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LETTER TO THE EDITOR

Broad *BRCA1* and *BRCA2* mutational spectrum and high incidence of recurrent and novel mutations in the eastern Spain population

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To the Editor,

It is well known that the pathogenic mutations in *BRCA1* or *BRCA2* genes are detected in 5–10% of total breast (BC) and ovarian cancer (OC) and in 20–30% of BC/OC found in families with strong history of cancer [1]. However, the incidence of the *BRCA1* and *BRCA2* pathogenic mutations depends on the criteria adopted to select the families to be studied [2], and the mutation spectrum varies considerable due to the influence of the ethnic groups [3]. In addition, ethnicity could substantiate the appearance of founder mutations coming from an old ancestor such as the recurrent mutations detected among Ashkenazim [4, 5].

The Program of Genetic Counselling in Cancer of Valencia Community was launched in March 2005 and we reported the results of the first 147 nonrelated families included, describing the preliminary mutational spectrum [6] and novel mutations [7]. However, since then, the number of subjects studied for *BRCA1* and *BRCA2* mutations has increased considerably having reached the 704 families at the end of 2008. This large number of individuals prompted us to update our series and to establish a consistent mutational spectrum of the population of Eastern Spain identifying the recurrent mutations and relevance of the novel mutations.

We studied 704 index patients (IP) (689 females and 15 males) who enrolled in the Program of Genetic Counselling in Cancer of the Valencian Community from March 2005 to December 2008. The IPs were selected by the Units of Genetic Counselling in Cancer (UGCC) according to the inclusion criteria established in the Program of Genetic Counselling in Cancer for familial BC/OC [8, 9]. All the individuals signed a written consent elaborated by the Consellería de Sanitat (Valencia Community) in accordance with the Helsinki Declaration (1964, amended in 1975 and 1983) [10].

BRCA1 and *BRCA2* mutations were detected by amplifying all the exons and the exon–intron boundaries of both genes by PCR using the primer pairs and the PCR conditions reported in the Breast Cancer Information Core (BIC) [11]. To identify the genetic variations, we carried out a pre-screening of the heteroduplexes of the PCR products by conformation sensitive gel electrophoresis (CSGE) [12] followed by the sequencing of the PCR products in which heteroduplexes were identified.

In *BRCA1*, we found 26 different deleterious mutations in 62 IPs (17 *frameshift*, 3 *splicing*, 4 *nonsense*, and 2 *missense* mutations; Table 1); 16 of these mutations have been

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Table 1 Pathogenic mutations detected in *BRCA1*

Exon	Mutation	Protein	Type	No. of families	References
2	c.187_188delAG[a]	Stop39	F	6	[6, 11, 13]
3	c.489_490delCT[a]	Stop99	F	2	[7]
5	c.330A>G[a]	p.R71G	M	3	[6, 11, 13, 15]
I-5	c.331+1G>A[a]	–	S	3	[6, 11, 13, 15]
7	c.443insA	Stop113	F	1	[11]
11	c.1623_1627delTTAAA[a]	Stop505	F	2	[6, 11, 14, 15]
	c.1689delG[a]	Stop531	F	1	[11]
	c.1806C>T	p.Q563X	N	1	[11]
	c.2072_2075delGAAA[a]	Stop699	F	1	[6, 11]
	c.2080delA[a]	Stop671	F	7	[6, 11, 13]
	c.3450_3453delCAAG[a]	Stop1115	F	3	[6]
	c.3700delC[a]	Stop1208	F	1	[6]
	c.3746dupA	Stop1218	F	2	[11]
	c.3814_3817delGTAA	Stop1242	F	1	[11]
	c.3889_3890delAG[a]	Stop1265	F	7	[6, 11, 15]
	c.3904C>A[a]	p.S1262X	N	1	[6, 11]
12	c.4280_4281delTC[a]	Stop1382	F	1	[6, 11]
13	c.4406C>A[a]	p.Y1429X	N	2	[6]
	c.4424_4425delCT	Stop1439	F	1	Novel
	c.4476delG	Stop1455	F	1	Novel
17	c.5149_5152delCTAA	Stop1678	F	1	[11]
18	c.5242C>A[a]	p.A1708E	M	9	[6, 11, 13–15]
I-18	c.5272+5G>A	–	S	1	[11, 13]
	c.5273_1G>A	Stop1729	S	1	[6, 11, 13–15]
19	c.5273G>A	p.W1718X	N	2	[11]
21	c.5430del23[a]	Stop1785	F	1	[7]

F frameshift, *I* intronic, *N* nonsense, *M* missense, *S* splicing

[a] Mutation included in our previous reports [6, 7]

included in our previous reports [6, 7]. The mutation with the highest recurrence was c.5242C>A *missense* mutation in exon 18, detected in nine families, followed by the frameshift mutations c.2080delA of exon 11 and c.187_188delAG of exon 2 found in seven and six families, respectively. These three mutations represent 35.5% (22/62) of all the mutations detected in this gene. If to these we add the missense c.330A>G and the splicing c.331+1G>A mutations, both in exon 5, each found in three families, the five mutations represent the 45.2% (28/62) of the mutations in this gene.

Besides, we found two novel *frameshift* pathogenic mutations c.4424_4425delCT and c.4476delG of exon 13, both present in single families (Table 1). The first one causes a stop at codon 1439, while the c.4476delG mutation causes the loss of the last nucleotide (G) of exon 13 and a stop at codon 1455. The FruitFly software [16] predicts that the latter mutation presumably affects the donor-splicing site.

In *BRCA2*, we found 36 different deleterious mutations in 71 IPs (30 *frameshift*, 1 *splicing*, and 5 *nonsense*; Table 2); 13 of these mutations had been previously reported [6, 7]. The recurrent mutations with highest incidence were all located in exon 23 such as the two *frameshift* mutations c.9254_9258delATCAT and c.9206_9219del14 found in 14 and 6 families, respectively, and a *nonsense* mutation c.9246C>A found in five families. Likewise, the c.3492insT mutation of exon 11 showed a high recurrence and was identified in five families. These four mutations cover 42.2% (30/71) of the families with the pathogenic mutations detected in this gene.

Besides in *BRCA2*, we found three novel mutations, two *frameshift* mutations c.1874_1877delAGGA in exon 10 and c.6118delA in exon 11 and a *splicing* mutation c.8860+2T>G in intron 20 (Table 2). The c.1874_1877delAGGA creates a stop at codon 556 with the consequent truncation of the translation of the *BRCA2* protein. The c.6118delA causes the interruption of the synthesis of the *BRCA2* protein at codon 2003. The *splicing* mutation c.8860+2T>G affects the second nucleotide of intron 20, suppressing the donor site as predicted by the FruitFly software [16].

Table 2 Pathogenic mutations detected in *BRCA2*

Exons	Mutation	Protein	Type	No. of families	References
3	c.373G>T	p.E49X	N	1	[11]
	c.489_490delCT	Stop99	F	2	[11]
5	c.598delA	Stop135	F	1	[11]
10	c.1354delT	Stop398	F	1	[11]
	c.1538_1541delAAGA	Stop458	F	1	[11, 13]
	c.1594_1595delGA	Stop460	F	1	[11]
	c.1676_1677delAGinsTTAC	Stop513	F	1	[11]
	c.1835insT[a]	Stop542	F	2	[7, 13]
	c.1873_1876delAGGA	Stop556	F	1	Novel
	c.2070dupT	Stop615	F	1	[11]
11	c.2929delC	Stop903	F	1	[17]
	c.3036_3039delACAA[a]	Stop958	F	2	[6, 11, 13–15]
	c.3492insT[a]	Stop1098	F	5	[6, 11, 14]
	c.4075_4076delGT	Stop1284	F	1	[11]
	c.4150G>T[a]	p.E1308X	N	3	[6, 11, 13, 15]
	c.5025delT[a]	Stop1616	F	3	[6, 7]
	c.5164_5167delGAAA[a]	Stop1668	F	1	[6, 11, 15]
	c.5340_5343delAATA	Stop1710	F	1	[11]
	c.5514_5515delTC	Stop1766	F	1	[11]
	c.5804_5807delTTAA	Stop1862	F	2	[11, 13, 14]
	c.5946_5949delCTCT	Stop1909	F	1	[11]
	c.6118delA	Stop2003	F	1	Novel
	c.6299delA	Stop2039	F	1	[11]
	c.6503_6504delTT[a]	Stop2099	F	2	[6, 11, 14, 15]
	c.6722delT[a]	Stop2167	F	1	[7]
	c.6857_6858delAA	Stop2223	F	1	[11]
14	c.7399insA	Stop2411	F	1	[11]
	c.7462insG	Stop2413	F	1	[11]
17	c.8091T>A[a]	p.Y2621X	N	1	[11]
18	c.8270_8271delCA	Stop2691	F	1	[11]
I-20	c.8860+2T>G	–	S	1	Novel
23	c.9206_9219del14[a]	Stop2975	F	6	[6, 13]
	c.9216_9218delATAinsTT	Stop3248	F	1	[11]
	c.9246C>A[a]	p.Y3006X	N	5	[6, 11, 13]
	c.9254_9258delATCAT[a]	Stop3015	F	14	[6, 11, 14, 15]
25	c.9694C>T[a]	p.Q3156X	N	1	[7]

F frameshift, *I* intronic, *N* nonsense, *M* missense, *S* splicing

[a] Mutation included in our previous reports [6, 7]

The current mutational spectrum of our population has broadened with regard to our previous report; recurrent mutations have emerged and five novel mutations have been identified. Thus, the mutational spectrum for *BRCA1* and *BRCA2* increased from the 16 and 13 mutations in our previous reports [6, 7] to 26 and 36 mutations, respectively, in the present report.

Eight of the mutations detected in *BRCA1* were also observed in recent studies carried out in the Spanish population [13–15]. However, our results are not as concordant as those expected with the larger study carried out in the Spanish population [13]. Only 7 of the 35 different mutations (20%) observed in the mentioned study, c.187_188delAG, c.330A>G, c.331+1G>A, c.2080delA, c.5242C>A, c.5272+5G>A, and c.5273-1G>A, were detected in our population. These mutations represent 48% (30/62) of the pathogenic mutations detected in our study. However, only two of them, c.187_188delAG and c.330A>G, were recurrent in both the studies.

For *BRCA2*, there is a coincidence of 11 mutations with other reports performed in the Spanish population [13–15]. The 2003 report of Diez et al. [13] is concordant with our study in 10 out of the 22 different mutations (45%), c.1538_1541delAAGA, c.3036_3039delACAA, c.3492insT, c.4150G>T, c.5804_5807delTTAA, c.6503_6504delTT, c.6857_6858delAA, c.9206_9219del14, c.9246C>A, and c.9254_9258delATCAT, which represent 70% (37/53) of the families reported by that study [13] and 58% of the families (41/71) of this study. The *frameshift* mutation c.9254_9258delATCAT, the most recurrent mutation of this study, also showed high incidence in the report of Diez et al. [13]. This recurrent mutation is considered characteristic of Eastern Spain. However, for most of *BRCA2* recurrent mutations, no correspondence was observed between both the studies.

We observed greater diversity and higher incidence for *BRCA2* mutations than for *BRCA1* (50.7% in *BRCA2* vs. 41.9% in *BRCA1*) in correspondence with our previous reports [6], and with other studies performed in the Spanish population [14–17], but in disagreement with the other one [13]. We think that the larger incidence of mutations in *BRCA2* could be a trait of the population of certain geographical areas of Spain, not related with the presence of the c.187_188delAG *BRCA1* Ashkenazim mutation, as suggested by Infante et al. [15], since in this study, we observed larger incidence of *BRCA2* mutations in spite of having high incidence of this mutation.

We describe five novel mutations (four *frameshift* and one *splicing*) in *BRCA1* and *BRCA2*, which in addition to the 10 novel pathogenic mutations of our previous reports [6, 7] represent a relevant percentage of 24% of novel mutations (18 novel mutations out of 62 different mutations), which attributes to the great heterogeneity of the Spanish population.

In summary, the population of Eastern Spain showed a great heterogeneity in the *BRCA1* and *BRCA2* mutations with slightly higher mutation incidence and heterogeneity for *BRCA2*. For *BRCA1*, we observed three major recurrent mutations—c.5242C>A, c.2080delA, and c.187_188delAG—which were present in 35% (22/62) of the families. For *BRCA2*, c.9254_9258delATCAT, c.9206_9219del14, and c.9246C>A are the major recurrent mutations present in 35% of the families (25/71). We detected in our population a total of 15 novel mutations, which represent the 24% of the different mutations. All these results attribute to the singularity of the population of Eastern Spain.

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References

1. Wooster R, Webwe BL (2003) Breast and ovarian cancer. N Engl J Med 23:2339–2347
2. Malone KE, Daling JR, Thompson JD et al (1998) BRCA1 mutations and breast cancer in the general population: analyses in women before age 35 years and in women before age 45 years with first-degree family history. JAMA 279:922–929
3. Szabo CI, King MC (1997) Population genetics of BRCA1 and BRCA2. Am J Hum Genet 60:1013–1020
4. Simard J, Tonin P, Durocher F et al (1994) Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families. Nat Genet 8:392–398
5. Neuhausen SL, Mazoyer S, Friedman L et al (1996) Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. Am J Hum Genet 58:271–280
6. Esteban E, Bolufer P, Palanca S et al (2008) Mutaciones de BRCA1 y BRCA2 en familias estudiadas en el Programa de Consejo Genético en el Cáncer de la Comunidad Valenciana. Med Clin (Barc) 130:121–126
7. Esteban E, Bolufer P, Palanca S et al (2007) Twenty-three novel BRCA1 and BRCA2 sequence alterations in breast and/or ovarian cancer families of Eastern Spain. Breast Cancer Res Treat 112:69–73
8. FESEO. Tercer Libro Blanco de la Oncología. Federación de Sociedades Españolas de Oncología (Biete Solá A, Palacios Eito A, Calvo Manuel FA eds) (2002) FESEO, Madrid
9. Conselleria de Sanitat (2003) Programa de Consejo Genético en el Cáncer. Grupo de Cáncer Hereditario, Plan Oncológico, Comunidad Valenciana, Valencia
10. Miki Y, Swensen J, Shattuck-Eidens D et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66–71
11. The Breast Cancer Information Core Database (BIC) http://research.nhgri.nih.gov/bic/Member/index.shtml. Accessed 17 Dec 2009
12. Ganguly A, Rock MJ, Prockop DJ (1993) Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. Proc Natl Acad Sci USA 90:10325–10329
13. Diez O, Osorio A, Duran M et al (2003) Analysis of BRCA1 and BRCA2 genes in Spanish breast/ovarian cancer patients: a high proportion of mutations unique to Spain and evidence of founder effects. Hum Mutat 22:301–312
14. Beristain E, Martínez-Bouzas C, Guerra I et al (2007) Differences in the frequency and distribution of BRCA1 and BRCA2 mutations in breast/ovarian cancer cases from the Basque country with respect to the Spanish population: implications for genetic counselling. Breast Cancer Res Treat 106:255–262
15. Infante M, Duran M, Esteban-Cardeñosa E et al (2006) High proportion of novel mutations of BRCA1 and BRCA2 in breast/ovarian cancer patients from Castilla-León (central Spain). J Hum Genet 51:611–617
16. Berkeley Drosophila Genome Project: FruitFly, http://www.fruitfly.org/seq_tools/splice.html. Accessed 17 Dec 2009
17. Miramar MD, Calvo MT, Rodriguez A et al (2008) Genetic analysis of BRCA1 and BRCA2 in breast/ovarian cancer families from Aragon (Spain): two novel truncating mutations and a large genomic deletion in BRCA1. Breast Cancer Res Treat 112:353–358