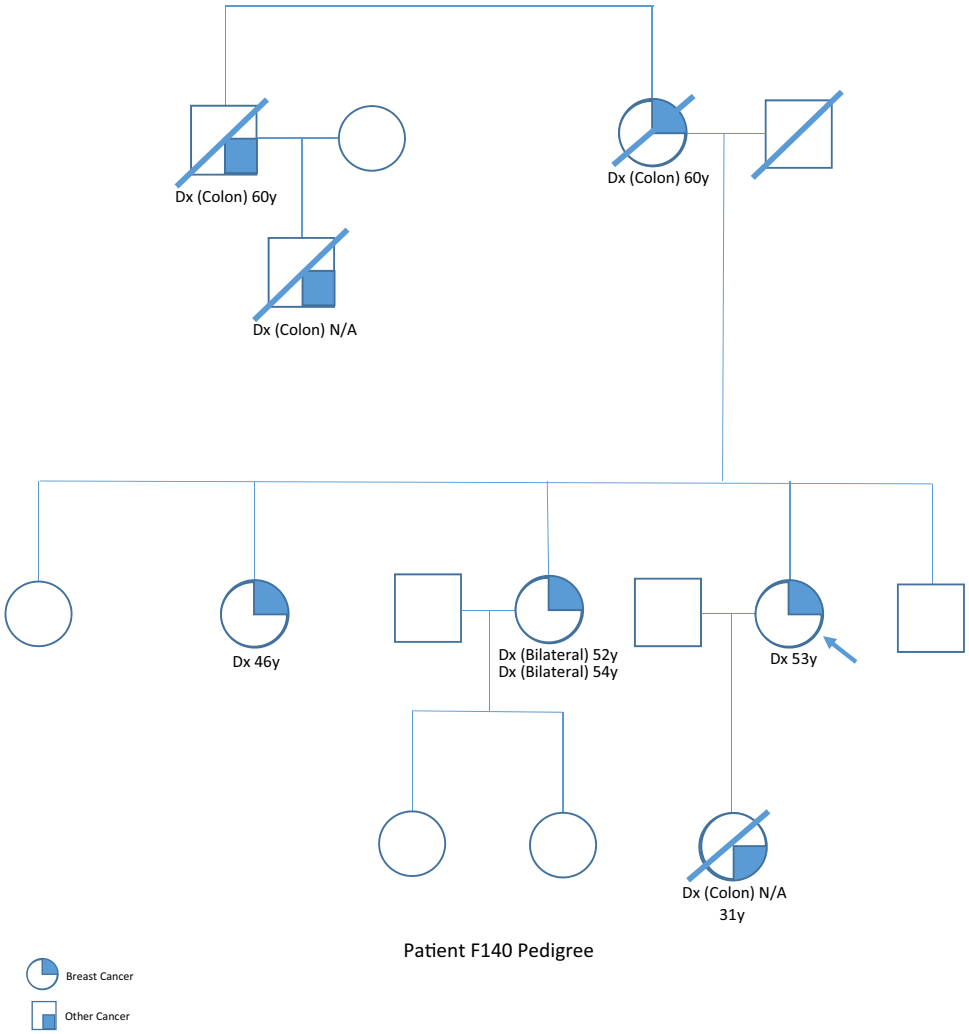


Fig. 1 (continued)



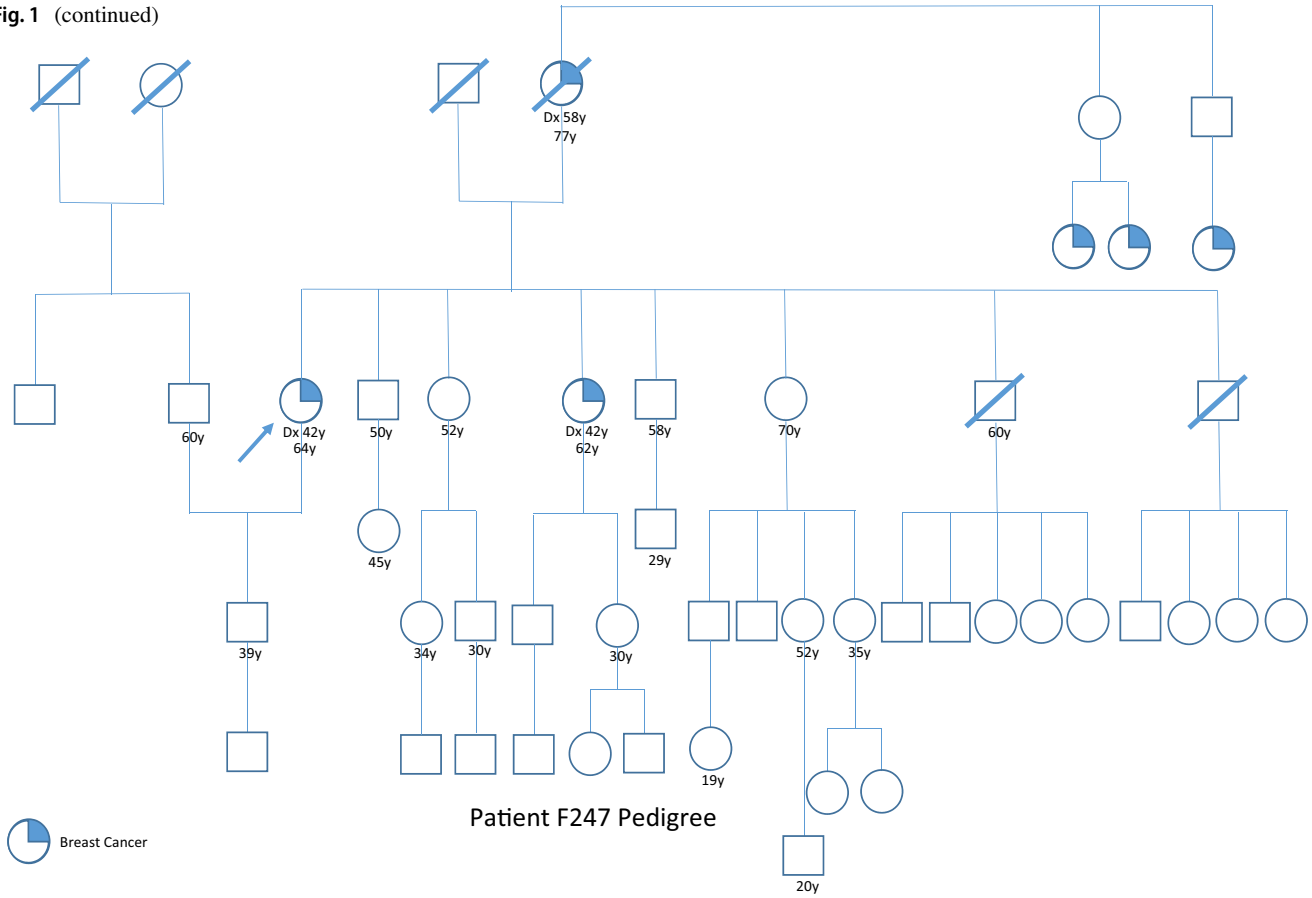
In our Argentinian population we identified one germline pathogenic variant in the *RAD51C* gene. To date, only a few breast cancer families have been described to carry deleterious *RAD51C* alterations, suggesting that compromised RAD51C function may result in both BC and OC [33, 34]. Because of the low *RAD51C* deleterious frequency, especially in BC-only families, large collaborative studies are needed to quantify the relative risk of *RAD51C* alterations in BC.

There are other breast cancer genes, such as *CHEK2* and *ATM*, for which we did not screen in this study's genetic panel. These omissions suggest that there may be more information to be found about the heredity of breast cancer within this population.

Conclusions

In this study, we demonstrated that a high percentage of Argentinian breast cancer patients (3.6%) carry a deleterious mutation. Our results suggest the importance of including *PALB2* screening as a standard, alongside *BRCA1* and *BRCA2*, for genetic testing in Argentinian women with breast cancer.

Fig. 1 (continued)



Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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