An evaluation of the polymorphisms Ins16bp and Arg72Pro in p53 as breast cancer risk modifiers in BRCA1 and BRCA2 mutation carriers

The close functional relationship between p53 and the breast cancer susceptibility genes BRCA1 and BRCA2 has promoted the investigation of various polymorphisms in the p53 gene as possible risk modifiers in BRCA1/2 mutation carriers. Specifically, two polymorphisms in p53, c.97-147ins16bp and p.Arg72Pro have been analysed as putative breast cancer susceptibility variants, and it has been recently reported that a p53 haplotype combining the absence of the 16-bp insertion and the presence of proline at codon 72 (No Ins-72Pro) was associated with an earlier age at the onset of the first primary tumour in BRCA2 mutation carriers (Osorio et al., 2006). Therefore, we have evaluated this association in a series of 2932 BRCA1/2 mutation carriers from the Spanish population. In this study, we have evaluated this association in a series of 2932 BRCA1/2 mutation carriers from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) and on behalf of CIMBA.

Keywords: BRCA1; BRCA2; p53; breast cancer

Given the involvement of p53 in cell cycle control, DNA repair and apoptosis, the role of this gene in cancer susceptibility has been extensively studied. Specifically, two polymorphisms in p53, c.97-147ins16bp and p.Arg72Pro have been analysed as putative breast cancer susceptibility variants, although not all studies have yielded consistent results (Weston et al., 1997; Wang-Gohrke et al., 1998; Espulsini et al., 2003; Damin et al., 2003; Dumont et al., 2007). The Arg72Pro single-nucleotide polymorphism (SNP) has gained special attention, as there is consistent evidence of functional differences in apoptotic rates between the Arg and Pro variants (Biros et al., 2002; Wu et al., 2002; Dumont et al., 2003). In addition, the close functional relationship between p53 and the breast cancer susceptibility genes BRCA1 and BRCA2 (Jonkers et al., 2001; Omouha et al., 2003; Liu et al., 2007) has promoted the investigation of the Arg72Pro SNP as a possible risk modifier in BRCA1/2 mutation carriers (Martin et al., 2003). Indeed, it was recently reported that a p53 haplotype combining the absence of the 16-bp insertion and the presence of proline at codon 72 (No Ins-72Pro) was associated with an earlier age at onset of the first primary tumour in BRCA2 mutation carriers (Osorio et al., 2006). In this study, we have evaluated this association in a series of 2932 BRCA1/2 mutation carriers from the Spanish population.
the Consortium of Investigators of Modifiers of BRCA1 and BRCA2 (CIMBA) (Chenevix-Trench et al, 2007).

MATERIALS AND METHODS

Patients

A total of 2088 BRCA1 mutation carriers, 841 BRCA2 mutation carriers and 3 carriers of mutations in both genes ascertained from eight centres participating in CIMBA were included in this study (Table 1). The inclusion criteria for subjects is described elsewhere (Chenevix-Trench et al, 2007).

Genotyping

Genotypes for the two polymorphisms – Ins16bp and Arg72Pro – were determined for each sample using previously described methodology (Osorio et al, 2006). In some cases, the Ins16bp SNP was genotyped by DHPLC on the WAVE HT system (Transgenomic, Omaha, NE, USA) using an acetonitrile gradient and profiles analysed with the Navigator™ software (Transgenomic), and the Arg72Pro SNP was genotyped by TaqMan (Applied Biosystems, Foster City, CA, USA). Hardy–Weinberg equilibrium (HWE) for each polymorphism was tested using the likelihood ratio test among unrelated individuals. The German Consortium of Hereditary Breast and Ovarian Cancer (GCHBOC) study gave 

| Study     | Country of residence | Ascertainment basis | Ins16bp N (%) | Arg72Arg N (%) | Arg72Pro N (%) |
|-----------|----------------------|---------------------|---------------|---------------|---------------|
| CNIO      | Spain and Greece     | Clinic              | 335 (74.12%)  | 281 (56.31%)  | 176 (35.27%)  |
| MBCSG     | Italy                | Clinic              | 190 (65.07%)  | 156 (50.81%)  | 135 (43.97%)  |
| DKFZ      | Germany, Pakistan, Colombia | Clinic | 128 (74.42%)  | 87 (51.18%)   | 67 (39.41%)   |
| GCHBOCb   | Germany              | Clinic              | 593 (75.16%)  | 474 (56.97%)  | 294 (35.34%)  |
| HBCS      | Finland              | Clinic              | 148 (78.72%)  | 96 (51.06%)   | 79 (42.02%)   |
| NCI       | United States        | Clinic              | 160 (73.06%)  | 96 (50.26%)   | 81 (42.41%)   |
| IHCC      | Poland               | Clinic              | 458 (67.25%)  | 328 (48.16%)  | 289 (42.44%)  |
| Total     |                      |                     | 2012 (72.04%) | 1518 (52.93%) | 1121 (39.09%) |

Abbreviations: GCHBOC = German Consortium of Hereditary Breast and Ovarian Cancer; HWE = Hardy–Weinberg equilibrium. aThe CNIO series consisted of samples from the Spanish Consortium for the Study of Genetic Modifiers of BRCA1 and BRCA2 and the NCSR Demokritos, Athens (Greece). bCases from the original study were included in the analysis (Osorio et al, 2006). cDeviation from HWE with P-values of 0.005 and 0.043 was observed for Ins16bp and Arg72Pro, respectively. dMissing genotypes are not included in the totals. Owing to technical difficulties, more failed genotypes were observed for the Ins16bp polymorphism.

RESULTS AND DISCUSSION

Genotype distributions and frequencies for the Ins16bp and Arg72Pro polymorphisms are shown in Table 1. Allele frequencies were similar to those previously published (Osorio et al, 2006), and genotype frequencies were consistent with HWE, except for the carriers from GCHBOC (see Materials and Methods). Haplotypes were inferred, and haplotype- and genotype-specific hazard ratios were estimated separately for each of breast (Table 2) and ovarian cancer (data not shown), among BRCA1 and BRCA2 mutation carriers. No evidence of association was found for any of the genotypes or haplotypes analysed with either breast or ovarian cancer risk, including the No Ins-72Pro haplotype, previously reported to be associated with an increased risk to develop a first primary tumour before 35 years of age in BRCA2 mutation carriers (Osorio et al, 2006).

To confirm that this negative result was not due to the different analytic approach performed in this study, we carried out a logistic regression analysis, as was done in the original study (Osorio et al, 2006), considering those with age at diagnosis younger than 35 as cases, and did not find a positive association between early diagnosis and this haplotype. In the original study, the result was corroborated by a functional assay (Osorio et al, 2006), in which a decrease in apoptotic rate was found to be associated with the No Ins-Pro72 haplotype. However, although concordant, both the genetic and the functional studies were limited by the small sample size (265 and 24 individuals, respectively), as reflected in the marginal statistically significant results described in that report.

In summary, the previously reported association of the No Ins-72Pro haplotype in p53 with an increased cancer risk in BRCA2 mutation carriers (Osorio et al, 2006) has not been validated in a larger series proceeding from the Consortium of Investigators of Modifiers of BRCA1 and BRCA2 (CIMBA). In this series of 2932 BRCA1/2 mutation carriers, no evidence of modification of breast or ovarian cancer risk by any of the two polymorphisms, Ins16bp and Arg72Pro, or their haplotype combinations has been detected. The lack of confirmation of a previously reported association found in a much smaller series highlights the necessity of international collaborative efforts aimed at achieving the statistical power required to reach reliable definitive conclusions in genetic association studies.
Table 2 Haplotype frequencies* by mutation and disease status and HR estimates for breast cancer

| BRCA1 mutation carriers | Unaffected (%) | Affected (%) | HR | 95% CI | P-value |
|-------------------------|----------------|--------------|----|--------|---------|
| p53 haplotype           |                |              |    |        |         |
| No Ins-Arg72/No Ins Arg72 | 49.60          | 50.50        | 1.00 |        |         |
| No Ins-72Pro             |                |              |    |        |         |
| One                     | 23.30          | 23.30        | 1.05 | 0.84–1.32 | 0.64   |
| Two                     | 2.80           | 2            | 0.80 | 0.47–1.38 | 0.42   |
| Ins16bp-72Pro            |                |              |    |        |         |
| One                     | 23.80          | 23.10        | 1.03 | 0.83–1.28 | 0.79   |
| Two                     | 2.20           | 2.10         | 1.16 | 0.54–2.50 | 0.70   |
| Ins16bp-Arg72            |                |              |    |        |         |
| One                     | 4              | 3.30         | 1.42 | 0.96–2.10 | 0.08   |
| Two                     |                |              |    |        |         |

| BRCA2 mutation carriers | Unaffected (%) | Affected (%) | HR | 95% CI | P-value |
|-------------------------|----------------|--------------|----|--------|---------|
| p53 haplotype           |                |              |    |        |         |
| No Ins-Arg72/No Ins Arg72 | 47.50          | 55.90        | 1.00 |        |         |
| No Ins-72Pro             |                |              |    |        |         |
| One                     | 26.50          | 23.60        | 0.82 | 0.53–1.26 | 0.35   |
| Two                     | 0.90           | 0.90         | 1.41 | 0.56–3.55 | 0.46   |
| Ins16bp-72Pro            |                |              |    |        |         |
| One                     | 26.50          | 19.40        | 0.81 | 0.52–1.27 | 0.36   |
| Two                     | 2.10           | 2.20         | 0.72 | 0.14–3.86 | 0.70   |
| Ins16bp-Arg72            |                |              |    |        |         |
| One                     | 2              | 2.70         | 1.11 | 0.42–2.97 | 0.83   |
| Two                     |                |              |    |        |         |

Abbreviations: CI = confidence interval; HR = hazard ratio. HRs corresponding to the haplotype associated with increased cancer risk in the original study are in bold. *Haplotypes were established or inferred only in those cases who had data for both polymorphisms. **Those individuals who were homozygous for the haplotype containing the common allele for both polymorphisms were considered as the reference group. ***Individuals harbouring at least one given haplotype (heterozygous or homozygous) for a given haplotype.

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