Breast Cancer Patients with p53 Pro72 Homozygous Genotype Have a Poorer Survival

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Abstract

Purpose: The p53 R72P polymorphism has been suggested to play a role in many cancers, including breast cancer. Our aim was to evaluate association of R72P with breast cancer risk as well as histopathologic features of the breast tumors and survival.

Experimental Design: The germ line R72P genotype was defined among 939 Finnish familial and 888 unselected breast cancer patients and 736 healthy population controls. The clinical and biological variables were tested for association by univariate analysis and the effects of several variables on survival by Cox's proportional hazards regression model.

Results: The distribution of the genotypes was similar in all groups studied, suggesting no association with breast cancer risk. Unselected breast cancer patients with 72P homozygous genotype presented significantly more often with lobular carcinoma, whereas R72 allele carriers had a significantly higher frequency of ductal carcinomas ($P = 0.004$). No significant association with other histopathologic variables, like tumor grade, hormone receptor status (estrogen and progesterone receptors), or tumor-node-metastasis stage, was observed. Survival analysis showed that unselected breast cancer patients with 72P homozygous genotype had significantly poorer survival than patients with other genotypes ($P = 0.003$). This effect on survival was independent of p53 expression in the tumors and multivariate analysis showed that 72P homozygous genotype was overall an independent prognostic factor (risk ratio of death, 2.1; 95% confidence interval, 1.4-3.3; $P = 0.001$).

Conclusions: These results suggest no effect of either R72P allele on breast cancer risk but a significantly reduced survival for 72P homozygous breast cancer patients. The finding of codon 72 genotype as an independent prognostic marker for breast cancer warrants further studies.

Common polymorphism in the tumor suppressor gene *TP53*, 215G > C in exon 4, has been suggested to play a role in several different cancer types. This base change results in an arginine-to-proline change in the protein sequence. These two variant protein forms may behave differently, as R72 variant has been shown to be a stronger and faster inducer of apoptosis than the 72P variant (1, 2). At least one mechanism underlying this greater efficiency at inducing apoptosis may be the enhanced localization of R72 variant to the mitochondria (3). The R72 variant is also more susceptible than the 72P variant to degradation induced by human papillomavirus E6 protein, which may result in an increased susceptibility to human papillomavirus–induced tumors in homozygous R72 individuals (4). Some studies report an overrepresentation of the R72 variant in cervical cancers compared with control samples (5, 6), whereas a larger meta-analysis study did not find such an association (7).

The R72 allele has been reported also to associate with increased risk of bladder and gastric cancer (8, 9). The *72P* allele, on the other hand, has been associated with incidence of squamous cell carcinoma of the head and neck (10), thyroid cancer (11), lung cancer (12, 13), and prostate cancer (14). The 72P allele has also been suggested to play a role in colorectal cancer pathogenesis: HNPCC patients with *MSH2* or *MLH1* germ line mutations and carrying 72P were found to develop colorectal cancer at a younger age than R72 homozygotes (15), whereas another study reported poorer survival among colorectal cancer patients with the 72P allele (16).

The R72 allele has been suggested as a candidate for a low-penetrance breast cancer susceptibility allele (17, 18). However, also 72P homozygosity has been associated with increased breast cancer risk (19). Studies that argue against the contribution

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Received 1/24/05; revised 3/24/05; accepted 4/25/05.

Grant support: The Academy of Finland, Finnish Cancer Society, Helsinki University Central Hospital Research Fund, and the Sigrid Juselius Fund.

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of the R72P polymorphism in breast cancer predisposition have also been reported (20). Association of R72 homozygous genotype with multiple cancers, especially in *BRCA1/2* families, has also been suggested (21).

To evaluate the effect of the p53 R72P polymorphism on breast cancer risk, bilateral breast cancer, multiple cancers, or the age of breast cancer diagnosis, we determined the frequency of the R72P polymorphism in an extensive analysis of DNA samples from 939 familial breast cancer patients, 888 unselected breast cancer patients, and 736 healthy population controls. In addition, *BRCA1* and *BRCA2* mutation carriers were studied to investigate whether this polymorphism modifies cancer risk in *BRCA1* or *BRCA2* mutation carriers. We also investigated the association of this polymorphism with the histopathologic features of the breast tumors, as well as survival among unselected breast cancer patients.

Materials and Methods

The germ line p53 codon 72 genotype was defined among Finnish familial and unselected breast cancer patients and healthy population controls. The series of 888 Finnish unselected breast cancer patients includes 626 consecutive newly diagnosed breast cancer patients collected in 1997 to 1998 at the Helsinki University Central Hospital and covers 87% of all breast cancer patients treated at the Department of Oncology during the collection period (described in detail in ref. 22). Additionally, the codon 72 genotype was determined in DNA samples collected from 262 consecutive newly diagnosed breast cancer patients at the Department of Oncology, Helsinki University Central Hospital in 2000. This cohort covers 65% of all breast cancer patients treated at the Department at that time period (23).

The familial series studied includes 939 familial breast cancer patients collected at the Helsinki University Central Hospital Departments of Oncology, Clinical Genetics, and Surgery (as described in ref. 24). This series includes 407 breast cancer patients with a stronger family history (three or more first- or second-degree relatives with breast or ovarian cancer in the family, including the proband), as verified through the Finnish Cancer Registry and Hospital records, and 532 unrelated breast cancer cases reporting only a single affected first-degree relative. Among these 939 familial patients, 804 have a family history of breast cancer only and 135 patients have a relative affected with ovarian cancer. For 627 of these familial patients, *BRCA1* and *BRCA2* mutations had been excluded as previously described (25, 26) and for 312 the *BRCA1/2* mutation status was unknown. Four patients known to carry a p53 germ line mutation were excluded from the analyses. The R72P was further evaluated also in 49 *BRCA1* and 48 *BRCA2* mutation carriers affected with breast cancer. Allele and genotype frequencies in the normal population were determined in 736 healthy population controls from the Finnish Red Cross Blood Transfusion Service.

Pathologic data were collected from pathology reports for all the primary breast tumors available among the 888 unselected breast cancer patients. Altogether, 46 bilateral breast cancer cases have been diagnosed among these 888 patients with histopathologic data available for a total of 932 tumors (932 of 934, 99.8% of all). The data set in this study includes information on tumor histology, grade, estrogen and progesterone receptor status, p53 immunohistochemical expression, tumor diameter, nodal status, and distant metastases. Grading was done according to Scarff-Bloom-Richardson modified by Elston and Ellis (27). In addition, p53 protein expression on 654 unselected breast tumors was studied by immunohistochemical staining of tumor tissue microarrays. The most representative area of the tumors was punched to produce breast cancer tissue microarrays including four cores (diameter, 0.6 mm) from each of the original blocks. The tissue microarray slides were stained with a mouse monoclonal anti-human p53-antibody (DAKO, Glostrup, Denmark) in a dilution of 1:300. Briefly, 5-μm sections were cut from paraffin-embedded blocks, deparaffinated in xylene, and dehydrated in a series of graded alcohols. The sections were pretreated in a microwave oven and incubated with antibody overnight. Samples were considered positive when 20% of the cancer cells were positive for the p53 staining. The data set for the unselected patients collected from 1997 to 1998 also includes the age at the time of (first) breast cancer diagnosis and survival (in months). The duration of follow-up ranged from 2.9 to 95.4 months (median, 80.0; mean, 73.3; SD, 18.4). Age at the time at diagnosis ranged from 22.3 to 95.6 years (median, 55.3; mean, 56.3; SD, 12.8).

The study was done with informed consents from the patients and permissions from the Ethics Committees of the Departments of Oncology and Obstetrics and Gynecology, as well as from the Ministry of Social Affairs and Health in Finland.

Genotyping. The genotyping of DNA samples from the different patient series and population controls was done using Amplifluor fluorescent genotyping (K-Biosciences, Cambridge, United Kingdom, http://www.kbioscience.co.uk). The genotyping was successful in 96.6% of unselected samples, 98.2% of familial samples, and 99.6% of the healthy population controls. The samples of the unselected cohort collected from 1997 to 1998 that failed to give a clear allele ratio in the first analysis were validated by reamplification and direct sequencing using BigDye Terminator Cycle Sequencing Kit and ABI 310 automated sequencer (Applied Biosystems, Foster City, CA).

Statistical analysis. The clinical and biological variables were tested for association by univariate analysis. Independent variables were compared with the χ^2 test. Univariate analyses of survival were done by calculating Kaplan-Meier survival curves and comparing subsets of patients using log-rank test. To explore the effects of several variables on survival, Cox's proportional hazards regression model was used. All P values are two sided, and due to multiple comparisons, $P < 0.01$ was considered significant. The data were analyzed using SPSS v12.0.1 for Windows (SPSS, Inc., Chicago, IL).

Results

The genotype frequencies in the unselected and familial breast cancer patient series and population controls are shown in Table 1. The familial breast cancer patients were further divided in subgroups by strength of family history and inclusion of ovarian cancer. The distribution of the genotypes was closely similar in all the groups studied, with no deviation from the Hardy-Weinberg equilibrium among the 733 population controls or among the total of 1,551 breast cancer patients studied ($P = 0.91$), or among any subgroups. The mean age at diagnosis for the RR homozygotes was 56.5 years, for RP heterozygotes was 56.6 years, and for PP homozygotes was 56.8 years among the unselected patients, and 54.5, 54.9, and 56.4 among the familial patients, respectively. No association of any of the genotypes with bilateral breast cancer or multiple cancers (breast cancer and at least one other non-breast cancer) was seen (Table 2).

Among the *BRCA1* and *BRCA2* carriers, the mean age at diagnosis for *BRCA1* carriers with RR genotype was 42.6 years, for RP heterozygotes was 44.9 years, and for PP homozygotes was 48.7, and for patients carrying a P allele (RP or PP; $n = 24$) 45.8 years and for RR homozygotes ($n = 25$) 42.6 years. The mean ages at diagnosis for *BRCA2* carriers were 50.1, 42.9, and 45.1, respectively. *BRCA2* carrier patients with a P allele ($n = 23$) tended to be diagnosed at a younger age than RR homozygotes ($n = 25$; mean age at diagnosis, 43.3 and 50.1 years, respectively; $P = 0.03$, t test for equality of means). No association of any genotype with bilateral breast cancer or multiple cancers was seen (Table 2).

Table 1. R72P genotype frequencies among population controls and unselected and familial breast cancer patients by family history

Study subjects	Total	RR (%)	RP (%)	PP (%)
Population controls	733	403 (55.0)	278 (37.9)	52 (7.1)
All breast cancer patients	1551	825 (53.2)	617 (39.8)	109 (7.0)
Unselected breast cancer patients	858	459 (53.5)	336 (39.2)	63 (7.3)
Familial breast cancer patients*	923	478 (51.8)	385 (41.7)	60 (6.5)
Breast cancer only	793	401 (50.6)	337 (42.5)	55 (6.9)
Including also ovarian cancer	130	77 (59.2)	48 (36.9)	5 (3.8)
Index with only one affected first-degree relative	526	263 (50.0)	231 (43.9)	32 (6.1)
Three or more affected in the family	397	215 (54.2)	154 (38.8)	28 (7.1)

*Familial patients (n = 230) also belong to the cohort of unselected breast cancer patients (these cases were included only once in the combined analysis of all breast cancer patients).

Histopathologic features among the unselected breast cancer patients are shown in Table 3. Analysis of the histopathologic features of the breast tumors from patients carrying different codon 72 genotypes shows that the different alleles are associated with specific histologic features of the tumors. Tumor histology of the PP homozygotes is significantly more often lobular than tumor histology of the other genotypes; the tumors of the RR homozygotes and heterozygotes have more often ductal histology (P = 0.004; Table 3). Carriers of the R allele also tended to have more often grade 3 tumors than PP homozygotes, whereas PP homozygotes had more frequently grade 1 tumors (P = 0.029). No association with hormone receptor status (estrogen and progesterone receptors) or tumor-node-metastasis stage was observed (Table 3). The codon 72 genotype does not correlate with p53 expression as evaluated by immunohistochemistry. All these results were similar also among patients diagnosed below or at or over 50 years of age.

Kaplan-Meier survival analysis showed that unselected breast cancer patients with PP homozygous genotype had poorer survival than patients with other genotypes (cumulative survival at 80 months follow-up, 74% and 88%, respectively; P = 0.003, log-rank test; n = 621; Fig. 1). This was more pronounced when only patients with p53-negative tumors were compared (P = 0.001, n = 356). For comparison, cumulative survival at 80 months follow-up was 74% among patients with p53-positive tumors and 92% among patients with p53-negative tumors (P < 0.001, n = 457; Fig. 1).

Multivariate analysis by Cox's proportional hazards regression model indicated tumor diameter, lymph node status, progesterone receptor expression, p53 expression, and PP

Table 2. Multiple cancer and bilateral breast cancer among unselected and familial breast cancer patients by R72P genotype

	Total (%)	RR (%)	RP (%)	PP (%)
Unselected breast cancer patients	858	459	336	63
Bilateral	54 (6.3)	26 (5.7)	23 (6.8)	5 (7.9)
Unilateral	804 (93.7)	433 (94.3)	313 (93.2)	58 (92.1)
Multiple cancer	86 (10.0)	48 (10.5)	34 (10.1)	4 (6.3)
Breast cancer only	772 (90.0)	411 (89.5)	302 (89.9)	59 (93.7)
Familial breast cancer patients	923	478	385	60
Bilateral	98 (10.6)	51 (10.7)	43 (11.2)	4 (6.7)
Unilateral	825 (89.4)	427 (89.3)	342 (88.8)	56 (93.3)
Multiple cancer	108 (11.7)	50 (10.5)	50 (13.0)	8 (13.3)
Breast cancer only	815 (88.3)	428 (89.5)	335 (87.0)	52 (86.7)
BRCA1 carrier patients	49	25	18	6
Bilateral	7 (14.3)	4 (16.0)	3 (16.7)	0 (0.0)
Unilateral	42 (85.7)	21 (84.0)	15 (83.3)	6 (100.0)
Multiple cancer	12 (24.5)	7 (28.0)	5 (27.8)	0 (0.0)
Breast cancer only	37 (75.5)	18 (72.0)	13 (72.2)	6 (100.0)
BRCA2 carrier patients	48	25	19	4
Bilateral	10 (20.8)	4 (16.0)	6 (31.6)	0 (0.0)
Unilateral	38 (79.2)	21 (84.0)	13 (68.4)	4 (100.0)
Multiple cancer	11 (22.9)	3 (12.0)	6 (31.6)	2 (50.0)
Breast cancer only	37 (77.1)	22 (88.0)	13 (68.4)	2 (50.0)

Table 3. Histopathologic characteristics of unselected breast tumors in R72P carriers

	Total (%)	RR (%)	RP (%)	PP (%)	*P*
Tumor histology (*n* = 852)					
Ductal carcinoma	664 (77.9)	361 (79.0)	263 (79.9)	40 (60.6)	0.004
Lobular carcinoma	138 (16.2)	65 (14.2)	55 (16.7)	18 (27.3)	0.004
Medullary carcinoma	13 (1.5)	8 (1.8)	3 (0.9)	2 (3.0)	NS
Other	37 (4.3)	23 (5.0)	8 (2.4)	6 (9.1)	NS
Grade (*n* = 809)					
1	221 (27.3)	110 (25.2)	88 (28.4)	23 (37.1)	0.029
2	349 (43.1)	198 (45.3)	124 (40.0)	27 (43.5)	NS
3	239 (29.5)	129 (29.5)	98 (31.6)	12 (19.4)	0.029
T (*n* = 878)					
1	536 (61.0)	283 (60.0)	210 (61.0)	43 (64.2)	NS
2	281 (32.0)	151 (32.3)	114 (33.1)	16 (23.9)	NS
3	30 (3.4)	18 (3.9)	9 (2.6)	3 (4.5)	NS
4	31 (3.5)	15 (3.2)	11 (3.2)	5 (7.5)	NS
1 + 2	817 (93.0)	434 (92.3)	324 (94.1)	59 (88.1)	NS
3 + 4	61 (6.9)	33 (7.1)	20 (5.8)	8 (12)	NS
N (*n* = 868)					
0	470 (54.1)	246 (52.7)	185 (55.1)	39 (60.0)	NS
1	379 (43.7)	210 (45.0)	145 (43.2)	24 (36.9)	NS
2	18 (2.1)	11 (2.4)	5 (1.5)	2 (3.1)	NS
3	1 (0.1)	0 (0.0)	1 (0.3)	0 (0.0)	NS
0 (negative)	470 (54.1)	246 (52.7)	185 (55.1)	39 (60.0)	NS
1 + 2 + 3 (positive)	398 (45.9)	221 (47.4)	151 (45.0)	26 (40.0)	NS
M (*n* = 862)					
Negative	823 (95.5)	441 (95.2)	321 (96.1)	61 (93.8)	NS
Positive	39 (4.5)	22 (4.8)	13 (3.9)	4 (6.2)	NS
ER status (*n* = 852)					
Positive	671 (78.8)	347 (76.9)	276 (81.7)	48 (76.2)	NS
Negative	181 (21.2)	104 (23.1)	62 (18.3)	15 (23.8)	NS
PR status (*n* = 853)					
Positive	580 (68.0)	293 (65.0)	244 (72.0)	43 (68.3)	NS
Negative	273 (32.0)	158 (35.0)	95 (28.0)	20 (31.7)	NS
p53 IHC (*n* = 650)					
Positive	131 (20.2)	70 (20.2)	53 (20.8)	8 (16.7)	NS
Negative	519 (79.8)	277 (79.8)	202 (79.2)	40 (83.3)	NS

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry; NS, not significant.

homozygous genotype as independent prognostic factors among the breast cancer patients with information of these variables available (n = 373; Table 4).

Discussion

Many studies have addressed association of p53 R72P polymorphism with increased risk for breast and other cancers. Most studies on breast cancer have included relatively small sample sets and yielded inconsistent, even contradictory results. We aimed to evaluate whether the R72P polymorphism associates with increased risk for breast cancer among extensive sets of 923 familial and 858 unselected breast cancer patients and 733 population controls. The genotype frequencies among all patient series, as well as in subgroups defined by different family history (including the 230 familial patients belonging also to the unselected series), bilateral breast cancer or multiple cancers, were closely similar. Similarly, no difference in the age at diagnosis was seen by R72P genotype. The results indicate that the p53 R72P genotypes are not associated with increased breast cancer risk among unselected or familial breast cancer patients.

We further evaluated R72P genotype frequencies and possible modifying effect on breast cancer risk among *BRCA1* and *BRCA2* mutation carriers. *BRCA2* carrier patients carrying a 72P allele (72P homozygotes and heterozygotes) tended to be diagnosed younger than the R72 homozygotes; Martin et al. (21) found a similar trend among *BRCA2* mutation carriers, but the difference was not significant in either study. They also found that presence of a 72P allele was associated with an earlier age of breast cancer diagnosis among *BRCA1* mutation carriers, which was not the case in our material. Association of R72 homozygous genotype with multiple primary cancers or family history of multiple primary cancers among *BRCA1/2* mutation

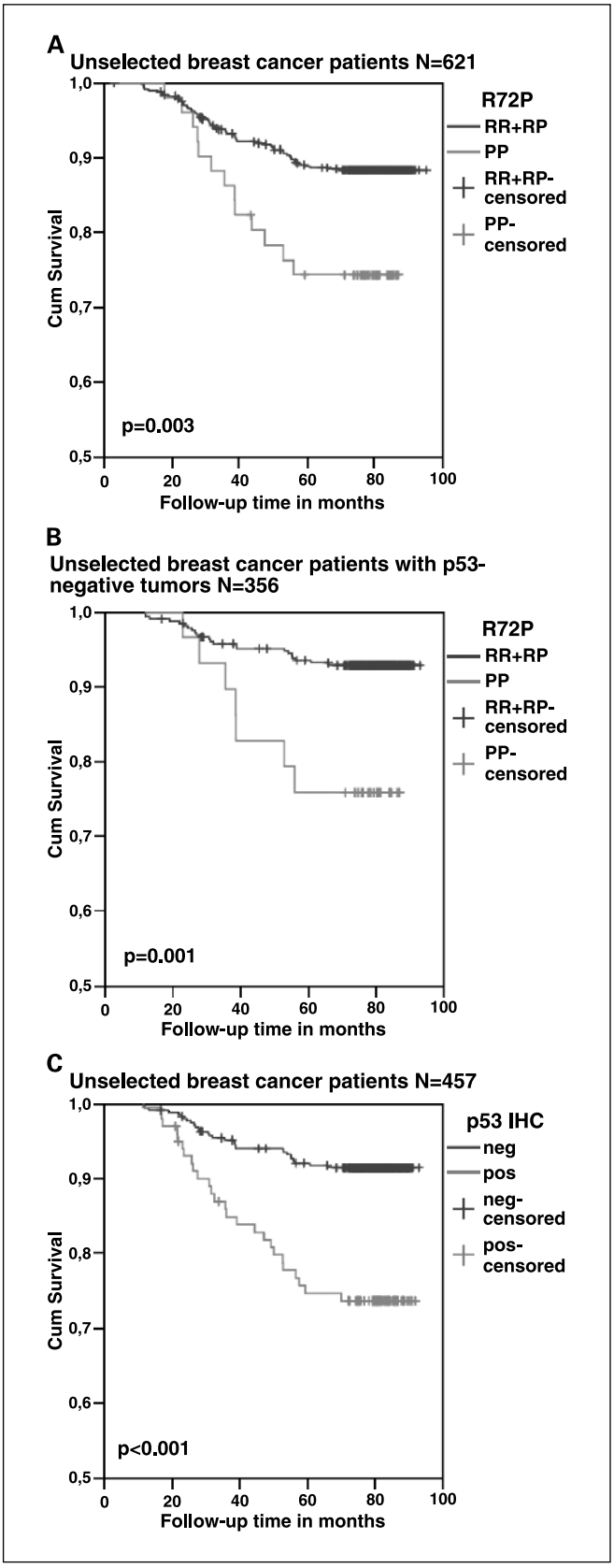

Fig. 1. Kaplan-Meier survival curves of unselected breast cancer patients. *A,* survival among all patients, by R72P genotype (PP homozygotes versus other genotypes). *B,* survival among patients with p53 immunonegative tumors, by R72P genotype. *C,* for comparison, survival among all patients, by p53 expression.

carrier women was also suggested (21), but no association of any of the genotypes with multiple cancers was seen in this study. As the numbers of *BRCA1/2* carrier patients in either study were quite small, larger studies will be needed to evaluate possible modifying effect of R72P genotype on cancer risk among *BRCA1* and *BRCA2* carriers.

Interestingly, analysis of histopathologic features of breast tumors and survival among unselected breast cancer patients revealed association of the genotypes with different histologic types of tumors and differential survival. The 72P homozygous breast cancer patients presented significantly more often with lobular carcinoma than patients carrying an R72 allele and had often grade 1 tumors, whereas R72 allele carriers had more frequently ductal carcinomas and often grade 3 tumors. Association of 72P homozygosity with lower grade is consistent with a higher frequency of lobular carcinomas among 72P homozygous patients as lobular carcinomas have been found to be more often of lower grade (28). However, no difference in survival has been found between patients with lobular and ductal infiltrating carcinomas (28), whereas p53 72P homozygotes were found here to have a significantly poorer survival than R72 homozygotes or heterozygotes ($P = 0.003$). Furthermore, this effect was even more pronounced when only patients with negative immunostaining for p53 on the tumors were included in the analysis ($P = 0.001$). Survival among 72P homozygotes with negative immunostaining for p53 was similar as survival among all patients with positive immunostaining for p53 on the tumors, with 74% cumulative survival at 80 months follow-up (as shown for comparison in Fig. 1). Multivariate analysis showed that 72P homozygous genotype was overall an independent prognostic factor, with a 2-fold increased risk of death. As 8.2% of all patients were homozygous for the 72P allele (8.1% among p53-negative

Table 4. Multivariable analysis (Cox's proportional hazards model) of prognostic factors ($N = 373$)

Variables	Risk ratio (95% CI)	*P*
Age at diagnosis	1.0 (1.0-1.0)	0.5
T		
2 versus 1	1.1 (0.5-2.3)	0.8
3 versus 1	2.7 (0.7-10.0)	0.1
4 versus 1	8.5 (2.5-29.2)	0.001
N		
1 versus 0	6.5 (2.7-16.0)	<0.001
2 versus 0	21.3 (4.7-97.1)	<0.001
M (positive versus negative)	4.9 (1.8-13.2)	0.002
Grade		
2 versus 1	3.1 (0.4-24.6)	0.3
3 versus 1	8.8 (1.1-72.4)	0.04
ER status (positive versus negative)	1.9 (0.7-5.2)	0.2
PR status (positive versus negative)	0.2 (0.1-0.5)	<0.001
p53 IHC (positive versus negative)	3.4 (1.7-6.9)	0.001
R72P (PP versus RP and RR)	2.1 (1.4-3.3)	0.001

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry.

cases), codon 72 genotype could be a useful additional prognostic factor among this subgroup of patients. The effect of 72P homozygous genotype on survival was similar both among patients with ductal and lobular carcinoma (data not shown).

The independent effect of 72P homozygous genotype on survival is also supported by findings that breast tumors of 72P homozygotes have a lower frequency of somatic p53 mutations than tumors of R72 homozygotes and heterozygotes (29, 30). These data suggest that the p53 Pro/Pro is functionally impaired per se, independently of somatic p53 mutations. Our finding is also consistent with the R72 variant of wild-type p53 being a more potent inducer of apoptosis than the wild-type 72P variant. It has been suggested that R72 homozygotes may respond more favorably to radiation or chemotherapy (3), and the superior activity of wild-type R72 in inducing apoptosis is reflected *in vivo* in more favorable outcome in patients whose cancers express the wild-type R72 variant, compared with those with the wild-type 72P, and receiving chemoradiotherapy for advanced squamous carcinomas of head and neck (31). These favorable effects of R72 allele may, however, be reversed by a somatic p53 mutation on this allele (31–33) and retention of the R72 allele with loss of the 72P allele in the tumor tissue has been associated with reduced survival in heterozygous breast cancer patients (34). In one breast cancer study, the 72P allele has been suggested to have a protective effect against death, with borderline significance, but this effect was reduced by inclusion of known prognostic variables in the analysis (35). The effect of the 72P allele on poorer survival is supported, however, also by poorer survival of 72P allele carriers reported among colorectal cancer patients (16).

In conclusion, these results suggest no effect of either allele on familial breast cancer risk or breast cancer risk in the population but an association with histopathologic features of the tumors and survival of the patients. The results present important novel findings also with clinical significance for the p53 codon 72 variant carriers. The finding of codon 72 genotype as an independent prognostic marker for breast cancer warrants further studies.

Acknowledgments

We thank the patients participating in this study, Nina Puolakka for her help in sample and data collection, and the Finnish Cancer Registry for cancer data.

References

1. Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G. Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol 1999;19:1092–100.
2. Pim D, Banks L. p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. Int J Cancer 2004;108:196–9.
3. Dumont P, Leu JI, Della Pietra AC III, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 2003;33:357–65.
4. Storey A, Thomas M, Kalita A, et al. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature 1998;393:229–34.
5. Zehbe I, Voglino G, Wilander E, Genta F, Tommasino M. Codon 72 polymorphism of p53 and its association with cervical cancer. Lancet 1999;354:218–9.
6. Zehbe I, Voglino G, Wilander E, et al. p53 codon 72 polymorphism and various human papillomavirus 16 E6 genotypes are risk factors for cervical cancer development. Cancer Res 2001;61:608–11.
7. Koushik A, Platt RW, Franco EL. p53 codon 72 polymorphism and cervical neoplasia: a meta-analysis review. Cancer Epidemiol Biomarkers Prev 2004;13:11–22.
8. Soulitzis N, Sourvinos G, Dokianakis DN, Spandidos DA. p53 codon 72 polymorphism and its association with bladder cancer. Cancer Lett 2002;179:175–83.
9. Shen H, Solari A, Wang X, et al. P53 codon 72 polymorphism and risk of gastric cancer in a Chinese population. Oncol Rep 2004;11:1115–20.
10. Shen H, Zheng Y, Sturgis EM, Spitz MR, Wei Q. P53 codon 72 polymorphism and risk of squamous cell carcinoma of the head and neck: a case-control study. Cancer Lett 2002;183:123–30.
11. Granja F, Morari J, Morari EC, Correa LA, Assumpcao LV, Ward LS. Proline homozygosity in codon 72 of p53 is a factor of susceptibility for thyroid cancer. Cancer Lett 2004;210:151–7.
12. Wang YC, Chen CY, Chen SK, Chang YY, Lin P. p53 codon 72 polymorphism in Taiwanese lung cancer patients: association with lung cancer susceptibility and prognosis. Clin Cancer Res 1999;5:129–34.
13. Fan R, Wu MT, Miller D, et al. The p53 codon 72 polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2000;9:1037–42.
14. Suzuki K, Matsui H, Ohtake N, et al. A p53 codon 72 polymorphism associated with prostate cancer development and progression in Japanese. J Biomed Sci 2003;10:430–5.
15. Jones JS, Chi X, Gu X, Lynch PM, Amos CI, Frazier ML. p53 polymorphism and age of onset of hereditary nonpolyposis colorectal cancer in a Caucasian population. Clin Cancer Res 2004;10:5845–9.
16. Starinsky S, Figer A, Ben-Asher E, et al. Genotype phenotype correlations in Israeli colorectal cancer patients. Int J Cancer 2004;114:58–73.
17. Papadakis EN, Dokianakis DN, Spandidos DA. p53 codon 72 polymorphism as a risk factor in the development of breast cancer. Mol Cell Biol Res Commun 2000;3:389–92.
18. Buyru N, Tigli H, Dalay N. P53 codon 72 polymorphism in breast cancer. Oncol Rep 2003;10:711–4.
19. Huang XE, Hamajima N, Katsuda N, et al. Association of p53 codon Arg^{72}Pro and p73 G4C14-to-A4T14 at exon 2 genetic polymorphisms with the risk of Japanese breast cancer. Breast Cancer 2003;10:307–11.
20. Suspitsin EN, Buslov KG, Grigoriev MY, et al. Evidence against involvement of p53 polymorphism in breast cancer predisposition. Int J Cancer 2003;103:431–3.
21. Martin AM, Kanetsky PA, Amirimani B, et al. Germline *TP53* mutations in breast cancer families with multiple primary cancers: is *TP53* a modifier of *BRCA1*? J Med Genet 2003;40:e34.
22. Syrjakoski K, Vahteristo P, Eerola H, et al. Population-based study of *BRCA1* and *BRCA2* mutations in 1035 unselected Finnish breast cancer patients. J Natl Cancer Inst 2000;92:1529–31.
23. Kilpivaara O, Bartkova J, Eerola H, et al. Correlation of CHEK2 protein expression and c.1100delC mutation status with tumor characteristics among unselected breast cancer patients. Int J Cancer 2005;113:575–80.
24. Eerola H, Blomqvist C, Pukkala E, Pyrhonen S, Nevanlinna H. Familial breast cancer in Southern Finland: how prevalent are breast cancer families and can we trust the family history reported by the patients? Eur J Cancer 2000;36:1143–8.
25. Vehmanen P, Friedman L, Eerola H, et al. Low proportion of *BRCA1* and *BRCA2* mutations in Finnish breast cancer families: strong evidence for additional susceptibility genes. Hum Mol Genet 1997;6:2309–15.
26. Vahteristo P, Eerola H, Tamminen A, Blomqvist C, Nevanlinna H. A probability model for predicting *BRCA1* and *BRCA2* mutations in breast and breast-ovarian cancer families. Br J Cancer 2001;84:704–8.
27. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991;19:403–10.
28. Molland JG, Donnellan M, Janu NC, Carmalt HL, Kennedy CW, Gillett DJ. Infiltrating lobular carcinoma: a comparison of diagnosis, management and outcome with infiltrating duct carcinoma. Breast 2004;13:389–96.
29. Langerod A, Bukholm IR, Bregard A, et al. The TP53 codon 72 polymorphism may affect the function of TP53 mutations in breast carcinomas but not in colorectal carcinomas. Cancer Epidemiol Biomarkers Prev 2002;11:1684–8.
30. Noma C, Miyoshi Y, Taguchi T, Tamaki Y, Noguchi S. Association of p53 genetic polymorphism (Arg^{72}Pro) with estrogen receptor positive breast cancer risk in Japanese women. Cancer Lett 2004;210:197–203.
31. Sullivan A, Syed N, Gasco M, et al. Polymorphism in wild-type p53 modulates response to chemotherapy *in vitro* and *in vivo*. Oncogene 2004;23:3328–37.
32. Bergamaschi D, Gasco M, Hiller L, et al. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell 2003;3:387–402.
33. Schneider-Stock R, Mawrin C, Motsch C, et al. Retention of the arginine allele in codon 72 of the p53 gene correlates with poor apoptosis in head and neck cancer. Am J Pathol 2004;164:1233–41.
34. Bonafe M, Ceccarelli C, Farabegoli F, et al. Retention of the p53 codon 72 arginine allele is associated with a reduction of disease-free and overall survival in arginine/proline heterozygous breast cancer patients. Clin Cancer Res 2003;9:4860–4.
35. Goode EL, Dunning AM, Kuschel B, et al. Effect of germ-line genetic variation on breast cancer survival in a population-based study. Cancer Res 2002;62:3052–7.