Detection of a large rearrangement in *PALB2* in Spanish Breast Cancer Families with male breast cancer

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ABSTRACT

It has been demonstrated that monoallelic *PALB2* (Partner and Localizer of BRCA2) gene mutations predispose to familial breast cancer. Some of the families reported with germline *PALB2* mutations presented male breast cancer as a characteristic clinical feature. Therefore, we wanted to investigate the contribution of germline *PALB2* mutations in a set of 131 Spanish *BRCA1/BRCA2*-negative breast/ovarian cancer families with at least one male breast cancer case. The analysis included direct sequencing of all coding exons and intron/exon boundaries as well as a Multiplex Ligation-dependent Probe Amplification-based analysis of genomic rearrangements. For the first time we have identified a genomic rearrangement of *PALB2* gene involving a large deletion from exon 7 to 11 in a breast cancer family. We have also identified several *PALB2* variants, but no other obvious deleterious *PALB2* mutation has been found. Thus, our study does not support an enrichment of *PALB2* germline mutations in the subset of breast cancer families with male breast cancer cases. The identification of intronic and exonic variants indicates the necessity of assessing the implications of variants that do not lead to *PALB2* truncation in the pathogenicity of the *PALB2* gene.

Keywords: Familial breast cancer; *PALB2*; Male Breast Cancer; genomic rearrangement; *BRCA1/BRCA2*-negative families, in silico analysis

INTRODUCTION

Breast cancer with an autosomal dominant pattern of inheritance accounts for 5-10% of all breast cancer cases. Between 20-25% of inherited breast cancer can be traced to germline mutations in *BRCA1* and *BRCA2* genes. Other genes participating in DNA damage response pathways such as *CHEK2*, *ATM* and *FANCI/BRIP1* are also involved in familial breast cancer [1].

The *PALB2* (for “partner and localizer of BRCA2”) gene encodes a protein that participates in genome maintenance through double strand break repair. *PALB2* protein binds to the *BRCA2* N-terminal end stabilizing it in key nuclear structures, which allows *BRCA2* to function in DNA repair and at the S-phase checkpoint [2]. *PALB2* co-localizes with *BRCA2* being essential for key *BRCA2* nuclear caretaker functions, implying that *PALB2* could be also considered a tumor-suppressor gene [3]. Recent studies have shown that *PALB2* not only interplay with *BRCA2*, but also mediates the interaction between *BRCA1* and *BRCA2* in the DNA-damage response [4]. *BRCA1* acts in this complex as an upstream regulator of *PALB2* and

BRCA2 in the DNA-damage response and is important for targeting PALB2 and BRCA2 to DNA-damage sites [4]. PALB2 and BRCA2 act together to deliver RAD51, another DNA double strand break repair, to the chromosomal lesion in order to initiate DNA repair. Moreover, it has been recently shown that PALB2 binds DNA and physically interacts with RAD51 exerting a stimulatory effect on the ability of RAD51 to make D-loops [5].

BRCA2 is also known as FANCD1, which is a Fanconi anemia protein. Biallelic mutations in BRCA2 and PALB2, respectively, underlie Fanconi anemia complementation groups FA-D1 and FA-N. However, clinical phenotype of these patients is strikingly different from that reported in classical Fanconi anemia [6]. In particular, FA-D1 and FA-N are associated with higher risks of solid childhood malignancies such as Wilms tumor and medulloblastoma, which occur very rarely in other subtypes of Fanconi anemia. Monoallelic *PALB2* mutations have also been confirmed to cause moderate-penetrance breast cancer susceptibility [2], accounting for about 1% of *BRCA1/BRCA2* negative familial and early breast cancer [7][8][9]. *PALB2* is responsible for 1-2% of young breast cancer patients unselected for breast family history in Chinese and white South African populations [10] [11]. None of the breast cancer cases with *PALB2* germline mutations reported to date have been found to show loss of heterozygosity (LOH) at this locus. This lack of LOH has raised speculation on alternative mechanisms of *PALB2* functional inactivation [7].

To date, studies examining *PALB2* gene in specific populations have been carried out, identifying recurrent mutations such as c.2323C>T (p.Q775X) in French Canadians [9], c.1592delT (p.L531fs) in Finnish [12] or c.751C>T (p.Q251X) in Chinese population [10].

Recently, a potential connection between *PALB2* truncating mutations and male breast cancer arose from several studies. Rahman et al. (2007)[6] detected that the prevalence of truncating mutations was higher in families with both male and female breast cancer (6.7%) than in families with only breast cancer cases in females (1%). Moreover, more recently Garcia et al. (2009)[7] analyzed *PALB2* germline mutations in a cohort of 95 *BRCA1/BRCA2*-negative Spanish breast/ovarian cancer families, identifying only one frameshift alteration (c.1056_1057delGA; p.K353IfsX7). Interestingly, the mutation was detected in a family with a male breast cancer, further supporting that male breast cancer is a clinical characteristic feature of *PALB2* autosomal dominant breast cancer syndromes.

The present study attempts to evaluate for the first time in the Spanish population, the contribution of germline *PALB2* mutations in 131 *BRCA1/BRCA2*-negative high-risk breast/ovarian cancer families with at least one case of male breast cancer.

MATERIAL AND METHODS

Patients

Index cases from 131 *BRCA1/BRCA2* mutation-negative Spanish high-risk breast/ovarian cancer families with at least one male breast cancer case were screened for mutations within the entire coding sequence and splicing sites of *PALB2* gene. Families were ascertained through 12 different Spanish centres (Supplementary Table 1). Clinical characteristics of the families are represented in Table 1. The ethics committee approvals as well as informed consent from the patients were obtained for the study.

All index cases had been previously screened for point mutations and large rearrangement mutations in *BRCA1* and *BRCA2* genes and were found to be negative.

Mutation analysis of PALB2 gene

DNA samples were obtained from peripheral blood leukocytes using standard procedures. The entire coding sequence of *PALB2* along with flanking intron boundaries were amplified using primers designed with Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Primer sequences and melting temperatures are shown in Supplementary table 2. A negative control was used in each PCR. PCR quality was checked on Agilent 2100 Expert Bioanalyzer (Agilent Technologies Inc. Santa Clara, CA USA). Then, PCR products were purified by “Exosap-it” (USB Corporation, Cleveland, Ohio) and bi-directional sequencing reaction was performed with “Big Dye Terminator v3.1 Cycles Sequencing Kit” (Applied Biosystems, Warrington, UK) on ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer’s instructions. Sequences were performed using the PCR primers with the exception of exon 13 (see supplementary table 2). Sequences were examined using Staden Package software (Open Source Technology Group, Inc).

Multiplex ligation-dependent probe amplification (MLPA) to detect genomic rearrangements of *PALB2* was performed using the FANCD2-PALB2 kit (P057) from MRC-Holland (Amsterdam, The Netherlands) in accordance with the manufacturer’s protocol. The samples were run and analyzed on the ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems) using fragment analysis tools. Analysis was repeated using an independent sample in an independent assay for positive DNA samples of a genomic rearrangement. Analysis of

MLPA results was performed visually and by Coffalyser MLPA-DAT from MRC-Holland using direct analysis as analysis method to normalization.

In silico analysis

Briefly, splicing predictions were performed with SpliceSiteFinder [13], MaxEntScan [14], NNSplice [15] and GeneSplicer [16] algorithms. Biological significance was suspected only if two algorithms predicted a splice site score variation greater than 10% [17]. Amino acid substitutions were tested with PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), SIFT (<http://sift.jcvi.org/>) and Align-GVGD (<http://agvgd.iarc.fr/>) tools. All in silico analysis were performed using Alamut- Mutation Interpretation Software v.1.54 (<http://www.interactive-biosoftware.com/alamut.html>).

Nomenclature and databases

Sequences used for *PALB2* nomenclature were obtained from the NCBI RefSeq database (NG_007406.1 for genomic, NM_024675.3 for mRNA and NP_078951.2 for protein) (<http://www.ncbi.nlm.nih.gov>). The nomenclature follows the recommendations from the Human Genome Variation Society (HGVS, www.hgvs.org/mutnomen/) [18].

RESULTS

We sequenced all exons and intronic boundaries of *PALB2* gene and we also carried out MLPA analysis in 131 index cases from *BRCA1/BRCA2*-negative high-risk Spanish breast/ovarian cancer families with at least one male breast cancer.

We identified a large deletion spanning from exon 7 to 11 (c.2587-?_3201+?del) (Figure 1), in a woman diagnosed with breast cancer at 70 years of age. The proband had a brother diagnosed both with breast cancer at 75 years and gastric cancer after the initial breast cancer diagnosis (Figure 2). To confirm segregation with disease, we tested for the presence of the *PALB2* deletion in the paraffined breast tumoral sample of the affected brother. MLPA analysis demonstrated the presence of the alteration. Interestingly, we observed a dosage reduction close to 75% for exons 7 to 11 together with a dosage reduction in the 25%-50% range for the remaining exons (the only exception being exon1, in which no dosage reduction was observed). The data might be compatible with partial loss of the wild type allele (exons 2 to 13) in the tumor (Figure 1).

In addition to this deletion, we also identified seventeen *PALB2* germline variants, although none of them was predicted to result in a truncated protein product (Table 2). Seven of them were novel (c.-200-27T>G; c.-158G>T; c.620C>G (p.Pro207Arg); c.1684+29A>G; c.2586+58C>T; c.2587-38C>G; c.2865T>A (p.Ser955Arg)) and all of them except c.2586+58C>T were found once in our samples. The variants c.-47G>A, c.212-58A>C, c.1010T>C (p.Leu337Ser), c.1194G>A (p.=), and c.1572A>G (p.=), c.1676A>G (p.Gln559Arg), c.2014G>C (p.Glu672Gln), c.2590C>T (p.Pro864Ser), c.2993G>A (p.Gly998Gln) and c.3300T>G (p.=) had been previously reported [7, 10, 12, 19-23].

The results of bioinformatic predictions for intronic and missense variants are represented in Table 3. Interestingly, three of the missense variants, c.620C>G (p.Pro207Arg), c.1010T>C (p.Leu337Ser), and c.2993G>A (p.Gly998Gln), were predicted to likely affect *PALB2* protein function by all the tested algorithms. Unfortunately, it was not possible to do segregation analysis in the families for any of these variants. The bioinformatic splicing analyses do not show variations in the consensus site scores. For variants c.-200-27T>G, c.-47G>A, c.1676A>G (p.Gln559Arg), and c.2590C>T (p.Pro864Ser), a reduction of 100% in a cryptic splice site score is predicted for at least two algorithms. MaxEntScan and NNSplice algorithms predicted an increase in the score of the cryptic site c.2597 in 11.2 and 22.8%, by the variant c.2590C>T (p.Pro864Ser).

There were six index cases sharing six different variants: c.212-58A>C, c.1676A>G (p.Gln559Arg), c.2014G>C (p.Glu672Gln), c.2586+58C>T, c.2993G>A (p.Gly998Gln) and c.3300T>G (p.=). The variants c.1676A>G (p.Gln559Arg) and c.2586+58C>T were also present in other cases, and the former is predicted by the bioinformatic software to probably affect *PALB2* protein function. In order to identify if these six variants were inherited together, co-segregation analysis in one of the carrier families was performed. Our results suggest that all the six variants constituted a haplotype (Supplementary figure 1) that is present in the two breast cancer patients of the analyzed family.

DISCUSSION

PALB2 mutations have recently been shown to confer a moderate risk to develop breast cancer [6]. It has been reported that families carrying protein-truncating *PALB2* mutations shown cases of male breast cancer [6, 7]. Given the increased risk of male breast cancer associated with carrying a germline *BRCA2* mutation and the functional relationship between *BRCA2* and *PALB2*, the male *PALB2* mutation carriers might be at an increased risk of breast cancer.

Until now more than 26 *PALB2* truncating mutations have been reported [20, 22, 24-26], 22 of them found in breast cancer families. Although large genomic rearrangements in *PALB2* have been analysed in studies of familial breast cancer [9, 20, 27, 28], the unique large genomic mutation detected in *PALB2* was c.71-?_561+?del. This genomic change was identified in a Fanconi Anemia patient, and it implies the deletion of exons 2, 3 and 4 [29]. In our series of 131 index cases from Spanish *BRCA1/BRCA2*-negative breast/ovarian cancer families with a male breast cancer case, we identified a large rearrangement in one breast cancer index case. The mutation c.2587-?_3201+?del detected in our population is a large deletion comprising exons 7 to 11. Our finding provides the first evidence that large genomic changes in *PALB2* also contribute to hereditary breast cancer in families *BRCA1/BRCA2* negatives.

To date, all *PALB2* mutations reported as pathogenic result in premature protein truncation [6], and were identified in about 1% of familial breast cancer. In breast cancer families with both female and male breast cancer cases, frequency of *PALB2* mutations increased to 6.7% (1/15) in the UK series of Rahman et al [6] and to 9% (1/11) in the Spanish series of Garcia et al [7]. Although these previous series of families with male breast cancer were small, results seemed to suggest a higher implication of *PALB2* mutations within this type of families. In the present study, we analysed 131 Spanish female and male breast cancer families extending the mutation detection analysis to detect large genomic rearrangements of *PALB2* by using Multiplex ligation-dependent probe amplification (MLPA). The frequency of *PALB2* mutations found was 0.75%, which is similar to the 1% frequency previously reported for breast cancer families not selected for male breast cancer. However, we cannot rule out the potential pathogenicity of non truncating *PALB2* variants. It has been shown that the N-terminal coiled-coil motif of *PALB2* (residues 1-319) interacts with the coiled-coil domain of *BRCA1* [4, 30], whereas the C-terminal WD40 repeats of *PALB2* (residues 836-1186) associate with the N-terminus of *BRCA2* [3, 4, 31]. Thus, *PALB2* missense variants in these domains might be pathogenic. From the missense variants identified in our study, c.620C>G (p.Pro207Arg) is located in the coiled-coil domain of *PALB2*, whereas c.2590C>T (p.Pro864Ser), c.2865T>A (p.Ser955Arg) and c.2993G>A (p.Gly998Gln) are located in the C-terminal domain of the protein. Moreover variants c.620C>G (p.Pro207Arg), c.1010T>C (p.Leu337Ser), and c.2993G>A (p.Gly998Gln) have been predicted as pathogenic by the three amino acidic algorithms (Table 3).

As for other genes, the possibility of intronic variants of *PALB2* being implicated in the splicing process should not be discarded. Besides, functional analysis for variants that could regulate gene expression should also be considered.

The MLPA analysis of the breast tumor sample FFPE tissue from the carrier of exon 7-11 deletion suggests partial loss of the wild type *PALB2* allele (exons 2 to 13) which in turn could support previous evidences that *PALB2* loss of function might also conform to the inactivation model of a classic tumor-suppressor gene [7]. Yet, great caution should be taken when interpreting MLPA analysis of FFPE tissue

In summary, our work identifies for the first time a genomic rearrangement of *PALB2* gene in a *BRCA1/BRCA2* mutation-negative Spanish cancer family with at least one male breast cancer case. If we considered only *PALB2* truncation variants as pathogenic, the frequency of *PALB2* mutations in high-risk families with male breast cancer is similar to that reported for high-risk families not selected by the presence of male breast cancer. However, more studies to assess the implication of *PALB2* variants that do not originate *PALB2* truncation (missense changes, small in-frame insertions or deletions and potential splice site alterations) are required.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1. Clinical characteristics of the 131 male and female breast cancer index cases

Clinical diagnosis and patient information	
Age in years at first diagnosis (years)(male/female)	58/55
Breast cancer (male/female)	83/36
Bilateral breast cancer (male/female)	5/4
Ovarian cancer	3
Family history of first degree relative with breast cancer (male/female)	40/77
Family history of second degree relative with breast cancer (male/female)	18/44
Other malignancies in family	
Gastric	21
Colon	17
Prostate	16
Lung	7
Ovarian	6
Leukaemia	5
Melanoma	5
Cerebral	4
Larynx	3
Kidney	3
Stomach	2
Pancreas	2
Osteosarcoma	2
Bladder	2
Throat	1
Uterus	1
Lymphoma	1
Non- Hodgkin Lymphoma	1
Esophagus	1
Lips	1
Cervix	1
Head-neck	1
Multiple malignancies	
Breast and prostate cancer	5
Breast and gastric cancer	3
Breast cancer and melanoma	2
Breast and colon cancer	2
Breast and ovarian cancer	1
Larynx and stomach cancer	1

Table 2. *PALB2* sequence variants identified in 131 Spanish breast/ovarian cancer families with male breast cancer cases

Location	Nucleotide change ^a	Protein change	Name of SNP	Genotypes			Allelic frequency (%) ^b	
				AA	AB	BB	A (A%)	B (B%)
5' upstream sequence	c.-200-27T>G	-	-	0	1	130	1 (0.38)	261 (99.62)
EXON1	c.-158G>C	-	-	0	1	130	1 (0.38)	261 (99.62)
	c.-47G>A	-	rs8053188	0	3	128	3 (1.14)	259 (98.86)
IVS3	c.212-58A>C	-	-	0	6	125	6 (2.29)	256 (97.71)
EXON 4	c.620C>G	p.Pro207Arg	-	0	1	130	1 (0.38)	261 (99.62)
	c.1010T>C	p.Leu337Ser	rs45494092	0	3	128	3 (1.14)	259 (98.86)
	c.1194G>A	p.Val398Val	rs61755173	0	1	130	1 (0.38)	261 (99.62)
	c.1572A>G	p.Ser524Ser	rs45472400	0	1	130	1 (0.38)	261 (99.62)
	c.1676A>G	p.Gln559Arg	rs152451	4	34	93	42 (16.03)	220 (83.97)
IVS4	c.1684+29A>G	-	-	0	1	130	1 (0.38)	261 (99.62)
EXON 5	c.2014G>C	p.Glu672Gln	rs45532440	0	6	125	6 (2.29)	256 (97.71)
IVS6	c.2586+58C>T	-	rs249954	7	60	64	74 (28.24)	188 (71.76)
	c.2587-38C>G	-	-	0	1	130	1 (0.38)	261 (99.62)
EXON 7	c.2590C>T	p.Pro864Ser	rs45568339	0	2	129	2 (0.76)	260 (99.24)
EXON 9	c.2865T>A	p.Ser955Arg	-	0	1	130	1 (0.38)	261 (99.62)
	c.2993G>A	p.Gly998Gln	rs45551636	0	6	125	6 (2.29)	256 (97.71)
EXON 12	c.3300T>G	p.Thr1100Thr	rs45516100	0	6	125	6 (2.29)	256 (97.71)

^a In bold variants not previously reported

^b 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.

Table 3. Results of bioinformatic analysis for *PALB2* variants

VARIANT	PROTEIN CHANGE	PREDICTION AMINO ACIDIC CHANGE				SPLICE SIGNAL DETECTION											
		POLYPHEN	SIFT	A-GVGD	Site ^a	SSF [0-100]			MaxEnt [0-12]			NNSPLICE [0-1]			GeneSplicer [0-15]		
						Consensus splice site score	Consensus splice mutation score ^b	Predicted variation of Cryptic Site Position and Score ^c	Consensus splice site score	Consensus splice mutation score ^b	Predicted variation of Cryptic Site Position and Score ^c	Consensus splice site score	Consensus splice mutation score ^b	Predicted variation of Cryptic Site Position and Score ^c	Consensus splice site score	Consensus splice mutation score ^b	Predicted variation of Cryptic Site Position and Score ^c
c.-200-27T>G	-	NA	NA	NA	A	-	-		-	-		-		c.-200-42 0.89 (+4.7%)	-	-	c.200-42 4.60 (+0.2%)
c.-158G>C	-	NA	NA	NA	A	-	-		-	-		-	-		-	-	
c.-47G>A	-	NA	NA	NA	A	-	-	c.-46 74.92 (-100%)	-	-	c.-46 5.71 (-100%)	-	-		-	-	
c.212-58A>C	-	NA	NA	NA	A	90.34	90.34		10.02	10.02		0.98	0.98		7.88	7.92 (+0.4%)	
c.620C>G	p.Pro207Arg	Probably damaging	Affect protein function	Class C65	A	90.34	90.34	c.623 79.40 (-100%)	10.02	10.02	c.623 0.27 (-94.9%)	0.98	0.98		7.88	7.88	
c.1010T>C	p.Leu337Ser	Possibly damaging	Affect protein function	Class C65	A	90.34	90.34		10.02	10.02		0.98	0.98		7.88	7.88	
					D	87.54	87.54		8.88	8.88		0.99	0.99		0.75	0.75	
c.1194G>A	p.Val398Val	NA	NA	NA	A	90.34	90.34		10.02	10.02		0.98	0.98		7.88	7.88	
					D	87.54	87.54		8.88	8.88		0.99	0.99		0.75	0.75	
c.1572A>G	p.Gln559Arg	Benign	Tolerated	Class C35	D	87.54	87.54		8.88	8.88		0.99	0.99		0.75	0.75	
c.1676A>G	p.Gln559Arg	Benign	Tolerated	Class C35	D	87.54	87.54	c.1679 76.25 (-1.1%)	8.88	8.88	c.1679 0.04 (-100%)	0.99	0.99		0.75	0.82 (+8.2%)	
c.1684+29A>G	-	NA	NA	NA	D	87.54	87.54		8.88	8.88		0.99	0.99		0.75	1.18 (+56.9%)	
c.2014G>C	p.Glu672Gln	Benign	Affect protein function	Class C25	D	71.73	71.73		7.09	7.09		0.95	0.95		2.36	2.36	
c.2586+58C>T	-	NA	NA	NA	D	84.48	84.48		6.80	6.80		0.81	0.81		-	-	
c.2587-38C>G	-	NA	NA	NA	A	75.57	75.57		3.31	3.31		-	-		-	-	
c.2590C>T	p.Pro864Ser	Probably damaging	Tolerated	Class C65	A	75.57	75.57		3.31	3.31	c.2597 1.89 (+11.2%)	-	-	c.2597 0.65 (+22.8%)	-	-	
c.2865T>A	p.Ser955Arg	Benign	Affect protein function	Class C65	A	77.22	77.22		8.14	8.14		0.54	0.54		2.70	2.62(-2.9%)	
c.2993G>A	p.Gly998Gln	Probably damaging	Affect protein function	Class C65	D	85.40	85.40		9.21	9.21		0.99	1.00 (+1.0%)		0.85	1.31 (+54.4%)	
c.3300T>G	p.Thr1100Thr	NA	NA	NA	D	100.0	100.0		-	-		-	-		-	-	

- No predicted Donor or Aceptor site

^a D=Donor site; A=Aceptor site

^b Variations in consensus splice sites are represented by its new score and in case of variation, the percentage of variation in parenthesis

^c Indicated are cryptic splice sites for which at least two algorithms predicted a splice site score variation, and in parenthesis the percentage of variation

LEGENDS

Figure 1. Bar charts of MLPA results. A: Profile of a normal control. B: Profile of the patient with the heterozygous deletion of exons 7-11 of *PALB2* gene. C: Profile of a paraffine tumor sample from male with BC relative of the *PALB2* index case, also carrying the deletion. The grey bars represent the exons with the deletion.

Figure 2. Pedigree of the breast cancer family carrying the c.2587-?_3201+?del *PALB2* mutation. Cancer diagnoses are indicated in those affected patients; in brackets the age at diagnosis or exitus. The arrow indicates the index case analysed. *PALB2* mutation carriers are indicated by the + sign. BiBC: bilateral breast cancer, GC: gastric cancer

Supplementary figure 1. Co-segregation analysis of variants c.212-58A>C, c.1676A>G, c.2014G>C, c.2586+58C>T, c.2993G>A, c.3300T>G in one of the six carriers families. Individuals analysed are indicated by + and – signs: carriers of all the six variants in heterozygous state are indicated with a + sign, individuals that were not carriers of any of the variants are indicated with a - sign. *One family member was heterozygous for variants c.1676A>G and c.2586+58C>T, only. Cancer diagnoses are indicated in the affected patient; in brackets the age at diagnosis or the actual age of the individual.

Supplementary table 1. Participating centres and families from Spain

Center/Hospital	Number of families
Hospital Universitario San Carlos (Madrid)	27
Hospital Universitari Vall d'Hebron (Barcelona)	14
Hospital de la Santa Creu i Sant Pau (Barcelona)	13
Fundación Pública Galega de Medicina Xenómica (Galicia)	12
Institut Català d'Oncologia	11
Hospital Infantil Miguel Servet (Zaragoza)	11
CNIO (Madrid)	10
Instituto de Biología y Genética Molecular (UVa-CSIC, Valladolid)	10
Hospital Universitario La Fe (Valencia)	10
Hospital Sant Joan (Reus)	8
Hospital de Cruces (Barakaldo)	4
Hospital Universitario Central de Asturias	1
Total	131

Supplementary table 2. Sequences of *PALB2* primers used for amplification and sequencing, length of the amplicon and melting temperature.

PCR-fragment	Sequence	Length	Melting Temperature (°C)
EXON 1	F-CAGAGATTCCGGCTACTTCC R-GACACAAAGCCAGGCCTAAA	450	56
EXONS 2&3	F-GTGCTACTCCCTGCCTCTTG R-CACACTGTGGGAAAAAGAACAA	470	56
EXON 4	F-GAACACATTCTCCTAAAGGTAACA R-GGAACTGTAGTCGCCCTGGT	750	58
	F-CAGAACCAGTTACAGAAATTAATGAAG R-TTGCATCCTTATTTTTATTTTAAACC	714	56
	F-TAGCCTGGAAGCAACCTCTC R-TTGAGCCAGGGTCTCACTCT	717	58
	F-GAAGCTCACTCTTTGTTGGGTA R-GTATGCTGGCTTTGCGAGTT	614	56
EXON 5	F-CGCATGGATACAGAAATGGA R-TCAAAGAAACACGTCAAACCA	653	56
EXON 6	F-GTTTGCATTTGGAGCTTTGC R-TAAAGAACAAGAAGCTATATGACTGAA	300	56
EXON 7	F-GCTCTTTCTTTTCACCTGCAT R-GCATGGTCATAGCTCCCAAT	398	56
EXON 8	F-TCCTTGACAGTGAGAATACAAAAGAA R-TGCACTTAAAACCAGCTGACA	260	56
EXON 9	F-AAAAGGTTACTCCTCACATCACC R-ATTACACCCCCAGCACAGAA	321	56
EXON 10	F-CAGTTCAACAATGCGGAGAA R-CAACAGCAACACAAAACCACA	328	56
EXON 11	F-TTTCCTGGTCACCTCCTAA R-CGGGGAAGGTTTGTTCAATTA	290	56
EXON 12	F-GAGCCTATCGGTCATTGCTT R-TTTTCCATTCTTCTAAGTGACACAA	361	56
EXON 13	F-TGGTTTTGGGAACATGGTTT† F- CCTGGAAGGTGACGTGAAAG* R-TGAAATTTACTTACTAGGCAAAGA	714	56

†Primer used only for amplification

*Primer used only for sequencing

Figure 1

A

B

C

Figure 2

Supplementary figure 1