D302H polymorphism delays the age of onset of breast cancer in and carriers

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PRECLINICAL STUDY

CASP8 D302H polymorphism delays the age of onset of breast cancer in *BRCA1* and *BRCA2* carriers

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Abstract The polymorphic genetic differences among individuals may modify the high risk for breast cancer (BC) and/or ovarian cancer (OC) susceptibility conferred by *BRCA1* and *BRCA2* mutations. In the present study we investigate the relevance of *RAD51* −135C > G, *TP53* R72P, *NQO1*2* and *CASP8* D302H polymorphisms as potential modifiers of BC and/or OC susceptibility conferred by these mutations. The study group encompasses 390 *BRCA1*/*BRCA2* mutation carriers (182 affected with BC and/or OC and 208 unaffected) of 131 unrelated families studied in the Program of Genetic Counselling on Cancer of Valencia Community. The polymorphisms were detected in genomic DNA by ASRA method or real time PCR using fluorescently labeled probes. We found similar incidence of *RAD51* −135C > G, *TP53* R72P and *NQO1*2* polymorphisms among affected and unaffected individuals considering *BRCA1*/*BRCA2* mutations together and separately. However, the *CASP8* D302H polymorphism was strongly associated with the absence of BC [OR = 3.41 (95% CI 1.33–8.78, $P = 0.01$)]. In fact, in the females with *CASP8* D302H polymorphism the BC appeared at a median age of 58 in opposition to the 47 years observed for the wild type subjects ($P = 0.03$). Furthermore, the *CASP8* D302H positive females showed a 50% probability of being free of BC by the age of 78 versus the 2% of the *CASP8* negative ones. Our results support that the presence of the *CASP8* D302H polymorphism diminishes the high risk of BC conferred by *BRCA1* and *BRCA2* mutations, making possible that some of the carriers could escape from suffering BC along their life span.

Keywords *BRCA1*/*BRCA2* mutation carriers · Breast cancer · *CASP8* D302H polymorphism

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Introduction

Breast cancer (BC) is the most common malignancy affecting women worldwide [1]. It is estimated that between 5 and 10% of all BC cases that show familial pattern of occurrence [2] are caused by inherited mutations in tumor suppressor genes [3]. *BRCA1* and *BRCA2* are the main genes responsible for most of the mutations currently

found in familial BC. Indeed, approximately 25% of these familial cancers show mutations in *BRCA1* or *BRCA2* genes [4]. The inheritance of a single *BRCA1* or *BRCA2* mutation confers a high risk of BC or ovarian cancer (OC) during lifetime (45–65% risk for BC and 11–39% for OC) [5]. However, these mutations exhibit incomplete penetrance and the age at diagnosis of BC and/or OC varies remarkably among mutation carriers [5, 6]. This incomplete penetrance has been attributed to lifestyle, diet and environmental factors as well as the influence of low penetrance predisposing genetic factors [7]. With regard to the genetic factors modifiers of BC and/or OC risk in *BRCA1* and *BRCA2* carriers, a wide spectra of single nucleotide polymorphisms (SNPs) has been explored [8], however, the results yielded in the different studies still remain controversial and most of them could not be confirmed with enough evidence in later reports.

Of these genetic factors one of the best studied is the SNP −135C > G (*rs1801320*) of *RAD51* gene implicated in repair of double-strand DNA breaks and it is known that functionally interacts with *BRCA*1 and *BRCA2* [9–11]. This polymorphism was first studied in Jewish carriers of Ashkenazi mutations and was found to be associated with increased BC risk in *BRCA2* carriers [12]. However, the results of this study still remain controversial. Thus, whereas in some reports this association was not found [13] in others the association is restricted to the homozygous carriers [14].

Likewise, several polymorphisms have been identified within the *TP53* gene, both in non-coding and coding regions [15]. One of them is the *TP53* R72P polymorphism (*rs1042522*), located in codon 72 on exon 4, leading to arginine-proline substitution. This SNP is placed in a proline-rich region of the gene homologous to an SH3-binding domain that is required for the growth suppression activity of *TP53* [16]. The potential role of this polymorphism in BC risk has been analyzed in some studies [17]. However, the results are still controversial and while some reports confirm its role as modifier that increases the BC risk in patients with a stronger family history [18, 19], other reports refuse it [20, 21].

The enzyme *NQO1* is involved in the protection against oxidative stress [22] and carcinogen metabolism [23], including stabilization of the *TP53* tumor suppressor [24]. *NQO1*-knockout mouses show reduced capacity for *TP53* induction and apoptosis and increased susceptibility to chemically induced tumors [25]. The c.558C > T missense variant in exon 6 of the *NQO1* gene, known as the *NQO1**2 polymorphism (*rs1800566*), that causes the substitution of proline for serine (P187S) abrogating the enzymatic activity has been associated with the increasing risk of cancer [26]. Although this polymorphism has been extensively explored as predisposing factor to leukemia [27], little is known about its contribution to the BC and/or OC predisposition [28, 29].

Finally, a recent study carried out in large series of sporadic BC and control population has shown the relevance of the *CASP8* D302H polymorphism (*rs1045485*) in the risk of BC [30]. The *CASP8* gene encodes one of the initiator caspases that transduce apoptotic signals from the death receptors at the cell surface. *CASP8* functions as cysteine aspartyl protease that cleaves numerous intracellular substrates initiating cell dissolution [31]. Suppression of apoptosis due to mutations involves an illegitimate cell survival and predisposition to cancer [32]. The *CASP8* D302H polymorphism has shown to be associated with a reduced risk of BC in a dose-dependent manner [33]. This finding has been confirmed in a subsequent study [34].

The present study tries to clarify the relevance of *RAD51* −135C > G, *TP53* R72P, *NQO1**2 and *CASP8* D302H polymorphisms as potential modifiers of BC and/or OC risk conferred by *BRCA1* and *BRCA2* mutations.

Materials and methods

Study population

The study group includes 390 *BRCA1*/*BRCA2* mutation carriers, 290 females and 100 males, (186 *BRCA1* mutations and 204 *BRCA2* mutations) of 131 unrelated families; 182 were affected with BC and/or OC (177 female and 5 male) and 208 were unaffected (113 females and 95 males). All the patients or subjects belonged to families with strong BC and/or OC history who were recruited by the Units of Genetic Counselling in Cancer (UGCC) established in the Program of Genetic Counselling on Cancer of Valencia Community. The status of the patients and family members (age of the patient at the moment of diagnosis of the cancer/s or the age of the unaffected subjects) was collected by the UGCC at the first visit. The types of tumors and demographic characteristics of the subjects are summarized in the Table 1.

All the patients or subjects signed an informed consent, following the guidelines of the institution's ethics committee in accordance with the Helsinki declaration (1964, amended in 1975 and 1983).

DNA extraction

The screening of *BRCA1* and *BRCA2* mutations and the study of polymorphisms were carried out on genomic DNA. The DNA was directly extracted from 500 μl of whole blood using the MagNA Pure LC DNA Isolation Kit, large volume (Roche, Mannheim, Germany) automated in the MagNA Pure LC System (Roche).

Table 1 Histological subtype and demographic characteristics of BC and OC cases

Histologycal subtype	Cases (%; $n = 182$)	Mean age at diagnosis ±SD
Ductal carcinoma in situ (DCIS)	4 (2.2%)	40.5 (±8.1)
Lobular carcinoma in situ (LCIS)	2 (1.1%)	52.5 (±13.4)
Invasive ductal carcinoma (IDC)	104 (57.1%)	42.1 (±11.8)
Invasive lobular carcinoma (ILC)	21 (11.5%)	41.7 (±8.4)
Medullary carcinoma (MC)	8 (4.4%)	40.1 (±7.5)
Papillary serous carcinoma (PSC)	16 (8.8%)	44.8 (±10.7)
Others (mucinous, endometrioid, clear cell…)	27 (14.9%)	40.9 (±11.5)

Screening for BRCA1 and BRCA2 germline mutations

To detect *BRCA1* and *BRCA2* mutations we amplified by polymerase chain reaction (PCR) all the exons of *BRCA1* and *BRCA2* genes including exon-intron boundaries, using the primer pairs and PCR conditions reported in the Breast Cancer Information Core (BIC; http://research.nhgri.nih.gov/bic/Member/index.shtml) [35]. Mutational screening was carried out by pre-screening the heteroduplex of the PCR products with conformation sensitive gel electrophoresis followed by the direct sequencing of the PCR products in which heteroduplexes were identified [36].

Polymorphisms genotyping

The methods used to detect *RAD51* and *TP53* polymorphisms were based on allele-specific restriction enzyme site analysis (ASRA) [37]. The assessment of *RAD51* −135C > G polymorphism was performed amplifying by PCR the 483 bp region around nucleotide 135 using the primers RAD51F (5′-GCAACTCATCTGGGTTGTGC-3′) and RAD51R (5′-TCAGGAATCCGGAAGCCCTG-3′) at 1.5 mM $MgCl_2$. The digestion of the PCR product with *Bst*OI yields fragments of 68, 161 and 258 bp for the wild type allele and 225 and 258 bp for the polymorphic one. The *TP53* R72P polymorphism was assessed following the method described by Osorio et al. [38] which consists of PCR amplification of a 311 bp region around nucleotide 215 where the polymorphism is placed. The genotyping is based on the fact that the presence of the polymorphisms abolishes the restriction site of the enzyme *Bst*UI.

The methods used to detect *NQO1* and *CASP8* polymorphisms were based on real-time PCR performed in the LightCycler 480 platform (Roche Mannheim, Germany), using primers and fluorescent-labeled hybridization probes.

The *NQO1*2* was performed following the method of Harth et al. [39] that briefly consists of a real-time PCR amplification of a 300 bp region on exon 6 of the gene using the primers NQO1F and NQO1R and the corresponding hybridization probes.

The *CASP8* D302H polymorphism was detected by PCR amplification of a 183 bp region on exon 13. PCR reactions were performed in 10 µl final volume using LightCycler® 480 Genotyping Master (Reference 04707524001, Roche Diagnostics) that contains Taq, nucleotides and $MgCl_2$. The primers designed CASP8F (5′-TGACTGTTCAAATTTCACTTTTCAGG-3′) and CASP8R (5′-CTTGTCTCCATGGGAGAGGATA-3′) were used at 0.2 µM. The hybridization probes designed for mutant allele (CASP8-sensor: 5′-CTCTACTGTGCAGTCATGGTGG-3′ and CASP8-anchor: 5′-GCTTGATCTCAAAATGAAGCTCTTCAAAGGT-3′) were used at 0.2 µM. PCR cycling conditions were 95°C for 10 min, followed by 45 cycles of 5 s denaturation at 95°C, 20 s annealing at 55°C and 15 s extension at 72°C. Melting curve analysis was performed after 60 s denaturation at 95°C and hybridization for 30 s at 50°C by increasing the temperature from 50 to 80°C with a slope of 2.2°C/min.

Each experiment included a blank in which the DNA was substituted by water, wild type, heterozygous and homozygous DNA samples.

The adequate performance of the methods used to assess the four polymorphisms was confirmed by direct sequencing the amplicon PCR of the wild type, heterozygous and homozygous samples. The sequencing was performed in the ABIprism 3130 (Applied Biosystems), using 5 µl of the purified PCR product following the instructions of the BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems).

Statistical analysis

SNPs genotype frequencies were compared in affected versus unaffected subjects using the χ^2 and Fisher′s exact tests (FET). The association of disease status of *BRCA1* and *BRCA2* carriers with the four SNPs, mutated gene *BRCA1*/*BRCA2*, gender and age was analyzed using logistic regression analysis with estimation of OR and 95% CI. Logistic regression was also employed to analyze the association of polymorphism genotype (positive or negative) and tumor type (BC, including unilateral and bilateral, and OC). Unadjusted time-to-event analysis was performed using the Kaplan–Meier method and log-rank tests for comparisons [40, 41]. In the analysis of affectation-free

survival (AFS), the age at diagnosis of the first malignancy was considered uncensored events. The analyzed outcome was BC only including unilateral and bilateral BC. In all analyzes, healthy carriers were censored at the age of participation in the study. All tests were two-sided and *P* values less than 0.05 were considered statistically significant. Computations were performed using the *SPSS* v11.0 statistical package (Chicago, IL).

Results

Polymorphisms and related cancers

The *RAD51* −135C > G polymorphism was detected in 15.4% of *BRCA1* and *BRCA2* carriers, being all of them heterozygous; *TP53* R72P was present in 38.4% of carriers (132 heterozygous and 18 homozygous); *NQO1* P187S was observed in 44.1% of carriers (146 heterozygous and 26 homozygous); and D302H *CASP8* was detected in 18.5% of carriers (69 heterozygous and 3 homozygous) (Table 2).

The *CASP8* D302H polymorphism was highly associated with the absence of cancer ($P = 0.003$; Table 2). Thus, this polymorphism was harbored by 24.0% of unaffected individual versus only 12.1% of the affected ones. However, the presence of *CASP8* D302H was only related with BC but not with OC [OR = 3.41 (95% CI 1.33–8.78, $P = 0.01$)]. The remaining polymorphisms showed similar incidence between affected and unaffected individuals (Table 2).

We analyzed the effect of the studied polymorphisms, the mutated gene (*BRCA1* or *BRCA2*), gender and age on the presence of the related malignancy (BC or OC or both) performing a multiple logistic regression. Gender (female), age and *CASP8* D302H were the only statistically significant parameters that influenced cancer risk; the *CASP8* D302H polymorphism decreased the risk (OR = 0.40; 95% CI: 0.22–0.75; $P = 0.004$; Table 3).

Table 3 Multivariate analysis of the studied polymorphisms, type of mutated gene, gender and age by logistic regression

	OR (95% CI)	*P*
Gene *BRCA1/BRCA2*	0.75 (0.47–1.22)	0.250
RAD51 −135 G > C	1.02 (0.52–1.97)	0.968
TP53 R 72P	0.91 (0.56–1.47)	0.698
NO01 P187S	0.84 (0.52–1.36)	0.483
CASP8 D302H	0.40 (0.22–0.75)	**0.004**
Age	1.04 (1.02–1.06)	**0.001**
Gender	0.03 (0.01–0.07)	**0.000**

The bold entries signify *P* values that are considered statistically significant

Since the polymorphisms studied only affect the females with BC (unilateral or bilateral) we estimated the logistic regression excluding males and females with OC. The logistic regression with these parameters reinforces the influence of *CASP8* D302H polymorphism lessening the risk of cancer (OR = 0.27; 95% CI = 0.13–0.57; $P = 0.001$).

Polymorphisms and age at cancer diagnosis

When we applied the Kaplan–Meier univariate model we did not find any significant association of the age at diagnosis of cancer with the *RAD51* −135C > G, *TP53* R72P and *NQO1*2* polymorphisms. Furthermore, we did not find influence of the *CASP8* polymorphism with the age of onset of OC in females or with the age at diagnosis of BC in males.

Table 2 Genotype distribution of the polymorphisms in *BRCA1* and *BRCA2* mutation carriers by participating study

Gene SNP (Entrez SNP ID)[a]	MAF[b]	Genotypes[c]	Cases (%; $n = 390$)	*BRCA1/BRCA2* carriers		OR (95% CI)	*P*
				Healthy (%; $n = 208$)	Affected (%; $n = 182$)		
RAD51	0.154	Negative	330 (84.6%)	175 (84.1)	155 (85.1)	0.9 (0.5–1.6)	0.779
−135 G > C (rs1801320)		Positive	60 (15.4%)	33 (15.9)	27 (14.9)		
TP53	0.478	Negative	240 (61.5%)	134 (64.4)	106 (58.2)	1.3 (0.9–2.0)	0.211
R72P (rs1042522)		Positive	150 (38.4%)	74 (35.6)	76 (41.8)		
NO01	0.251	Negative	218 (55.9%)	115 (55.3)	103 (55.6)	1.0 (0.6–1.4)	0.796
P187S (rs1800566)		Positive	172 (44.1%)	93 (44.7)	79 (44.4)		
CASP8	0.192	Negative	318 (81.5%)	158 (76.0)	160 (87.9)	0.4 (0.3–0.8)	**0.003**
D302H (rs1045485)		Positive	72 (18.5%)	50 (24.0)	22 (12.1)		

[a] Entrez SNP reference ID number (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db5snp)

[b] Minor allele frequency

[c] Positive: including heterozygous and homozygous

The bold entry signifies *P* value that is considered statistically significant

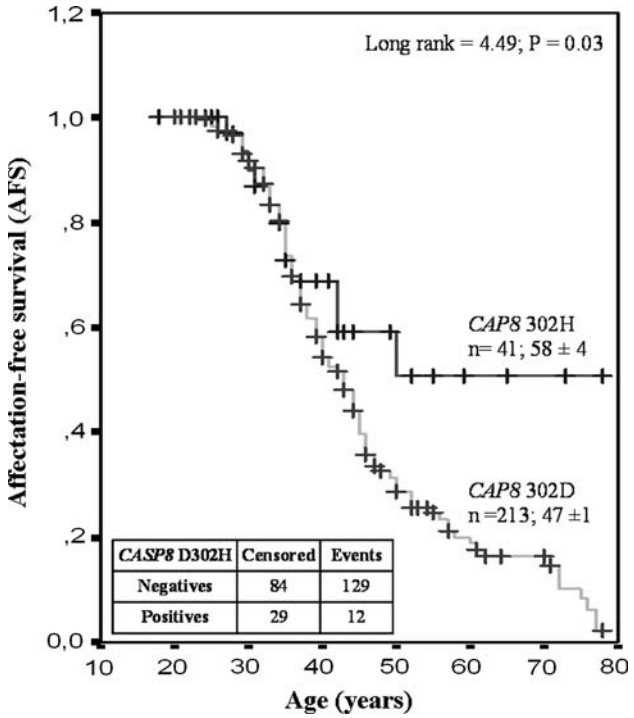

Fig. 1 Breast cancer free-survival and *CASP8* D302H polymorphism

We analyzed the possible effect of the presence of *CASP8* D302H polymorphism in females with the age at diagnosis of BC or the age of the unaffected subjects at the first visit, applying the Kaplan–Meier stochastic model. The analysis showed that the *CASP8* D302H polymorphism was significantly associated with the late onset of BC (long rank = 4.49; $P = 0.03$) in comparison with the patients negative for this polymorphism (Fig. 1). The patients with *CASP8* D302H polymorphism had a median age of onset of BC of 58 years (range: 49–66) versus 47 years (range: 44–49 years) of the subjects negatives for the polymorphism. Furthermore, the *CASP8* D302H positive subjects showed a 50% probability of being free of BC by the age of 78 versus the 2% of the *CASP8* negative ones.

The multivariate Cox analysis relating the presence of the studied polymorphisms and the mutated gene (*BRCA1* or *BRCA2*) with the age at diagnosis of BC of the patients or the age of the unaffected subjects at the first visit, corroborates the *CASP8* D302H polymorphism as the single independent parameter associated with the age of onset of BC [HR = 0.54; (95% CI: 0.29–0.98; $P = 0.04$)].

Discussion

Our results support that the presence of *CASP8* D302H polymorphism decreases the BC risk of *BRCA1*/*BRCA2* mutation carriers (OR = 0.27; 95% CI = 0.13–0.57) delaying the age of onset of BC. To this regard, a recent study carried out in large series of sporadic BC and controls (11,391–18,290 cases and 14,753–22,670 controls) in which nine SNPs were considered, showed that *CASP8* D302H polymorphism was associated with a moderate reduction in the BC risk (OR = 0.89; 95% CI = 0.85–0.94) [30]. Concerning familial BC, Frank et al. [42] analyzed *CASP10* V410I and *CASP8* D302H polymorphisms in 511 familial BC negative for *BRCA1* and *BRCA2* mutations and 547 normal controls, concluding that *CASP10* V410I and *CASP8* D302H variants are linked to a highly significant reduction of familial BC risk. In another case-control study carried out in 580 Italian familial BC index cases, that were also tested negative for *BRCA1* and *BRCA2* mutations, a statistically significant association of the *CASP8* −652 6 N deletion promoter polymorphism with the advanced age of diagnosis of BC was found [43], confirming the results obtained in previous Europeans studies [44]. Even more, the lack of association of *CASP8* D302H polymorphism with OC risk showed in the present study is in accordance with the current report of Ramus et al. [45] carried out in a large series of 4,624 invasive epithelial ovarian cancer cases and 8,113 controls.

The *CASP8* gene plays an important role as initiator of apoptosis activated by external death signals and in response to DNA damage. Recruitment of *CASP8* to death-inducing signaling complex (DISC) and apoptosome leads to their activation by dimerization while the suppression of apoptosis due to mutations involves an illegitimate cell survival and predisposition to cancer [32]. It has been mentioned that genes involved in the initiation of apoptosis might act as low-penetrance familial BC susceptibility genes. However, the specific mechanism of action of the *CASP8* D302H polymorphism is not yet known. Further experiments are required to establish whether the polymorphism is itself causative or is in strong linkage disequilibrium with other unidentified causative variants.

No association was found of the *RAD51* −135C > G polymorphism with the related cancers (BC or OC), both considering *BRCA1* and *BRCA2* mutation together and separately. Our results are in agreement with some studies [13] but in opposition with others [12, 14]. The latter pointed out that *RAD51* −135C variant increased the risk of BC and/or OC for *BRCA2* mutation carriers, but in the large study of Antoniou et al. [14] the relationship of the polymorphism with the BC and/or OC risk was only evident in the homozygous for *RAD51* −135C polymorphism group. The absence of homozygous for *RAD51* −135C polymorphism in our series may be the rationale for not finding any association of this polymorphism with the risk of cancer.

We found similar incidence for *TP53* R72P polymorphism in the affected and unaffected subjects. These results are concordant with those reported in similar studies in which no association of the *TP53* R72P polymorphism with

the risk of BC or OC was found [20, 21]. Although it has been reported that the *TP53* Arg homozygous genotype could be a potential genetic risk factor for cancer [18], not all the studies concur. In fact, our results are in agreement with the report of Osorio et al. [20] carried out in 2,932 *BRCA1* or *BRCA2* Spanish mutation carriers that showed no evidence of modification of BC and/or OC risk for R72P and Ins16 bp variants of the *TP53* studied or their haplotype combinations.

Moreover, no differences were found in the incidence of *NQO1*2* polymorphism between the patients with cancer (BC or OC) and the unaffected subjects. This result is not surprising taking into account that *NQO1* is a two-electron reductase engaged in protection against oxidative stress and carcinogen metabolism [22, 23]. Hence, the *NQO1*2* that decreases the enzymatic activity in heterozygous and abolishes it in homozygous has been linked to the predisposition to leukemia [27] and particularly in the treatment related to this disease [26]. In the field of BC the *NQO1*2* polymorphism was associated with the response to anthracycline-based adjuvant therapy [28], however, regarding BC predisposition we only found relation of the polymorphism with the histological type [29] but not with the presence of tumor.

In conclusion, the *CASP8* D302H polymorphism decreases the BC risk in *BRCA1/BRCA2* mutation carriers and delays the age of onset of BC; therefore it will be advisable to assess this polymorphism in all *BRCA1/BRCA2* mutations carriers for a better prediction of their particular risk. The presence of the polymorphism could attenuate the high risk conferred by *BRCA1* and *BRCA2* mutations in predisposed individuals, making possible that some of them escape from suffering BC.

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References

1. Boyley P, Ferlay J (2005) Cancer incidence and mortality in Europe 2004. Ann Oncol 16:481–488. doi:10.1093/annonc/mdi098
2. Rowell S, Newman B, Boyd J et al (1994) Inherited predisposition to breast and ovarian cancer. Am J Hum Genet 55:861–865
3. Antoniou AC, Easton DF (2003) Polygenic inheritance of breast cancer: implications for design of association studies. Genet Epidemiol 25:190–202. doi:10.1002/gepi.10261
4. Wooster R, Bignell G, Lancaster J et al (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789–792. doi:10.1038/378789a0
5. Antoniou A, Pharoah PD, Narod S et al (2003) Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 72:1117–1130. doi:10.1086/375033
6. Simchoni S, Friedman E, Kaufman B et al (2006) Familial clustering of site-specific cancer risks associated with BRCA1 and BRCA2 mutations in the Ashkenazi Jewish population. Proc Natl Acad Sci USA 103:3770–3774. doi:10.1073/pnas.0511301103
7. Chenevix-Trench G, Milne RL, Antoniou AC et al (2007) An international initiative to identify genetic modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: the consortium of investigators of modifiers of BRCA1 and BRCA2 (CIMBA). Breast Cancer Res 9:104. doi:10.1186/bcr1670
8. Dunning AM, Dunning AM, Healey CS et al (1999) A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 8:843–854
9. Karran P (2000) DNA double strand break repair in mammalian cells. Curr Opin Genet Dev 10:144–150. doi:10.1016/S0959-437X(00)00069-1
10. Wong AK, Pero R, Ormonde PA et al (1997) RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene brca2. J Biol Chem 272:31941–31944. doi:10.1074/jbc.272.51.31941
11. Scully R, Chen J, Plug A et al (1997) Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell 88:265–275. doi:10.1016/S0092-8674(00)81847-4
12. Levy-Lahad E, Lahad A, Eisenberg S et al (2001) A single nucleotide polymorphism in the RAD51 gene modifies cancer risk in BRCA2 but not BRCA1 carriers. Proc Natl Acad Sci USA 98:3232–3236. doi:10.1073/pnas.051624098
13. Blasiak J, Przybylowska K, Czechowska A et al (2003) Analysis of the G/C polymorphism in the 5′-untranslated region of the RAD51 gene in breast cancer. Acta Biochim Pol 50:249–253
14. Antoniou AC, Sinilnikova OM, Simard J et al (2007) RAD51 135G > C modifies breast cancer risk among BRCA2 mutation carriers: results from a combined analysis of 19 studies. Am J Hum Genet 81:1186–1200. doi:10.1086/522611
15. Olivier M, Eeles R, Hollstein M et al (2002) The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat 19:607–614. doi:10.1002/humu.10081
16. Walker KK, Levine AJ (1996) Identification of a novel p53 functional domain that is necessary for efficient growth suppression. Proc Natl Acad Sci USA 93:15335–15340. doi:10.1073/pnas.93.26.15335
17. Buyru N, Tigli H, Dalay N (2003) P53 codon 72 polymorphism in breast cancer. Oncol Rep 10:711–714
18. Tommiska J, Eerola H, Heinonen M et al (2005) Breast cancer patients with p53 Pro72 homozygous genotype have a poorer survival. Clin Cancer Res 11:5098–5103. doi:10.1158/1078-0432.CCR-05-0173
19. Ohayon T, Gershoni-Baruch R, Papa MZ et al (2005) The R72P P53 mutation is associated with familial breast cancer in Jewish women. Br J Cancer 92:1144–1148. doi:10.1038/sj.bjc.6602451
20. Osorio A, Pollán M, Pita G et al (2008) An evaluation of the polymorphisms Ins16 bp and Arg72Pro in p53 as breast cancer risk modifiers in BRCA1 and BRCA2 mutation carriers. Br J Cancer 99:974–977. doi:10.1038/sj.bjc.6604624

21. Costa S, Pinto D, Pereira D et al (2008) Importance of TP53 codon 72 and intron 3 duplication 16 bp polymorphisms in prediction of susceptibility on breast cancer. BMC Cancer 8:32. doi:10.1186/1471-2407-8-32
22. Siegel D, Gustafson DL, Dehn DL et al (2004) NAD(P)H:quinone oxidoreductase 1: role as a superoxide scavenger. Mol Pharmacol 65:1238–1247. doi:10.1124/mol.65.5.1238
23. Nebert DW, Roe AL, Vandale SE et al (2002) NAD(P)H:quinone oxidoreductase (NQO1) polymorphism, exposure to benzene, and predisposition to disease: a HuGE review. Genet Med 4:62–70. doi:10.1097/00125817-200203000-00003
24. Asher G, Lotem J, Cohen B et al (2001) Regulation of p53 stability and p53-dependent apoptosis by NADH quinone oxidoreductase 1. Proc Natl Acad Sci USA 98:1188–1193. doi:10.1073/pnas.021558898
25. Long DJ, Waikel RL, Wang XJ et al (2000) NAD(P)H:quinone oxidoreductase 1 deficiency increases susceptibility to benzo(a)-pyrene-induced mouse skin carcinogenesis. Cancer Res 60:5913–5915
26. Larson RA, Wang Y, Banerjee M et al (1999) Prevalence of the inactivating 609C > T polymorphism in the NAD(P)H:quinone oxidoreductase (NQO1) gene in patients with primary and therapy-related myeloid leukemia. Blood 94:803–807
27. Bolufer P, Barragan E, Collado M et al (2006) Influence of genetic polymorphisms on the risk of developing leukemia and on disease progression. Leuk Res 30:1471–1491. doi:10.1016/j.leukres.2006.01.016
28. Fagerholm R, Hofstetter B, Tommiska J et al (2008) NAD(P)H:quinone oxidoreductase 1 NQO1*2 genotype (P187S) is a strong prognostic and predictive factor in breast cancer. Nat Genet 40:844–853. doi:10.1038/ng.155
29. Siegelmann-Danieli N, Buetow KH (2002) Significance of genetic variation at the glutathione S-transferase M1 and NAD(P)H:quinone oxidoreductase 1 detoxification genes in breast cancer development. Oncology 62:39–45. doi:10.1159/000048245
30. Cox A, Dunning AM, Garcia-Closas M et al (2007) A common coding variant in CASP8 is associated with breast cancer risk. Nat Genet 39:352–358. doi:10.1038/ng1981
31. Hengartner MO (2002) The biochemistry of apoptosis. Nature 407:770–776. doi:10.1038/35037710
32. Evan GI, Vousden KH (2001) Proliferation, cell cycle and apoptosis in cancer. Nature 411:342–348. doi:10.1038/35077213
33. MacPherson G, Healey CS, Teare MD et al (2004) Association of a common variant of the CASP8 gene with reduced risk of breast cancer. J Natl Cancer Inst 96:1866–1869
34. Frank B, Bermejo JL, Hemminki K et al (2005) Re: association of a common variant of the CASP8 gene with reduced risk of breast cancer. J Natl Cancer Inst 97:1012–1013
35. The Breast Cancer Information Core Database BIC. http://research.nhgri.nih.gov/bic/Member/index.shtml. Accessed 1 Jan 2009
36. 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. doi:10.1073/pnas.90.21.10325
37. Todd AV, Ireland CM, Radloff TJ et al (1991) Analysis of N-ras gene mutations in acute myeloid leukemia by allele specific restriction analysis. Am J Hematol 38:207–213. doi:10.1002/ajh.2830380310
38. Osorio A, Martinez-Delgado B, Pollan M et al (2006) A haplotype containing the p53 polymorphisms Ins16 bp and Arg72Pro modifies cancer risk in BRCA2 mutation carriers. Hum Mutat 27:242–248. doi:10.1002/humu.20283
39. Harth V, Brüning T, Abel J et al (2001) Real-time genotyping of cytochrome P4501A1 A4889G and T6235C polymorphisms. Mol Cell Probes 15:93–97. doi:10.1006/mcpr.2001.0349
40. Kaplan EL, Meier P (1958) Nonparametric estimations from incomplete observations. J Am Stat Assoc 53:457–481. doi:10.2307/2281868
41. Mantel N (1966) Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50:163–170
42. Frank B, Hemminki K, Wappenschmidt B et al (2006) Association of the CASP10 V410I variant with reduced familial breast cancer risk and interaction with the CASP8 D302H variant. Carcinogenesis 27:606–609. doi:10.1093/carcin/bgi248
43. De Vecchi G, Verderio P, Pizzamiglio S, et al. (2008) Evidences for association of the CASP8 −652 6N del promoter polymorphism with age at diagnosis in familial breast cancer cases. Breast Cancer Res Treat (in press)
44. Sun T, Gao Y, Tan W et al (2007) A six-nucleotide insertion-deletion polymorphism in the CASP8 promoter is associated with susceptibility to multiple cancers. Nat Genet 39:605–613. doi:10.1038/ng2030
45. Ramus SJ, Vierkant RA, Johnatty SE et al (2008) Consortium analysis of 7 candidate SNPs for ovarian cancer. Int J Cancer 123:380–388. doi:10.1002/ijc.23448