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LETTER TO THE EDITOR

Detection of the *CHEK2* 1100delC mutation by MLPA *BRCA1/2* analysis: a worthwhile strategy for its clinical applicability in 1100delC low-frequency populations?

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Dear Editor,

We would like to add some information and thoughts in relation to the letter of Martínez-Bouzas et al. [1] published in your journal, dealing with the presence of the *CHEK2* 1100delC mutation in Spanish families.

Germ-line mutations in the *CHEK2* gene (OMIM 604373) have been identified in breast cancer (BC) families negative for *BRCA1/2* mutations. The 1100delC variant was initially identified as a susceptibility allele to BC from a study in 718 Western-European and North-American *BRCA1/2* negative families. The variant was found in 5.1% of these families and in 1.1% of healthy individuals [2]. The authors estimated that the variant results in a 2-fold increase of BC risk in women, accounting for about 1% of all female BC. This estimate has been confirmed in another population-based study from Denmark [3]. Additionally, having a personal or familial history of bilateral BC increased the probability of finding the 1100delC mutation [4]. Recent data suggest that premenopausal carriers of this variant have a 2-fold increased risk of developing a second BC (mostly contralateral) and a worse 10-year recurrence-free survival [5]. It has also been suggested a 10-fold increased risk for male BC for 1100delC carriers and that this mutation accounts for as much as 9% of all male BC in the population [2]. Although increased risk of prostate cancer has not been confirmed, some data suggest that risks of colon and lung cancer might be higher in female carriers between ages 20 and 50 years than non carriers [6]. Overall, these reports suggest that *CHEK2* acts as a low-penetrance tumour suppressor gene, and that it makes a significant contribution to familial clustering of BC, including families with only two affected relatives. In some populations, the variant has been identified in approximately 3–4% of young BC patients unselected for family history, and between 3% and 6% of non-*BRCA1/2* BC families [2, 4, 7, 8].

Nevertheless, a wide variation in the frequency of 1100delC mutation in *CHEK2* has been observed in different populations. The 1100delC mutation accounts for a part of BC susceptibility in populations from Northern Europe (Netherlands, Denmark, Finland, UK, Poland), and North America. But it does not seem to play a role in Ashkenazi Jewish families, and in some other countries, such as Australia, Italy and Spain, this association is not so clear [reviewed in 9]. Osorio et al. [10] did not find this mutation in 400 non-*BRCA1/2* Spanish BC families, 56 patients with BC diagnosed before 40 years of age without family history, and 400 healthy individuals. The authors stated that its low penetrance and low frequency make this variant irrelevant for the clinical practice in our population. These results were confirmed by two other studies [11, 12]. Bellosillo et al. [11] studied the *CHEK2* gene in 28 high-risk and 53 moderate-risk Spanish BC cases negative for *BRCA1/2* mutations, and in none of them could the 1100delC mutation nor other variants previously described be detected. Sánchez de Abajo et al. [12] analysed 148 Hereditary non-Polyposis Colorectal Cancer (HNPCC) and 32 Hereditary Breast and Colon Cancer (HBCC) Spanish families and found that this variant was of no clinical relevance in this population.

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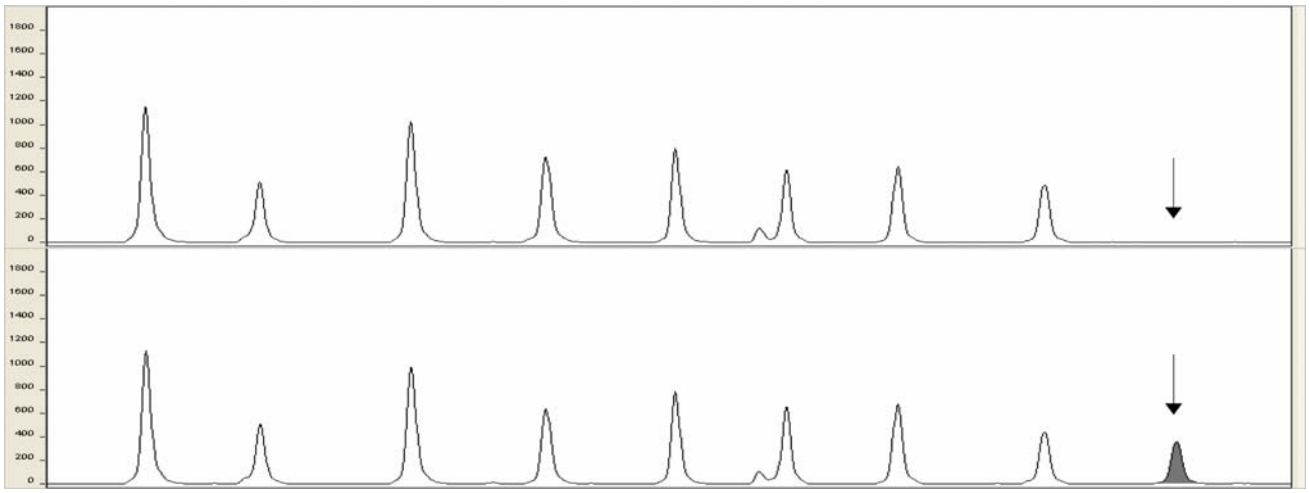

Fig. 1 Capillary electrophoresis pattern from samples analyzed with SALSA MLPA kit *BRCA2*. Each peak represents one *BRCA2* exon recognised by a specific fragment size. The arrows indicate the CHEK2 1100delC peak: this peak will only appear if the CHEK2 1100delC is present in the DNA sample

In contrast, Martínez-Bouzas et al. [1] found the *CHEK2* 1100delC in two (0.93%) unrelated patients from a cohort of 214 individuals with familial non-*BRCA1/2* BC. The mutation was not present in 120 control individuals from the same region, the Basque Country, in the North of Spain. These data suggested that the variant was not completely absent in this population and the prevalence was in accordance with that from UK or Germany.

The present report confirms the existence of *CHEK2* 1100delC in the Spanish population. We identified a *CHEK2* 1100delC mutation carrier in a BC family in the context of a screening for large *BRCA1/2* rearrangements in breast/ovarian cancer families previously found negative by conventional methods of analysis [13]. The *BRCA1/2* analysis was carried out by MLPA (multiple ligand probe amplification analysis) according to the instructions provided by the manufacturer (MRC Holland, The Netherlands). The *BRCA2* P045/*CHEK2* probe mix kit contains probes for the coding exons of the *BRCA2* gene. From lot 02–04 onwards, three probes for the *CHEK2* gene on chromosome 22q12.1 are included. One of these probes will only result in an amplification product in case the DNA sample contains the *CHEK2* 1100delC mutation. A MLPA profile compatible with the *CHEK2* 1100delC deletion was detected in a patient with BC at age 46 (Fig. 1). Her mother had been diagnosed with bilateral BC at 59 and 66 years of age. In both cases tumour characteristics were invasive ductal carcinoma, low grade, and with positive hormone receptors. A proband's daughter was diagnosed with a possible thymoma at age 11. A maternal uncle and cousin were diagnosed with lung cancer at ages 60 and 36, respectively.

MCR-Holland and our laboratory had noticed in previous kits that a *CHEK2* 1100delC peak spuriously appeared in known normal controls as well as all samples. Therefore, the presence of the mutation was confirmed by sequencing the corresponding DNA fragment.

While further studies should evaluate whether *CHEK2* 1100delC analysis should be offered to specific risk groups, such as women having a personal or familial history of bilateral BC [4], health care professionals from countries with a low prevalence of this mutation should also reconsider its clinical applicability.

According to current data on risk of contralateral BC and recurrence-free survival, identification of 1100delC carriers might lead to a more intensive individualised follow up of BC patients compared to non-carriers. In contrast with screening all *BRCA1/2* genes, *CHEK2* 1100delC analysis is a single genotyping test with a low cost. For those BC families undergoing genetic counselling and testing for *BRCA1/2* genes, identification of a low-penetrance BC allele might be clinically more relevant than a non-informative result from *BRCA* testing. The analysis of large rearrangements in the *BRCA1/2* genes in BC families by the MLPA kit allows the detection of the *CHEK2* 1100delC alteration simultaneously. This can be a useful strategy in populations such as the Spanish, in which *CHEK2* 1100delC seems to account for a small proportion of familial BC cases.

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