The TP53 Arg72Pro and MDM2 309G>T polymorphisms are not associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers
The TP53 pathway is crucial for tumour suppression, acting through regulation of cell-cycle control, apoptosis, senescence and DNA repair. The TP53 gene and its negative regulator MDM2 are central to this pathway, promoting polyubiquitination and degradation of TP53, and also controlling the TP53 synthesis (Toledo and Wahl, 2006; Candeias et al., 2008). Inactivation of the TP53 pathway has an important role in BRCA1- and BRCA2-associated tumourigenesis. BRCA1 and BRCA2 mutations are associated with genomic instability caused by defective cell-cycle checkpoint and DNA damage repair (Deng, 2006). Mouse model studies have highlighted functional links between these genes. Biallelic inactivation of BRCA1 and BRCA2 in mice have shown that embryonic lethality because of growth retardation can be partially rescued in a TP53 null background (Evers and Jonkers, 2006). The development of mammary tumours in conditional BRCA1 and BRCA2 knockout mice was considerably accelerated in a TP53 knockout background (Evers and Jonkers, 2006). In addition, a high incidence of TP53 mutations has been found in breast tumours of human BRCA1 and BRCA2 mutation carriers (Greenblatt et al., 2001; Manie et al., 2009). The observed interactions between TP53 and BRCA pathways are integral to the progression of tumourigenesis in breast cancer. A TP53 polymorphism (rs1042522) has been found to be of functional significance, with the Pro72 allele being less efficient than Arg72 at inducing apoptosis, mainly due to weaker binding and ubiquitination by MDM2 of the Pro72 variant protein (Dumont et al., 2003; Osorio et al., 2006). An SNP in the promoter region of MDM2 (309T > G, rs2279744) has been shown to increase MDM2 transcriptional activity, thus attenuating the TP53 pathway (Bond et al., 2004). This latter SNP was associated with an earlier onset of breast cancer in Li–Fraumeni patients carrying TP53 mutations (Bougard et al., 2006; Ruijs et al., 2007). The effect on breast cancer risk of the TP53 Arg72Pro and the MDM2 309T > G polymorphisms, separately and in combination, was investigated in a large case–control study by the Breast Cancer Association Consortium (BCAC), but no association was detected (Schmidt et al., 2007). However, several smaller studies examined these polymorphisms in BRCA1 and BRCA2 mutation carriers (Martin et al., 2003; Tommiska et al., 2005; Copson et al., 2006; Osorio et al., 2006; Wasilewski et al., 2007; Yarden et al., 2008), and some suggested an association between the TP53 Pro72 and the MDM2 309G alleles with an earlier age at breast cancer diagnosis (Martin et al., 2003; Tommiska et al., 2005; Osorio et al., 2006; Yarden et al., 2008). We therefore investigated the associations between breast cancer risk and these TP53 and MDM2 polymorphisms in a large series of BRCA1 and BRCA2 mutation carriers from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) (Chenevix-Trench et al., 2007).

MATERIALS AND METHODS

Study sample

Eligibility was restricted to female carriers with pathogenic mutations in BRCA1 or BRCA2 who were ≥18 years. Data were obtained from 13 CIMBA studies (Table 1). The majority of carriers were recruited through cancer genetics clinics offering genetic testing, and enrolled into national or regional studies. Information collected included the year of birth; mutation description; age at last followup; ages at breast and ovarian cancer diagnosis; and age at bilateral prophylactic mastectomy. Information was also available on the country of residence, which was defined to be the country of the clinic at which the carrier family was recruited for the study. Related individuals were identified through a unique family identifier. Further details of the information collected on the BRCA1 and BRCA2 mutation carriers and other details of the CIMBA initiative can be found elsewhere. Additional specific acknowledgements to the CIMBA collaborating centres are included in the Supplementary Appendix. (http://www.srl.cam.ac.uk/consortia/cimba/index.html) (Chenevix-Trench et al., 2007). All carriers participated in clinical and research studies at the host institutions under IRB-approved protocols.

Genotyping

We pooled genotype data from studies within CIMBA that had previously genotyped polymorphisms rs1042522 and rs2279744 (see Table 1). Deviation from Hardy–Weinberg equilibrium among unrelated subjects was evaluated separately for each SNP and study. There was evidence for deviation for only one study (P = 0.03), but cluster plot examination did not show any unusual
pattern and the study was included in the analysis. Where available study specific genotyping quality control data were examined and data were included if the call rate was over 95% and the concordance among duplicates was over 98%.

Statistical analysis

Mutation carriers were classified according to their age at diagnosis of breast cancer or their age at last follow up. For this purpose, individuals were censored at the age of first breast cancer diagnosis, ovarian cancer diagnosis, bilateral prophylactic mastectomy or the age at last observation. Only individuals censored at breast cancer diagnosis were assumed to be affected (Table 2).

To correct for a potential bias related to the fact that BRCA1 and BRCA2 mutation carriers are not randomly sampled with respect to their disease status, the data were analysed within a survival analysis framework, by modelling the retrospective likelihood of their disease status, the data were analysed within a survival analysis framework, by modelling the retrospective likelihood of the observed genotypes conditional on the disease phenotypes. A detailed description of the retrospective likelihood approach has been published (Antoniou et al., 2007). We used a Cox proportional hazards model, where the effect of each SNP was modelled either as a per-allele hazard ratio (HR) or using separate HRs for heterozygotes and homozygotes. To assess the combined effects of the SNPs, we fitted a model in which a separate HR parameter was estimated for each multilocus genotype. More details of the statistical analysis can be found elsewhere (Antoniou et al., 2008).

RESULTS

In total, 7011 BRCA1 and BRCA2 mutation carriers were genotyped for TP53 Arg72Pro and 2222 carriers were genotyped for MDM2 309T>G (Table 1). Table 2 shows summary statistics for the cohort of BRCA1 and BRCA2 mutation carriers with an observed genotype for either the TP53 or MDM2 polymorphism. There was no evidence of an association between either SNP and breast cancer risk in BRCA1 or BRCA2 mutation carriers combined or analysed separately (TP53 Arg72Pro: $P_{\text{trend}} = 0.89$, 0.77 and 0.83, respectively; MDM2 309T>G: $P_{\text{trend}} = 0.60$, 0.54 and 0.88, respectively) (Table 3). There was no evidence for heterogeneity in the HRs between studies (TP53 Arg72Pro: $P = 0.22$ and 0.93; MDM2 309T>G: $P = 0.11$ and 0.82 for BRCA1 or BRCA2 mutation carriers respectively). The HRs for the 9 TP53–MDM2 combined genotypes, estimated separately in BRCA1 and BRCA2 mutation carriers, ranged between 0.72 and 1.31, but none of them were significant.

DISCUSSION

To our knowledge, this is the largest study to investigate the hypothesis that TP53 Arg72Pro and MDM2 309T>G influence breast cancer risk in BRCA1 and BRCA2 mutation carriers individually or in combination. Our findings of no association...
mutation-associated breast tumours are ER-negative (Lakhani et al, 2005), the absence of an association in our study of breast cancer with the TP53 and MDM2 SNPs in BRCA1 mutation carriers is consistent with the lack of an association with ER-negative cancers in the general population.

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