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Two founder *BRCA2* mutations predispose to breast cancer in young women

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Abstract The mutation spectrum of *BRCA1* and *BRCA2* presents a wide range of unique mutations in breast/ovarian cancer patients but recurrent mutations with founder effects have also been described. *BRCA2* 5344delAATA and 9538delAA are recurrent mutations in Castilla-León (Spain) representing 10.6% of *BRCA2* positive families. By genotyping eleven chromosome 13 markers (4.3 Mb) we demonstrate that each mutation shows core haplotypes of 1.66 and 0.87 Mb, respectively, supporting a common ancestor in Castilla-León. Furthermore, both mutations are associated with earlier onset of breast cancer (5344delAATA: 37.4 years, $P = 0.033$; 9538delAA: 39.4 years, $P = 0.008$). The identification of founder effects improves the genetic screening strategy to be followed and facilitates the clinical management of asymptomatic carriers

Keywords *BRCA1* · *BRCA2* · Founder mutations · Hereditary breast cancer

Introduction

Germ-line mutations in the breast cancer-susceptibility genes, *BRCA1* (MIM# 113705) [1] and *BRCA2* (MIM# 600185) [2], are responsible for approximately 16% of the familial risk of breast cancer [3]. *BRCA1/2* mutations are spread throughout their entire coding regions and splice sites, which makes mandatory the complete scanning of both genes. Each population shows a particular mutation spectrum in *BRCA1* and *BRCA2*, where specific mutations can occur more frequently as a result of founder effects [4, 5]. The detection of such events is a useful tool to establish a routine workflow that could reduce time and cost of the screening [5]. In previous works, we reported the mutational spectrum of *BRCA1* and *BRCA2* in breast/ovarian cancer patients from the east of Castilla-León, where we showed several recurrent *BRCA* mutations [6–8]. We have recently described the presence of founder effects in two mutations, *BRCA1* 5272-1G>A and *BRCA2* 5374delTATG [9]. We also found deleterious *BRCA2* mutations 5344delAATA (c.5116_5119delAATA, premature stop at codon 1710) of exon 11 and 9538delAA (c.9310_9311delAA, premature stop at codon 3109) of exon 25 in three and five families, respectively, that had not been reported in any other populations supporting a putative founder effect in Spain. Mutation 5344delAATA was described by our group for the first time [6], whereas 9538delAA had already been reported as the third most prevalent Spanish *BRCA2* mutation (the BIC database, <http://research.nhgri.nih.gov/projects/bic/>) [10]. We aimed to evaluate the presence of a unique ancestry for each of these mutations in

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order to outline a cost-effectiveness algorithm in Spanish families and to correlate clinical characteristics of the disease with each mutation.

Patients and methods

Selection criteria of breast/ovarian cancer patients of the Inherited Cancer Prevention Programme of Castilla y León (788 families-1312 patients and family members) were previously published [6]. Mutation detection of *BRCA1* and *BRCA2* was carried out by heteroduplex analysis in capillary array electrophoresis [11]. A total of eight breast/ovarian cancer families harbouring the *BRCA2* mutations 5344delAATA (three families) and 9538delAA (five families) were collected for this study. Another three 5344delAATA families were recruited from other Spanish centres: Hospital de la Santa Creu i Sant Pau (Barcelona), Hospital Universitario San Carlos (Madrid) and Hospital La Fe (Valencia) (Table 1). Information about the type of cancer in each family, age of diagnosis, age of death or current age and geographical origin was also obtained (Table 1). Basic statistical calculations (*t*-test) were performed in Excel.

At least one affected member was genotyped except for family BU-627 (9538delAA), where all cancer patients died, and family BU-59 (also 9538delAA) without any available DNA sample. Therefore, haplotype construction was performed in 28 and 32 individuals from six 5344delAATA and four 9538delAA families, respectively. Additionally, 75 control individuals were genotyped to estimate the allele frequencies of each marker. Previous written informed consent was obtained from all participants.

Ten short tandem repeats (STR) markers (D13S260, D13S1699, D13S1698, D13S1697, D13S1701, D13S171, D13S1695, D13S1694, D13S267 and D13S220) and the SNP 1342C>A of *BRCA2* exon 10 were selected to construct a haplotype that spans 4.3 Mb of the chromosome 13 region containing the *BRCA2* gene (Table 1). Primer sequences of microsatellite markers were obtained from the Ensembl database and were amplified by fluorescent-PCR [9]. The amplification products were separated on an ABI3130 DNA sequencer and analyzed with the GeneMapper v3.7 software (Applied Biosystems, Foster City, USA). The SNP 1342C>A (rs144848) was typed by a TaqMan assay in a 7500 Real Time apparatus (Applied Biosystems) following the manufacturers' protocol. Allele designation was arranged according to a previous report [9]. Subsequently, we determined the date of the most recent common ancestor with the equation $G = \log \delta / \log(1 - \theta)$, based on the calculation of the linkage disequilibrium between the mutation and linked markers [12].

Results and discussion

BRCA1/2 mutations in Castilla-León (Spain)

The *BRCA1* and *BRCA2* genes of 788 unrelated families were scanned for mutations. We identified 120 families carrying a deleterious mutation, 44 *BRCA1* (*BRCA1*+) and 76 *BRCA2* positive families (*BRCA2*+) . A total of 55 different deleterious mutations were identified, of which 35 were unique and 20 occurred in more than one family, including *BRCA2* 5344delAATA (three families) and 9538delAA (five families) that accounted for 10.6% of *BRCA2*+ families (Fig. 1).

Construction of the ancestral haplotype

The panel of 11 markers between D13S260 to D13S267 was genotyped in six independent 5344delAATA families (Table 1). The disease associated haplotype could be unambiguously deduced in five families and it was present in BU-749 (only index case available). All of them share a conserved haplotype along nine markers (1,660 Kb) that cosegregated with the mutation in all the 18 positive carriers, including families M-355 and V-273 from outside of Castilla y León, which support the presence of a unique founder effect for this mutation. This core haplotype was detected neither in non-carrier family members nor in 75 controls. The estimation of mutation age was 76.18 generations (1904 years, assuming 25 years per generation) with recombinant distal marker D13S220, and 8.28 generations (207 years) with D13S260. The latter date is consistent with the result obtained with Finnish mutations 8555T>G (p.L2776X) and 9342-2A>G that share the same chromosomal region between D13S260 and D13S267 and were dated 7–11 generations ago [13]. In addition, this *BRCA2* region (nucleotides 5,100–6,100) seems to be a hotspot for mutations as eight different frameshift mutations have been identified in our cohort of patients (22/76 *BRCA2*+ families: 28.9%). Moreover, only 30 nucleotides downstream there is another Spanish founder mutation, 5374delTATG, that is responsible for 14.5% of our *BRCA2*+ families [9].

With regard to 9538delAA mutation, only four families were available for DNA typing (Table 1). All the families showed a conserved haplotype along 870 kb, from D13S1697 to D13S1694, also indicating a unique founder effect for this mutation. The estimation of the mutation age was 54.6 generations (1,365 years) for recombinant marker D13S1698 and 37.37 generations (934 years) with D13S220. Both dates would have allowed the mutation to spread significantly in Spain, which is supported by the fact that 9538delAA is one of the most prevalent *BRCA2* Spanish mutations and distributed in several regions [10]. In any case, calculation of age in both mutations

Table 1 Haplotypes associated with 5344delAATA and 9538delAA mutations and clinical characteristics of families

Haplotypes associated with each mutation													
Marker		Chromosome 13 position (Mb)											
		D13S260	D13S1699	D13S1698	D13S1697	1342C>A	D13S1701	D13S171	D13S1695	D13S1694	D13S267	D13S220	
		31.33	31.50	31.60	31.76	31.80	32.04	32.15	32.42	32.67	33.16	35.56	
5344delAATA													
Family	Onset age	Family history of cancers (onset age) ^b											
BU-339	34	2 BC (26, 38), Hepatic (<u>54</u>)											
BU-366	40 ^{OC}	1 BC (44); 2 OC (<u>59</u> , 72), CRC (<u>49</u>)											
BU-749	42-59 ^{Bil} 66 ^{OC}	1 BC (69); Gallbladder (72)											
M-355	55	2 BC (44, 46-54 ^{Bil}); CRC (47); Lung (40)											
SP-135	35-35 ^{Bil}	2 BC (<u>31</u> , 34); 2 OC (47,52); CRC 4											
V-273	29-30 ^{Bil}	1 BC (<u>36-42</u> ^{Bil}); Osteosarcoma 8											
		Allele frequencies in controls (%) 4 (2)											
9538delAA													
Family	Index case onset age	Family history of cancers (onset age) ^b											
BU-59	51 ^{OC} -52	2 BC (38,other); Larynx											
BU-138	38-40 ^{Bil}	2 BC (<u>34</u> , 55); Pancreas (50); Bladder (66)											
BU-403	50	2 BC (45, 47)											
BU-545	38	2 BC (<u>39</u> , <u>42</u>); 2 MBC (<u>69</u> , <u>76</u>)											
BU-627	0 (70) ^a	6 BC (29-33 ^{Bil} , <u>31</u> , <u>32</u> ^{Bil} , <u>41</u> , <u>60</u> , 80-85 ^{Bil})											
		Allele frequencies in controls (%) 9 (18)											
			2 (56)	12 (23)	3 (78)	C (28)	5 (21)	8 (9)	3 (3)	4 (43)	9 (45)	5 (18)	

Types of cancer: BC breast cancer; OC ovarian cancer; Bil bilateral breast cancer; MBC male breast cancer; CRC colorectal cancer. Core haplotypes of both mutations are italicized

^a Bilateral prophylactic mastectomy performed, the current age is within brackets^b Carriers or obligate carriers of the familial mutation are underlined^c Allele 7 of family BU-403 was probably generated by a mutational event since proximal and distal markers maintain the core haplotype

Fig. 1 Pedigrees of families BU-339 and BU-545 carrying the *BRCA2* truncating mutations 5344delAATA (**a**) and 9538delAA (**b**), respectively. Pedigree symbols: *black figures* affected individuals; *half-filled figures* asymptomatic carriers; *diagonal slash* deceased individuals; *arrows* index cases; *MBC* male breast cancer. *Positive*

and *negative signs* after the name of each mutation denote carrier or non-carrier status, respectively. The sequencing electropherograms corresponding to mutations 5344delAATA (**a**) and 9538delAA (**b**) are shown below each pedigree

(5344delAATA and 9538delAA) can be considered a rough estimate principally due to the small number of families. In addition, this value is prone to changes as a result of several factors [14], such as differences between the frequencies of founder and recombinant alleles since this is the rationale of the estimate. Actually, differences between 5344delAATA and 9538delAA in estimations with the same recombinant marker (D13S220) are due to the different frequencies of the founder allele of both mutations (allele #8 = 0.45 of 5344delAATA vs. allele #5 = 0.18 of 9538delAA). Despite the plausible uneven

estimations and given the conservation of the core haplotypes of 5344delAATA and 9538delAA, we can conclude that each mutation arose from a common ancestor in Castilla-León and expanded to other Spanish regions.

Correlation genotype–phenotype

Among 5344delAATA carriers, seven women had developed breast cancer (four of them bilateral) at an average age of 37.4 (range 29–55 years old) that is significantly lower than the average of *BRCA2* mutation carriers (47.5 years;

$P = 0.033$) [15]. Therefore, we can conclude that this deletion confers predisposition to early onset breast cancer despite this mutation lies in the *BRCA2* ovarian cancer cluster region (OCCR; nucleotides 3,035–6,629) that has been associated with a reduced risk to breast cancer [15, 16]. Three out of ten proven carrier women (40, 59 and 66 years old) developed ovarian cancer that is very similar to the lifetime risk of ovarian cancer in *BRCA2* carriers (27%). Mutation 9538delAA was associated with 11 breast cancer cases, including one breast and ovarian cancer and two male breast cancers (family BU-545, Fig. 1b). However, no evidence for a correlation between mutation position in *BRCA2* and risk of male breast cancer has been reported [17]. The medium age for female BC was 39.4 years (range 29–60) that is also significantly below the average of *BRCA2* carriers ($P = 0.008$) [15].

Concluding remarks

We have provided evidence of a unique origin of two *BRCA2* mutations, which involves an improvement of the cancer genetic counselling in our population. Together with previous results, we have reported three *BRCA2* founder mutations that are in part responsible for the disequilibrium towards *BRCA2*+ families (36.7% *BRCA1*+ vs. 63.3% *BRCA2*+) in our population. Another factor that may influence in this result is that, conversely, the most prevalent *BRCA1* mutation in the rest of Spain (187delAG) [10] has not been found in our population until now. In conclusion, each of these mutations likely arose from a common ancestor that could be traced to a small area of the region of Castilla y León. The constant migratory movements of the Castilla-León population during the twentieth century may have helped these mutations to spread to the rest of Spain.

These mutations also contribute to clarify the workflow to launch in Spanish families, reducing costs and accelerating the diagnosis in high risk BOC families. Based on these results, we can define a panel of four *BRCA2* Spanish proven founder mutations together with the highly prevalent *BRCA2*-3036delACAA (a priori of multiple origin) [18] that would allow to identify nearly 50% of *BRCA2*+ families with only 5 PCR reactions. Furthermore, in these mutations associated with early breast cancer it is essential to detect rapidly asymptomatic carriers who may benefit from prevention protocols since survival rates of breast cancer are worse than in older women.

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