Letter to the Editor

Large genomic rearrangements in mutation-negative BRCA families: a population-based study

To the Editor:

Approximately 20% of hereditary breast and ovarian cancer (HBOC) syndrome cases are associated with mutations in BRCA1 and BRCA2 because an elevated percentage of the individuals who inherited a mutation are likely to develop the disease (1). The large deletions, duplications or large genomic rearrangements (LGRs) of the gene sequence were described in 1997 (2). The precise consequences of these mutations cannot be determined in the absence of functional assays but intragenic rearrangements led either to protein truncations or to in-frame duplications or deletions when these mutations covered extensive regions of the gene, and thus it is likely that they are causative of cancer susceptibility in carriers. It has been reported that they may account for a low percentage of all BRCA1 and BRCA2 mutations. The frequency of LGRs of these genes varies among populations. In Spain, this proportion is around 2% for BRCA1 (3, 4) and 1.5% for BRCA2 (3).

To estimate the frequency of the LGRs in the Tarragona province population (Catalonia), we used 207 families, fulfilling classical HBOC criteria that were identified through the Genetic Counselling Unit of the Hospital Universitari Sant Joan de Reus, which is the referral centre in Tarragona. These index cases were selected to detect BRCA LGRs because they had been previously tested for the presence of point mutations in both genes by direct sequencing of the entire coding sequence, including intron/exon boundaries, and no deleterious mutations were found.

Recently, multiplex ligation-dependent probe amplification (MLPA, whose protocol of work is available at www.mlpa.com) has been widely used as a highly sensitive method for detecting the relative copy number of BRCA1 and BRCA2 exons in a high-throughput format (5). All three sets of probes used [P002B and P087 for BRCA1 and P045B for BRCA2, commercially available from MRC-Holland (Amsterdam, The Netherlands)] allow screening for deletions and duplications of all BRCA1 and BRCA2 exons and the 1100delC CHEK2 mutation in two polymerase chain reactions (PCRs). For the results of statistical analysis, the individual peak heights were analysed using Coffalyser from MRC-Holland. Expected allele dosage for LGR non-carriers is 1, for carriers of a heterozygous deletion is 0.5 because of the 50% reduced relative peak area of the amplification product of that probe, and for carriers of a heterozygous duplication is 1.5; but when the DNA samples show a dosage less than 0.7 or greater than 1.30, these samples are tested again (6). The positive MLPA results and false-positive deletions or duplications of single exons were confirmed or resolved by repeated testing of independent DNA samples from the patient.

The BRCA MLPA analysis revealed two different LGRs, one copy number alteration was found within each BRCA gene. We did not detect a CHEK2 deletion in the samples analysed and as in other previous studies done in Spain (7, 8), it does not seem to play a role in Catalan families because of the minimal presence previously described and the absence of it in our study.

The family 333 presented two breast cancer cases of patients younger than 50 years of age. The index case, showed an MLPA profile suggestive of a deletion encompassing exons 16 and 17 in the BRCA1 gene (Fig. 1a,b). This in-frame deletion has been previously described (9) as a deletion which produces stable mRNA lacking exons 16 and 17 but retaining the correct frame in the splicing of exons 15–18. Although retaining an intact minimal transactivation domain, it results in loss of function, as described by Carvalho et al., because deletion of exon 17 removes a part of the region of the BRCA1 protein which is thought to be involved in DNA repair because of the lost of a part of the BRCT domain. The family 154 presents three breast cancer cases of patients less...
Fig. 1. Results of the MLPA analysis (a) MLPA electropherograms. Note the decreased peak heights of deleted exons. (b) MLPA statistical analysis. In family 333 a large genomic deletion in BRCA1 of exons 16 and 17 was found. In family 154 a large genomic deletion in BRCA2 involving exons 1 to 24 was detected.
than 50 years of age. The proband showed an MLPA profile suggestive of a deletion spanning exons 1–24 of the BRCA2 gene (Fig. 1a,b). Three of her relatives were also deletion carriers, and one of them was affected by breast cancer at the age of 30. This large deletion, previously described in Catalonia (10), removes most of the gene including the promoter (11), thereby preventing the transcription of BRCA2 (12).

LGRs were detected in approximately 1% of the Catalan high-risk families previously designated as BRCA1/2 negative for point variations and small insertions/deletions, showing a 0.48% prevalence of BRCA1 and 0.48% of BRCA2. The ratios of large BRCA1 deletions described in Europe vary from about 36% in the Netherlands to 3.8% in Danish families (13, 14). However, when we look for large rearrangements of BRCA2 in the literature, the majority of publications discuss the absence of this kind of mutations in this gene. In Spain, the percentages described were within 2% for both genes. Therefore, the proportion of large deletions characterized in this study is lower than that found in other publications.

MLPA is a rapid, highly sensitive and inexpensive compared to other methods and also has the advantage that the analysis is carried out on genomic DNA, eliminating the need for cell lines or RNA isolation, and despite the very low percentage of detected alterations, we support the inclusion of this technique as a first step in the mutational study of BRCA genes in families with criteria of HBOC.

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Conflict of interest
The authors declare that there are no conflicts of interest.

References

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