ELECTRONIC LETTER

Germline *TP53* mutations in breast cancer families with multiple primary cancers: is *TP53* a modifier of *BRCA1*?

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Somatic mutations in *TP53* are the most frequent events in human cancer and lead to inactivation of the gene, loss of tumour suppressor function, and in some cases generation of a dominant negative form of p53.[1–3] Eleven exons make up the primary transcript of *TP53*, of which exons 2-11 encode the protein. Five conserved domains exist in exons 1, 4, 5, 7, and 8,[4] which are considered essential for normal p53 function. Approximately 90% of disease associated mutations occur in these domains, with mutations in five codons (175, 245, 248, 249, and 273) accounting for approximately 20% of all mutations reported to date.

Germline mutations in *TP53* cause Li-Fraumeni syndrome (LFS), a familial association of childhood leukaemia, brain cancer, soft tissue sarcoma, and adrenal cortical carcinoma,[5 6] as well as other cancers such as breast cancer, melanoma, germ cell tumours, and carcinomas of the lung, pancreas, and prostate.[7 8] Cancers characteristically develop at unusually early ages and multiple primary tumours are frequent. Susceptibility to cancer in these families follows an autosomal dominant pattern of inheritance[7] and among families with a known germline *TP53* mutation the probability of developing any invasive cancer (excluding carcinomas of the skin) approaches 50% by the age of 30, compared to an age adjusted population incidence of cancer of 1%. It is estimated that more than 90% of *TP53* mutation carriers will develop cancer by the age of 70.[9]

In addition to the numerous mutations, *TP53* also contains several polymorphisms that may alter its activity. In particular, at nucleotide 215 (codon 72) there is a single base pair variant (g.215G>C) in the coding region, which results in a substitution of proline for arginine in the protein sequence.[10] The frequency of this polymorphism varies from 26-35%[11–13] and it appears to affect protein function. The R72 variant of TP53 is believed to be more sensitive to human papillomavirus (HPV) induced degradation by the E6 oncoprotein than the 72P variant, and is thought to be of functional significance in HPV associated tumours[14] such as cervical tumours.[15–17] Furthermore, some, but not all studies document an over-representation of R72 variant in cervical cancer patients compared to a control population.[18 19] However, other reports suggest the association of the 72P variant with incidence of squamous cell carcinoma of the head and neck[20] and lung adenocarcinomas in smokers.[21]

In families with multiple cases of breast cancer that do not fit the criteria for LFS, the frequency of *TP53* germline mutations has been investigated in multiple studies,[22–28] documenting that *TP53* mutations account for <1% of site specific breast cancer families.[23] However, among LFS families, there is a very high incidence of early onset breast cancer. Taken together, these data suggest that germline *TP53* mutations are strongly associated with hereditary breast cancer susceptibility but almost exclusively in the context of LFS.

Because of the high penetrance of early onset breast cancer and the known increased incidence of multiple primary cancers in LFS families (50% by 30 years of age),[9] we investigated whether deleterious germline mutations in *TP53* and/or the R72P polymorphism were associated with multiple primary cancers (in which one was breast cancer) in families with ⩾2 breast cancers but no evidence of LFS. One previous study investigated the frequency of germline *TP53* mutations with bilateral breast cancer[29] and found no *TP53* mutations; however, only 19 samples were tested. In the current study we determined the frequency of deleterious *TP53* germline mutations in 172 breast cancer families, with and without multiple primary cancers. Germline *BRCA1* or *BRCA2* mutation status was known in all subjects[30]; 43% of women with multiple primary cancers and 12% of women with breast cancer only had

Key points

- Eighty eight women with breast cancer and a personal or family history of multiple primary cancers (MPC) (including ovarian cancer) and 84 women with a personal and family history of breast cancer only (BC) were studied. All women had been previously screened for germline *BRCA1* and *BRCA2* mutations; 38 (43%) of MPC women and 10 (12%) of BC women had a mutation in one of these two genes.
- We determined the frequency of deleterious germline *TP53* mutations, as well as the common R72P polymorphism in *TP53* and investigated the association of this polymorphism with the development of cancers in the entire study set. We also evaluated the association between R72P and breast cancer penetrance in the subset of women with known *BRCA1* or *BRCA2* mutations.
- One woman, from a family with breast cancer only, was found to have a deleterious *TP53* mutation (exon 7, G245S); no deleterious *TP53* mutations were detected in the families with cases of multiple primary cancers. The common R72P polymorphism was seen at a frequency of 41% in the entire sample. MPC women were more likely to be homozygous for R72 compared to BC women (p=0.05, OR 2.83, 95% CI 1.2 to 6.9), an association that was more striking in women with a *BRCA1* or *BRCA2* mutation (OR 6.1, 95% CI 1.4 to 26.4).
- We also found that the presence of a 72P allele was associated with an earlier age of breast cancer diagnosis among *BRCA1* mutation carriers (p=0.05), suggesting that the R72P polymorphism may be a modifier of *BRCA1* penetrance.

Abbreviations: MPC, multiple primary cancers; BC, breast cancer only; LFS, Li-Fraumeni syndrome; HPV, human papillomavirus

Table 1 Non-breast primary cancers in families with multiple primary cancers

Cancer	No of families with cancer*
Ovarian	29
Colorectal	13
Non-melanoma skin	11
Thyroid	6
Endometrial	6
Cervix	5
Leukaemia	4
Lymphoma	3
Others†	13

*Five patients had two or more non-breast cancers, so the number of cancers does not equal the number of patients.
†Other primary cancers include melanoma, brain, head/neck, sarcoma, lung, kidney, and pharynx.

either a *BRCA1* or *BRCA2* mutation. In addition, we established the frequency of the common exon 4 polymorphism (R72P) in this sample and evaluated whether this polymorphism may be a modifier of breast cancer penetrance in the presence of *BRCA1* or *BRCA2* mutations.

MATERIALS AND METHODS

Patient population

All families were recruited from clinics at the University of Michigan (1993-1995) and the University of Pennsylvania (1995-1998). Patients were either self- or physician referred because of a perceived risk of inherited susceptibility to breast cancer. All women consented to genetic testing for clinical and/or research purposes. Personal and family histories of all cancers were recorded, including age of diagnosis of all cancers and the number of related women in each family at risk for breast cancer (age ⩾20 years). Pathology reports were obtained on all probands and on other family members when possible. The testing protocol was approved by duly constituted institutional review boards at both the University of Michigan and the University of Pennsylvania.

Eighty-eight women were from families with at least two cases of breast cancer and at least one woman affected with both a primary breast cancer and a primary non-breast cancer (denoted MPC). Eighty-four (95%) MPC women had two primary cancers, and four MPC women (5%) had three or more primary cancers. All non-breast malignancies were considered, including non-melanoma skin cancers. An additional 84 women were from families with at least two cases of breast

Table 2 Description of relatives providing sample for testing

Sample number tested (relative)	Age of breast cancer in relative	Average age of diagnosis of additional cancer(s) in multiply affected subject	Second primary cancer in multiply affected subject	Relationship of subject providing DNA for testing
229	30	58	Ovarian	Daughter
522	56	63	Ovarian	Niece
743	69	74	BCC, cervical NHL	Niece
673	40	43	BCC	Daughter
513	38	30	Cervical	Daughter
641	44	30	Colon	Cousin
813	36	44	Ovarian	Niece
852	56	53	BCC, endometrial	Niece
834	45	40	Cervical	Sister
1019	50	63	Ovarian	Daughter
842	27	47	Ovarian	Sister
975	57	50	BCC	Sister
1965	57	25	Thyroid	Daughter
910	74	55	Thyroid	Daughter
1946	44	84	Leukaemia	Daughter
1901	30	65	Lung	Niece
1907	46	86	Colon, rectal	Daughter
1208	41	51	Ovarian	Sister
1708	72	69	Skin, cervical, endometrial	Sister
1785	51	49	Colon	Granddaughter
1844	52	67	Colon	Daughter
1773	50	51	Cervical	Niece
1762	45	74	Colon	Daughter
1797	36	50	Ovarian	Daughter
1748	42	72	Hodgkin's lymphoma	Granddaughter
1722	50	83	Endometrial	Granddaughter
1719	40	23	Ovarian	Sister
1718	35	36	Ovarian	Granddaughter
1763	41	56	Colon	Daughter
1794	40	40	Ovarian	Cousin
1783	53	53	Throat	Double cousin
1853	57	57	Brain	Grandniece
320	40	40	Pituitary	Niece
1987	55	55	Ovarian	Daughter
1909	70	70	Leukaemia	Granddaughter
1954	61	61	Thyroid, colon	Sister
1851	43	74	Leukaemia	Cousin
1806	49	49	Melanoma	Daughter
1863	39	39	Ovarian	Daughter
1993	67	67	Colon	Cousin
2074	95	95	Colon	Niece
2038	50	52	Ovarian	Daughter
2067	50	50	BCC	Daughter
2233	41	42	ALL	Niece
2241	61	52	Ovarian	Cousin

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Table 3 Primer sequences

Exon	Primer sequences	Annealing temp (°C)	Reference
2/3	Forward: 5′-ggatccccacttttcctctt-3′ Reverse: 5′-agcatcaaatcatccattgc-3′	57	
4	Forward: 5′-gacctggtcctctgactgct-3′ Reverse: 5′-atacggccaggcattgaagt-3′	54	
5/6	Forward: 5′-tgccctgactttcaactctgt-3′ Reverse: 5′-ttaacccctcctcccagaga-3′	54	
7	Forward: 5′-tgccacaggtctccccaagg-3′ Reverse: 5′-aggggtcagcggcaagcaga-3′	55	Evans *et al*[53]
8/9	Forward: 5′-caagggtggttgggagtaga-3′ Reverse: 5′-acttgataagaggtcccaag-3′	54	
10	Forward: 5′-atgttgcttttgatccgtca-3′ Reverse: 5′-ctttccaacctaggaaggca-3′	54	
11	Forward: 5′-agccacctgaactcaaaaa-3′ Reverse: 5′-aatggcaggggagggagaga-3′	55	Evans *et al*[53]

cancer but no cases of multiple primary cancers (denoted BC). DNA was available from at least one woman with multiple primary cancers in 43 families. In the remaining 45 multiple primary cancer families, the multiply affected woman was dead (n=33) or unavailable (n=12). In these families, *TP53* screening was undertaken using DNA from the closest female relative diagnosed with breast cancer. Table 1 provides a description of the cancers reported in subjects with multiple primary cancers and table 2 is a detailed description of the female relatives of a multiply affected woman, who provided samples for testing. All samples were previously screened for germline mutations in *BRCA1* and *BRCA2*[30]; 38 MPC women and 10 BC women had a *BRCA1* or *BRCA2* germline mutation.[30]

Mutation analysis

DNA was extracted from peripheral blood mononuclear cells and stored in TE at 4°C. The entire 10 exon coding domain and flanking splice site regions of *TP53* were amplified using seven PCR primer sets (table 3). PCR amplification was performed in a final volume of 20 µl containing 80 ng of DNA, 1.5 mmol/l $MgCl_2$, 10 mmol/l Tris-HCl (pH 8.3), 50 mmol/l KCl, 0.2 mmol/l each of dCTP, dATP, dTTP, dGTP (Amersham Pharmacia Biotech), each primer at 1.0 µmol/l, and 1.0 unit of *Taq* polymerase (Boerhinger Manheim). Annealing temperatures were optimised for each primer set and ranged from 55-60°C. Variants were identified by conformation sensitive gel electrophoresis (CSGE) as previously described[30] and characterised by direct sequencing using the ABI Prism 377 after reamplification from source DNA. All mutation nomenclature is reported using the recommendations of den Dunnen and Antonarakis.[31]

Statistical analysis

Differences in *TP53* mutation frequency between the MPC and BC groups were assessed using χ^2 analysis. Odds ratios (OR) and 95%t confidence intervals (95% CI) were reported. In those instances where expected cell counts fell below five, we used exact methods to determine the 95% CI.[32] Furthermore, we used the Mann-Whitney U-Wilcoxon rank sum test to determine whether *TP53* genotypes altered the median age of first breast cancer diagnosis within categories defined by *BRCA1* or *BRCA2* mutation status.

RESULTS

DNA from one woman from a BC family and no *BRCA1* or *BRCA2* mutation showed an abnormal CSGE profile in *TP53* exon 7. Sequence analysis of this variant showed a G to A transition at the first nucleotide in codon 245 resulting in a glycine-serine change at this position (G245S). No presumed deleterious *TP53* mutations were seen in the MPC group (MPC=0%, BC=1.2%, p=0.31).

The proline allele of the R72P polymorphism was seen at a frequency of 41% in the entire sample. The distribution of R72P genotypes within groups is presented in table 4. Owing to the small number of homozygous 72P genotypes, all 72P alleles were combined into one group (P/*). When we performed subgroup analyses in women with *BRCA1* or *BRCA2* mutations, MPC women were six times more likely to have the homozygous R72 genotype than BC women (OR=6.1, 95% CI 1.4 to 26.4) (table 5). However, because of the small sample size, a statistically significant association between the homozygous R72 genotype and MPC could not be confirmed separately in an analysis of only *BRCA1* mutation carriers or only *BRCA2* mutation carriers.

Table 4 *TP53* exon 4 R72P genotypes in subjects with multiple primary cancers (MPC) compared to subjects with breast cancer only (BC)

TP53 genotype	MPC (n=43)		BC (n=84)		
	No	(%)	No	(%)	
R/R	35	(81)	51	(61)	
R/P	6	(14)	28	(33)	
P/P	2	(5)	5	(6)	
P/	8	(19)	33	(39)	OR=2.83 (1.2, 6.9)

P/ is the combined genotypes of R/P and P/P used for statistical analysis owing to the rarity of the P allele.

Table 5 *TP53* R72P genotypes in MPC and BC families by *BRCA1/2* mutation status

TP53 genotype	MPC (n=43)		BC (n=84)		
	No	(%)	No	(%)	OR 95% CI
BRCA1 or *BRCA2* mutation					
R/R	19	(83)	7	(44)	6.1 (1.4, 26.4)
P/*	4	(17)	9	(56)	1.0
BRCA1 mutation					
R/R	16	(80)	3	(43)	5.3 (0.83, 34.1)
P/*	4	(20)	4	(57)	1.0
BRCA2 mutation					
R/R	5	(83)	4	(44)	6.3 (0.50, 77.5)
P/*	1	(17)	5	(56)	1.0
No detectable mutation					
R/R	16	(80)	44	(65)	2.2 (0.66, 7.3)
P/*	4	(20)	24	(35)	1.0

P/* = R72P or P72P.
Three women in the sample set had both a *BRCA1* and a *BRCA2* mutation.

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Table 6 *TP53* R72P genotypes by age of diagnosis of breast cancer and *BRCA1/2* mutation status

TP53 genotype	Median age	(IQR)	p value
BRCA1 or *BRCA2* mutation			
R/R (n=26)	42	(35-51)	0.01
P/* (n=13)	32	(30-38)	
BRCA1 mutation			
R/R (n=19)	46	(35-51)	0.05
P/* (n=8)	32	(30-41.5)	
BRCA2 mutation			
R/R (n=9)	39	(35-46)	0.39
P/* (n=6)	35	(30-43)	
No detectable mutation			
R/R (n=60)	49	(37-61.5)	0.98
P/* (n=28)	50	(42.5-57)	

Three women in the sample set had both a *BRCA1* and a *BRCA2* mutation.

In an evaluation of the R72P polymorphism as a modifier of breast cancer penetrance in women with germline *BRCA1* or *BRCA2* mutations, we found that in the combined *BRCA1* and *BRCA2* mutation carrier analysis, the presence of any 72P allele was associated with an earlier median age of breast cancer diagnosis (median age=32, interquartile range (IQR) 30-38) compared with the homozygous R72 genotype (median age=42, IQR 35-51, $p<0.01$) (table 6). This association was limited to *BRCA1* mutation carriers (median age=32, IQR 30-41.5, $p<0.05$) and was not seen in *BRCA2* mutation carriers (median age=35, IQR 30-43, $p<0.39$) (table 6).

DISCUSSION

In this study, we screened all 10 coding exons of *TP53* in women with a personal history of breast cancer with or without a personal or family history of multiple primary cancers. These women previously had been characterised for *BRCA1* and *BRCA2* mutations.[30] We identified one potential deleterious missense mutation (G245S) in a member of a family with a history of site specific breast cancer only. This patient did not carry a germline mutation in either *BRCA1* or *BRCA2*. The G245S missense mutation has been reported previously in the germline of a woman with breast cancer[23] and the germline of a man with sarcoma.[33] Our proband was diagnosed with breast cancer at the age of 29. In addition, her sister was diagnosed with breast cancer at the age of 27 and went on to develop a second primary breast cancer at 31 years of age (fig 1). However, the G245S mutation was not detected in DNA from the sister's first breast tumour. In addition, there was no allelic loss of flanking *TP53* in that tumour (data not shown). Thus it is possible that either the proband's sister is a phenocopy or the *TP53* mutation is not the relevant source of breast cancer susceptibility in this family, but this would need to be confirmed by testing the germline DNA, which was unavailable. Thus, we conclude that germline *TP53* mutations are not an important cause of multiple primary cancers outside the setting of LFS.

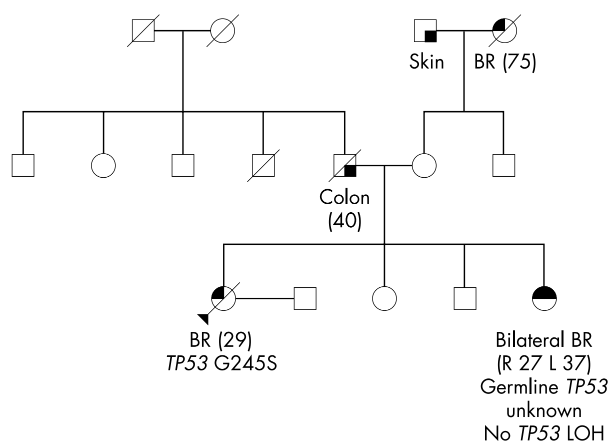

Figure 1 Pedigree of a patient with germline *TP53* mutation. Numbers in parentheses indicate age of cancer diagnosis.

In this sample set the 72P allele was found at a frequency of 41%, somewhat higher than the previously reported 26-35%.[11–13] Nonetheless, we observed a six-fold higher frequency of the homozygous R72 genotype among MPC women with *BRCA1* or *BRCA2* mutations compared to BC women with a *BRCA1* or *BRCA2* mutation. These data suggest that women who are homozygous for the R72 allele and have a mutation in *BRCA1* or *BRCA2* may be at increased risk for developing multiple primary cancers. Although contrary to the study by Brose *et al*,[34] who showed that *BRCA1* mutations do not confer an increased risk of most additional primary cancers, nonetheless this present study did not explore the combined association of both a *BRCA1* mutation and the *TP53* R72P polymorphism and its potential role as a modifier of *BRCA1* associated breast cancer risk.

Other studies associating the homozygous R72 allele and increased cancer risk have been reported. In the first such example, the association between *TP53* polymorphisms and human papillomavirus (HPV) associated cervical cancer was examined, suggesting that women who were homozygous for the R72 allele were seven times more susceptible to HPV related cervical cancer than with at least one 72P allele.[35] However, these data have been difficult to replicate and an equal number of studies have either confirmed[36–38] or disputed[39–43] the R72 association with cervical cancer.

In the subset analysis of the R72P polymorphism as a candidate modifier of breast cancer penetrance in *BRCA1/2* mutation carriers, we observed that the presence of a 72P allele was associated with an earlier age of breast cancer diagnosis among women with a *BRCA1* mutation. One possible explanation for the association of 72P with earlier onset breast cancer in *BRCA1* mutation carriers and R72 with MPC would be excess or earlier mortality among women with an earlier age of diagnosis of breast cancer (that is, those with the 72P). Thus if women homozygous for R72 may live longer, they may have a greater likelihood of developing a second cancer.

BRCA1 physically interacts with p53 in vitro and both BRCA1 and BRCA2 physically interact with p53 in vivo resulting in enhanced p53 mediated transcription.[44–46] There are two p53 binding sites in BRCA1; one is close to the nuclear localisation signal in the N-terminal region of exon 11[45] and one is in the most C-terminal BRCT domain.[47] Deletion of the N-terminal exon 11 p53 binding site prevents in vitro interaction of the two proteins and abrogates the coactivation effect of BRCA1 on p53 responsive promoters such as bax, p21, and GADD45.[45, 48] In addition, a truncation mutant of BRCA1 that retains the p53 interacting site but removes the C-terminal BRCA1 transactivation domain acts as a dominant inhibitor of p53 dependent transcription.[45] Finally, *TP53* mutations are more common in *BRCA1* associated breast cancers than sporadic or *BRCA2* associated tumours. Somatic *TP53* mutations have been reported in as many as 80% of *BRCA1* associated tumours,[49, 50] leading to the speculation that *TP53* mutations, or another component of the relevant pathway, maybe required before *BRCA1* related tumorigenesis can proceed.[51] Recent data from murine models strongly support this hypothesis.[52]

Our data provide additional support for a critical role of the p53/BRCA1 interaction in tumorigenesis, suggesting an association between *TP53* variants and cancer risk in women with *BRCA1* mutations. Thus, it is possible that the R72P polymorphism in *TP53* subtly alters the p53/BRCA1 interaction and in turn alters BRCA1 associated tumorigenesis.

In summary, we provide evidence that germline mutations in *TP53* are rarely associated with the presence of multiple

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primary cancers in breast cancer families and support previous studies suggesting that *TP53* mutations account for less than 1% of hereditary susceptibility to breast cancer. However, we found presence of the homozygous R72 allele was associated with a six-fold increased risk for the development of multiple primary cancers among subjects with a germline *BRCA1* or *BRCA2* mutation. Finally, we provide preliminary evidence that the arginine allele of R72P in exon 4 of *TP53* may modify *BRCA1* associated breast cancer risk, using age of diagnosis as a surrogate for penetrance.

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