Research article

Combined effects of single nucleotide polymorphisms TP53 R72P and MDM2 SNP309, and p53 expression on survival of breast cancer patients

Marjanka K Schmidt¹, Johanna Tommiska², Annegien Broeks¹, Flora E van Leeuwen¹, Laura J Van't Veer¹, Paul DP Pharoah³, Douglas F Easton³, Manjeet Humphreys³, Thilo Dörk⁴, Scarlett A Reincke⁴, Rainer Fagerholm², Carl Blomqvist⁵ and Heli Nevanlinna²

¹Departments of Epidemiology, Experimental Therapy and Pathology, Netherlands Cancer Institute, The Netherlands, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
²Department of Obstetrics and Gynecology, Helsinki University Central Hospital (HUCH), Haartmaninkatu 8, 00290 Helsinki, Finland
³Strangeway's Research Laboratory, Worts Causeway, CB1 8RN Cambridge, UK
⁴Hannover Medical School, Departments of Gynaecology and Radiation Oncology, Carl-Neuberg-Straße 1, 30625 Groß-Buchholz, Hannover, Germany
⁵Department of Oncology, Helsinki University Central Hospital (HUCH), Haartmaninkatu 8, 00290 Helsinki, Finland

Corresponding author: Heli Nevanlinna, Heli.Nevanlinna@hus.fi

Received: 7 Jul 2009 Revisions requested: 3 Sep 2009 Revisions received: 9 Dec 2009 Accepted: 18 Dec 2009 Published: 18 Dec 2009

Breast Cancer Research 2009, 11:R89 (doi:10.1186/bcr2460)
This article is online at: http://breast-cancer-research.com/content/11/6/R89
© 2009 Schmidt et al.; licensee BioMed Central Ltd.
This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Introduction Somatic inactivation of the TP53 gene in breast tumors is a marker for poor outcome, and breast cancer outcome might also be affected by germ-line variation in the TP53 gene or its regulators. We investigated the effects of the germ-line single nucleotide polymorphisms TP53 R72P (215G>C) and MDM2 SNP309 (-410T>G), and p53 protein expression in breast tumors on survival.

Methods We pooled data from four breast cancer cohorts within the Breast Cancer Association Consortium for which both TP53 R72P and MDM2 SNP309 were genotyped and follow-up was available (n = 3,749). Overall and breast cancer-specific survival analyses were performed using Kaplan-Meier analysis and multivariate Cox's proportional hazards regression models.

Results Survival of patients did not differ by carriership of either germ-line variant, R72P (215G>C) or SNP309 (-410T>G) alone. Immunohistochemical p53 staining of the tumor was available for two cohorts (n = 1,109 patients). Survival was worse in patients with p53-positive tumors (n = 301) compared to patients with p53-negative tumors (n = 808); breast cancer-specific survival: HR 1.6 (95% CI 1.2 to 2.1), P = 0.001. Within the patient group with p53-negative tumors, TP53 rare homozygous (CC) carriers had a worse survival than G-allele (GG/GC) carriers; actuarial breast cancer-specific survival 71% versus 80%, P = 0.07; HR 1.8 (1.1 to 3.1), P = 0.03. We also found a differential effect of combinations of the two germ-line variants on overall survival; homozygous carriers of the G-allele in MDM2 had worse survival only within the group of TP53 C-allele carriers; actuarial overall survival (GG versus TT/TG) 64% versus 75%, P = 0.01; HR (GG versus TT) 1.5 (1.1 to 2.0), P = 0.01. We found no evidence for a differential effect of MDM2 SNP309 by p53 protein expression on survival.

Conclusions The TP53 R72P variant may be an independent predictor for survival of patients with p53-negative tumors. The combined effect of TP53 R72P and MDM2 SNP309 on survival is in line with our a priori biologically-supported hypothesis, that is, the role of enhanced DNA repair function of the TP53 Pro-variant, combined with increased expression of the Mdm2 protein, and thus overall attenuation of the p53 pathway in the tumor cells.

ABCS: Amsterdam Breast Cancer Study; ER: estrogen receptor; HABCS: Hannover Breast Cancer Study; HEBCS: Helsinki Breast Cancer Study; HR: hazard ratio; SD: standard deviation; SEARCH: Studies of Epidemiology and Risk Factors in Cancer Heredity; TMA: tissue micro array.
Introduction
Breast cancer outcome may be affected by germ-line variants in genes that play a role in DNA damage control and repair such as TP53 (R72P) and MDM2 (SNP309) [1,2]. The Mdm2 protein is a negative regulator of the tumor suppressor protein p53 [3]. The R72P (215G>C) polymorphism of the TP53 gene is located in a proline-rich region of p53 suggested to be required for the growth suppression activity of p53 [4] and for its ability to induce apoptosis [5]. The two variant protein forms, R72 (arginine) and 72P (proline), have been shown to differ in their biological functions: the R72 variant is a stronger and faster inducer of apoptosis than the 72P variant [6,7]. The 72P variant also binds more efficiently to iASPP, an inhibitor of pro-apoptotic function of p53, which may be another reason for the inferiority in apoptosis induction of this variant [8]. The 72P variant has been found to be more efficient in inducing cell-cycle arrest [7] and DNA repair [9] than the R72 variant which may protect tumor from chemotherapy-induced apoptosis.

Previous studies have shown that the R72P polymorphism is not associated with increased breast cancer risk [1,10,11]. However, an association of R72P with breast cancer survival has been suggested, though with inconsistent results and possibly only in patients with p53-negative tumors [10-16]. It has also been suggested that patients with the Pro/Pro genotype are less sensitive to anthracycline-based treatment than those with the Arg/Pro or Arg/Arg genotype [14,16], in line with the Pro-allele being more efficient in cell-cycle arrest [7] and DNA repair [9] induction.

A common single nucleotide polymorphism in the MDM2 promoter region, a T to G change at nucleotide 309 in the first intron (-410G>T; named SNP309), has been shown to create an improved Sp1 binding site, leading to increased expression of the Mdm2 protein and thus attenuation of the p53 pathway and accelerated tumor formation in individuals carrying a germ-line p53 mutation [17-19]. A number of small studies revealed an inconsistent association between SNP309 and breast cancer risk (see overview in [1], and [20,21]). However, we have shown in a large pooled analyses of the Breast Cancer Association Consortium series that there is no general association of SNP309 with breast cancer, nor if stratified by estrogen receptor (ER) [1].

In two small studies no association between breast cancer survival and MDM2 SNP309 genotype alone was found [13,22]. However, the results of one of those studies suggested a differential effect of MDM2 SNP309 genotype by tumor p53 status (mutant p53 or aberrant protein expression) on breast cancer survival [22]. Though MDM2 SNP309 has been implicated to affect survival in other tumors (for example, [23]), as far as we know there are no other publications on breast cancer outcome and this polymorphism, except for a recent publication in BRCA1/2 carriers of Ashkenazi origin [24]. Our aim was to investigate the combined effects of MDM2 SNP309 and TP53 R72P polymorphisms and p53 protein expression on breast cancer survival.

Materials and methods
Clinico-pathologic data and genotyping
Breast cancer cases from four European studies within the Breast Cancer Association Consortium were included in this analysis (Table 1) [1,25]. Patients that were genotyped for MDM2 SNP309 and TP53 R72P from studies with follow-up data were included [1]. Patient selection criteria, participation rates and information on the collection of follow-up and clinical data are shown in Table 1. P53 protein expression data were available for two of the four studies (Table 1). Immunohistochemical staining of TMA slides was performed with a mouse monoclonal anti-human p53-antibody (DO-7, DAKO) (Table 1). Missing p53 data could be attributed to missing tumor blocks, loss of cores in the slicing or staining process or cores not containing enough tumor material. P53 protein expression scoring and MDM2 SNP309 and TP53 R72P genotyping were performed blinded to the survival status of the patients. Genotyping assays were performed by each group separately [1] (see Table 1 for assay description). Primer (and probe) sequences are available from the authors upon request. Methods and results in this paper are reported following the REMARK recommendations [26]. All studies were approved by the appropriate (Medical) Ethical Research Committees.

Statistical analyses
Univariate analyses of survival were performed by calculating Kaplan-Meier survival curves and comparing subsets of patients using log-rank test. To explore the effects of several variables and their combined effects on survival, multivariate Cox’s proportional hazards regression models were used (reported as Hazard Ratio (HR) with 95% confidence interval). Results are reported for one polymorphisms stratified by the other polymorphisms or p53 expression, adjusted for other covariates. Interaction terms were tested by Cox regression models including the main effects (2df each), interaction terms, for example, four interaction terms for both polymorphisms, and other covariates. Covariates included were prognostic factors for breast cancer survival, that is, age, stage, grade and ER and p53 protein expression. In order to run models including all patients, missing value categories were included for each separate variable with missing information. Polymorphisms were included as categorical variables (with the homozygous common allele group as reference), or as a continuous variable in the per-allele analyses. All pooled analyses were adjusted for study, that is, ABCS, HABCS, HEBCS, SEARCH, included as a categorical variable. Breast cancer-specific survival was defined as survival until death from breast cancer, with breast cancer being the underlying cause of death; death due to other causes was censored (these analy-
ses included the ABCS and HEBCS studies, see Table 1). Overall survival was defined as survival until death of any cause. In all analyses, follow-up time was censored at 10 years. All statistical tests used were two-sided and \( P \) values < 0.05 were considered statistically significant. All analyses were performed using SPSS 15.0 (SPSS Inc, Chicago, IL, USA).

| Contributing studies | Design | Description of case subjects and ascertainment (age range) | Participation rates | Follow-up | P53 IHC* | Genotyping platform(s) |
|----------------------|--------|----------------------------------------------------------|---------------------|-----------|---------|----------------------|
| ABCS: Amsterdam Breast Cancer Study, The Netherlands [41] | Hospital-based consecutive cases | All operable breast cancer patients aged < 50 years diagnosed 1974-1994 in four Dutch hospitals (Amsterdam and Leiden) (23 to 50 years) | All patients with paraffin-embedded tissue blocks available (normal tissue) from the Pathology archives and successful DNA isolation (approximately 85%) | Active follow-up through the medical registries and general practitioners | By IHC staining of TMAs* as previously described [25]; p53 positive defined as > 10% of cells with positive nuclear staining. | Taqman |
| HABCS: Germany: Hannover Breast Cancer Study and bilateral breast cancer patients [42,43] | Hospital-based case-control studies | Case patients who received radiotherapy for breast cancer at Hannover Medical School between 1997 and 2003 (27 to 91 years) | Approximately 80% of case subjects contacted agreed to give a blood sample | Active follow-up at the Department of Radiation Oncology, Hannover Medical School | NA | Restriction enzyme-based assays |
| HEBCS: Helsinki Breast Cancer Study [10,44] | Hospital-based case-control study | Consecutive incident cases from the Department of Oncology, Helsinki University Central Hospital 1997-1998 (22 to 96 years) | 79% of the case subjects | Active follow-up of the medical records until five years and annual linkage to the nation-wide Finnish Cancer Registry | By IHC staining of TMAs* as previously described [10] and data for 23 cases derived from the pathology reports; p53 positive defined as > 20% of cells with positive nuclear staining. | RFLP (MDM2 SNP309) Amplifluor(tm) fluorescent genotyping (Kbiosciences) (TP53 R72P) |
| SEARCH: Studies of Epidemiology and Risk Factors in Cancer Heredity, Cambridge, UK [45] | Population-based case-control study | Two groups of case patients (prevalent and incident) identified through East Anglian Cancer Registry: patients diagnosed before age 55 years in 1991 to 1996 and still alive when study started in 1996 and patients diagnosed before age 70 years since 1996 (25 to 65 years) | 64% of eligible case subjects provided a blood sample | Combination of passive follow-up through national death registrations and active follow up every five years by the cancer registry | NA | Taqman |

*IHC = immunohistochemistry; TMA = Tissue Micro Array; NA = not applicable (no p53 data available).

Results
Patient characteristics
Breast cancer patients with follow-up and TP53 R72P and MDM2 SNP309 genotypes from three hospital-based and one population-based study within the Breast Cancer Association Consortium were included for analysis (n = 3,749) (Table 1). Frequencies of TP53 R72P and MDM2 SNP309 and clinicopathologic characteristics of the breast cancer patients in the four studies are shown in Table 2. We have described and discussed earlier the small difference in MDM2...
Table 2

Germ-line variants and clinicopathologic characteristics of breast cancer patients by study

|                  | ABCS N = 1076 | HABCS N = 152 | HEBCS N = 599 | SEARCH N = 1922 | P value* |
|------------------|---------------|---------------|---------------|-----------------|---------|
| **MDM2 SNP309**  |               |               |               |                 |         |
| TT               | 444           | 41.3%         | 36.2%         | 30.6%           | 774     | 40.3   |
| GT               | 487           | 45.3%         | 48.0%         | 51.9%           | 913     | 47.5   |
| GG               | 145           | 13.5%         | 15.8%         | 17.5%           | 235     | 12.2%  < 0.001 |
| **TP53 R72P**    |               |               |               |                 |         |
| GG               | 570           | 53.0%         | 55.9%         | 52.4%           | 1052    | 54.7   |
| GC               | 422           | 39.2%         | 36.2%         | 39.4%           | 733     | 38.1   |
| CC               | 84            | 7.8%          | 7.9%          | 8.2%            | 137     | 7.1%   0.9 |
| **Stage**        |               |               |               |                 |         |
| 1                | 341           | 31.9%         | 69.2%         | 36.9%           | 861     | 52.4   |
| 2                | 581           | 54.4%         | 30.0%         | 53.1%           | 713     | 43.4   |
| 3                | 146           | 13.7%         | 0.8%          | 10.1%           | 69      | 4.2%   < 0.001 |
| Missing          | 8             | 32%           | 7%            | 8%              | 43      | 279%   |
| **Differentiation grade** | | | | | |
| 1                | 338           | 35.8%         | 8.7%          | 24.6%           | 368     | 25.4   |
| 2                | 317           | 33.6%         | 53.4%         | 43.2%           | 647     | 44.7   |
| 3                | 288           | 30.5%         | 37.9%         | 32.2%           | 431     | 29.8%  < 0.001 |
| Missing          | 133           | 49%           | 37%           | 476             |         |
| **ER status tumor** | | | | | |
| Negative         | 240           | 34.2%         | 15.4%         | 23.2%           | 175     | 19.8   |
| Positive         | 461           | 65.8%         | 84.6%         | 76.8%           | 708     | 80.2%  < 0.001 |
| Missing          | 375           | 35%           | 18%           | 1035            |         |
| **p53 status tumor** | | | | | |
| Negative         | 473           | 70.4%         | 335%          | 76.7%           |         |
| Positive         | 199           | 29.6%         | 102%          | 23.3%           | 0.02    |
| Missing          | 404           | 152%          | 162%          | 1922            |         |
| **Vital status patient** | | | | | |
| Alive            | 694           | 64.5%         | 84.9%         | 77.1%           | 1596    | 83.0%  |
| Deceased, all    | 362           | 35.5%         | 15.1%         | 22.9%           | 326     | 17.0%  < 0.001 |
| Deceased, breast cancer | 337 | 20% | 105 | | |
| **Years of diagnosis** | | | | | |
| Range            | 1974 to 1994  | 1997 to 2003  | 1997 to 1998  | 1991 to 1996    |         |
| **Age at diagnosis** | Mean ± SD | 42.8 ± 5.2 | 56.8 ± 11.3 | 56.4 ± 12.8 | 50.1 ± 7.7 | 7.7% < 0.001 |
| **Follow-up**    | Mean ± SD     | 10.5 ± 5.7   | 6.5 ± 1.9    | 6.3 ± 2.1       | 2.1 ± 6.3 | 2.1% < 0.001 |

*P value of comparison of either categories of non-missing data among studies (by chi-square) or comparison of continuous data (by t-test).
SNP309 allele frequencies between European populations [1] while difference in patient characteristics between studies can mostly be attributed to differences in patient selection criteria (Table 1). Mean follow-up was 7.7 years (SD 4). A small number patients (n = 26) were carriers of the homozygous rare variants for both polymorphisms (Table 3).

**Breast cancer survival by TP53 R72P, MDM2 SNP309 genotype, and p53 tumor status**

Overall survival of patients did not differ by carriership of either germ-line variant, R72P or SNP309, alone in the pooled analyses (Table 4). Tumor p53 status was available for 1109 patients from the ABCS and HEBCS series (Table 1). In both series, the patients with p53-positive tumors showed poorer overall survival than the patients with p53-negative tumors (pooled HR 1.5 (1.2-1.9)), overall survival than the patients with p53-negative tumors (p = 0.002; Table 4).

**Differential effect of TP53 R72P on breast cancer survival stratified for p53 tumor status**

In the patient group with p53-negative tumors, the actuarial breast-cancer-specific survival for the patients carrying the TP53 CC genotype (Pro/Pro) was worse, though not statistically significantly, at 10 years of follow-up as compared to those carrying TP53 GG/GC (Arg/Arg; Arg/Pro) (71% versus 80% P = 0.07; Figure 1). The interaction terms between p53 expression and TP53 R72P were not significant in a multivariate Cox regression analysis, but considering the difference seen in the actuarial curves we still considered it useful to perform Cox analyses stratified for p53 expression. Patients with the TP53 CC genotype had worse breast-cancer specific survival (HR adjusted for study, age, stage, grade and ER: 1.79 (1.05 to 1.96), P = 0.001. In multivariate analyses (adjusting for study, age, stage, grade and ER) stratified for TP53 GC and MDM2 GG was significant (P = 0.028), also if additional interaction terms for TP53 R72P and p53 expression were included (P = 0.027). The multivariate models (adjusting for study, age, stage, grade and ER) stratified for TP53 R72P (analogue to Figure 2) showed that MDM2 GG carriers had significantly worse survival compared with MDM2 GT carriers only within the TP53 C-allele carriers; more specifically, within TP53 CG carriers: HR 1.43 (1.05 to 1.96), P = 0.02; within TP53 CC carriers HR 1.39 (0.56 to 3.48), P = 0.48 (Table 6); within TP53 CG and CC carriers combined: HR (adjusted for study, age, stage, grade and ER) 1.46 (1.09 to 1.96), P = 0.01.

**Discussion**

In the survival analyses including 3,749 breast cancer patients from Finland, The Netherlands, Germany and United Kingdom, we showed combined effects of two germ-line polymorphisms, TP53 R72P, MDM2 SNP309, and p53 tumor expression (by immunohistochemistry). Firstly, we confirmed our earlier observation in Finnish patients [10] that TP53 R72P homozygous carriership predicts a worse survival in patients with p53-negative tumors, also when adjusted for clinical prognostic variables. Thus, in the absence of inactivating p53 mutations in the tumor, the 72P variant form of p53 protein may have a compromising effect on the p53 apoptotic function, leading to reduced survival of the patients. Similarly, a study of 414 Chinese breast cancer patients reported that the 72P homozygous (CC) genotype was associated with both poorer five-year overall survival (five to eight percentile difference, P = 0.04) and poorer disease-free survival among the patients with a wild-type p53 in their tumors (n = 346) [16]. In line with other studies published we did not observe an effect of carriership of R72P alone on survival of patients [12-16].

No significant difference in survival by TP53 R72P carriership was observed among the patients with p53-positive tumors, who showed a worse survival overall compared to p53-nega-
Table 4

HR estimates of overall survival* by \textit{TP53} R72P, \textit{MDM2} SNP309 and p53

|          | HR       | Lower and upper limit 95% CI | \(P\) value |
|----------|----------|------------------------------|-------------|
| \textit{TP53} R72P** |          |                              |             |
| ABCS     |          |                              |             |
| GC       | 0.99     | 0.79                         | 1.26        | 0.95       |
| CC       | 0.72     | 0.45                         | 1.16        | 0.17       |
| HABCS    |          |                              |             |
| GC       | 1.40     | 0.60                         | 3.45        | 0.46       |
| CC       | 2.54     | 0.69                         | 9.34        | 0.16       |
| HEBCS    |          |                              |             |
| GC       | 1.17     | 0.82                         | 1.67        | 0.40       |
| CC       | 1.72     | 1.00                         | 2.98        | 0.05       |
| SEARCH   |          |                              |             |
| GC       | 1.18     | 0.95                         | 1.49        | 0.14       |
| CC       | 0.93     | 0.59                         | 1.48        | 0.77       |
| Pooled†  |          |                              |             |
| GC       | 1.11     | 0.96                         | 1.28        | 0.18       |
| CC       | 1.00     | 0.76                         | 1.31        | 0.97       |
| \textit{MDM2} SNP309** |          |                              |             |
| ABCS     |          |                              |             |
| TG       | 0.93     | 0.73                         | 1.18        | 0.54       |
| GG       | 0.99     | 0.70                         | 1.40        | 0.97       |
| HABCS    |          |                              |             |
| TG       | 0.60     | 0.25                         | 1.46        | 0.26       |
| GG       | 0.36     | 0.08                         | 1.65        | 0.19       |
| HEBCS    |          |                              |             |
| TG       | 0.76     | 0.53                         | 1.11        | 0.16       |
| GG       | 0.91     | 0.56                         | 1.47        | 0.69       |
| SEARCH   |          |                              |             |
| TG       | 1.03     | 0.82                         | 1.31        | 0.78       |
| GG       | 1.43     | 1.03                         | 1.97        | 0.03       |
| Pooled†  |          |                              |             |
| TG       | 0.93     | 0.80                         | 1.08        | 0.34       |
| GG       | 1.11     | 0.90                         | 1.37        | 0.31       |
| p53 status tumor ** |          |                              |             |
| ABCS     |          |                              |             |
| p53 positive | 1.31    | 0.96                         | 1.80        | 0.09       |
| HEBCS    |          |                              |             |
| p53 positive | 1.93    | 1.27                         | 2.94        | 0.002      |
| Pooled†  |          |                              |             |
| p53 positive | 1.50    | 1.16                         | 1.93        | 0.002      |
| Breast cancer-specific survival Pooled† |          |                              |             |
| p53 positive | 1.57    | 1.20                         | 2.05        | 0.001      |

*Overall survival including all studies unless otherwise specified; ** HRs of heterozygous and homozygous rare allele groups have been calculated by comparison to the reference categories of common alleles: \textit{TP53} R72P = GG; \textit{MDM2} SNP309 = TT; p53 = negative tumors; †Pooled analyses have been adjusted for study.
production of which is increased by the SNP309 G-variant ant [6,7] and the attenuation of the p53 pathway by mdm2, the and GG group combined (80% versus 71%, P = 0.07).

However, we found an 11 percentile survival difference for patients in our study and two other, smaller studies [13,22].

Conclusions
We have shown here that TP53 R72P may have additional prognostic value especially among patients with p53-negative tumors. However, the effect of p53 on outcome may be influence by adjuvant systemic therapy (for example, [31,36], reviewed in Bertheau [37]) and larger studies will be needed to address this question. Our study is one of the few that have shown an interaction of germ-line variants, that is, TP53 R72P and MDM2 SNP309, in breast cancer survival. The results, showing a statistically significant interaction of the p53 Pro-variant and the GG genotype of MDM2 SNP309, are in line with our a priori biologically-supported hypothesis, which is, the role of enhanced DNA repair function of the Pro-variant, combined with increased expression of the Mdm2 protein, and thus overall attenuation of the p53 pathway in the tumor cells. These results suggest that even subtle differences in p53 apoptotic function caused by synergistic polymorphisms may affect patient’s survival, possibly by modifying treatment response. Altogether, our findings are in line with biological evidence in literature, and in the future, may have also clinical significance for models of breast cancer prognosis or treat-
Table 5

HR estimates of overall and breast cancer-specific survival by TP53 R72P, in p53 negative and positive tumors (multivariate models)

| TP53 R72P | HR     | Lower and upper limit 95% CI | P value |
|-----------|--------|------------------------------|---------|
| Overall survival                      |        |                              |         |
| p53 negative tumors                  |        |                              |         |
| GC    | 1.11   | 0.80                         | 1.52    | 0.54   |
| CC    | 1.63   | 0.97                         | 2.74    | 0.06   |
| p53 positive tumors                  |        |                              |         |
| GC    | 0.82   | 0.53                         | 1.27    | 0.37   |
| CC    | 0.86   | 0.34                         | 2.18    | 0.75   |
| Breast cancer-specific survival      |        |                              |         |
| p53 negative tumors                  |        |                              |         |
| GC    | 0.97   | 0.68                         | 1.37    | 0.85   |
| CC    | 1.79   | 1.05                         | 3.05    | 0.03   |
| p53 positive tumors                  |        |                              |         |
| GC    | 0.90   | 0.57                         | 1.42    | 0.65   |
| CC    | 1.00   | 0.39                         | 2.55    | 1.00   |

| MDM2 SNP309  | HR     | Lower and upper limit 95% CI | P value |
|--------------|--------|------------------------------|---------|
| Overall survival                      |        |                              |         |
| p53 negative tumors                  |        |                              |         |
| TG    | 0.84   | 0.60                         | 1.17    | 0.30   |
| GG    | 1.28   | 0.85                         | 1.94    | 0.24   |
| p53 positive tumors                  |        |                              |         |
| TG    | 0.78   | 0.49                         | 1.24    | 0.30   |
| GG    | 0.78   | 0.41                         | 1.48    | 0.45   |
| Breast cancer-specific survival      |        |                              |         |
| p53 negative tumors                  |        |                              |         |
| TG    | 0.92   | 0.64                         | 1.31    | 0.63   |
| GG    | 1.41   | 0.90                         | 2.19    | 0.13   |
| p53 positive tumors                  |        |                              |         |
| TG    | 0.76   | 0.47                         | 1.23    | 0.27   |
| GG    | 0.69   | 0.35                         | 1.39    | 0.30   |

Pooled analyses for studies with p53 information (ABCS and HEBCS); HRs of heterozygous and homozygous rare allele groups have been calculated by comparison to the reference categories of the homozygous common allele: TP53 R72P = GG, and MDM2 SNP309 = TT; analyses have been adjusted for study, age, stage, grade and ER.
Figure 2

Cumulative overall survival of breast cancer patients by MDM2 SNP309 and TP53 R72P genotypes. Each figure shows Kaplan Meier survival curves of MDM2 SNP309 genotypes within one group of TP53 R72P genotype. (a) TP53 GG genotype (ns); (b) TP53 GC genotype ($P = 0.006$); (c) TP53 CC genotype (ns). The numbers at start of follow-up were: Figure A: TT n = 798, TG n = 939, GG n = 281; B: TT n = 545, TG n = 698, GG n = 200; C: TT n = 110, TG n = 144, GG n = 25. Within the TP53 C-allele carriers (Figure A and B combined), MDM2 GG carriers had significantly worse survival compared to TT/TG carriers combined: 64% versus 75%, $P = 0.001$. 

Available online http://breast-cancer-research.com/content/11/6/R89
The combined effect of TP53 R72P and MDM2 SNP309 on breast cancer survival and we cannot exclude a chance finding, other studies to confirm this will be necessary. Larger studies will also be needed to investigate the effect of specific treatment modalities on the survival by TP53 R72P and MDM2 SNP309.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MKS, TD and HN took final responsibility for the decision to submit the paper for publication; all other authors read and approved the manuscript. MKS, JT, FEVL, LJTV, PDPP, DFE, TD, CB, HN were responsible for the study design. MKS, JT, AB, MS, MH, TD, SAR, RF were responsible for data acquisition and collection. MKS and JT did the data analyses. Data interpretation was carried out by MKS, JT, AB, RF, CB, and HN. MKS, AB, TD, CB, and HN wrote the paper. All authors read and approved the manuscript.

Acknowledgements
We wish to thank Dr Hannaleena Eerola and Dr Kirsimari Aaltonen and Nina Puolakka RN for their help with the Finnish patient data. The Finnish Cancer registry is gratefully acknowledged for the cancer data. The Helsinki study has been financially supported by the Helsinki University.

Table 6

| TP53 R72P | HR Lower and upper limit 95% CI | P value | HR Lower and upper limit 95% CI | P value | HR Lower and upper limit 95% CI | P value |
|-----------|---------------------------------|---------|---------------------------------|---------|---------------------------------|---------|
| **MDM2 SNP309 TT** | | | | | | |
| TG | 1.0 (Ref) | | | | | |
| GG | .90 (.73 1.11 .33) | | | | | |
| **p53 negative** | | | | | | |
| positive | 1.37 (.96 1.96 .08) | | | | | |
| missing | 1.63 (1.16 2.28 .005) | | | | | |
| **Stage 1** | | | | | | |
| 2 | 2.55 (1.88 3.46 <.001) | | | | | |
| 3 | 7.56 (5.18 11.02 <.001) | | | | | |
| missing | 2.55 (1.54 4.21 <.001) | | | | | |
| **Grade 1** | | | | | | |
| 2 | 1.12 (.77 1.62 .55) | | | | | |
| 3 | 2.39 (1.65 3.46 <.001) | | | | | |
| missing | 1.39 (0.87 2.22 .17) | | | | | |
| **ER negative** | | | | | | |
| positive | .61 (.44 .83 .002) | | | | | |
| missing | .65 (.46 .91 .01) | | | | | |
| **Age** | | | | | | |
| 1.02 | 1.01 | 1.03 | .001 | 1.00 | .99 | 1.02 | .54 | 1.01 | .98 | 1.04 | .70 |

Models have also been adjusted for study.

---

Combined effect of TP53 R72P and MDM2 SNP309 on breast cancer survival and we cannot exclude a chance finding, other studies to confirm this will be necessary. Larger studies will also be needed to investigate the effect of specific treatment modalities on the survival by TP53 R72P and MDM2 SNP309.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MKS, TD and HN took final responsibility for the decision to submit the paper for publication; all other authors read and approved the manuscript. MKS, JT, FEVL, LJTV, PDPP, DFE, TD, CB, HN were responsible for the study design. MKS, JT, AB, MS, MH, TD, SAR, RF were responsible for data acquisition and collection. MKS and JT did the data analyses. Data interpretation was carried out by MKS, JT, AB, RF, CB, and HN. MKS, AB, TD, CB, and HN wrote the paper. All authors read and approved the manuscript.

Acknowledgements
We wish to thank Dr Hannaleena Eerola and Dr Kirsimari Aaltonen and Nina Puolakka RN for their help with the Finnish patient data. The Finnish Cancer registry is gratefully acknowledged for the cancer data. The Helsinki study has been financially supported by the Helsinki University.

---

Table 6

HR estimates of multivariate analyses for MDM2 SNP309 stratified by TP53 R72P

| TP53 R72P | HR Lower and upper limit 95% CI | P value | HR Lower and upper limit 95% CI | P value | HR Lower and upper limit 95% CI | P value |
|-----------|---------------------------------|---------|---------------------------------|---------|---------------------------------|---------|
| **MDM2 SNP309 TT** | | | | | | |
| TG | 1.0 (Ref) | | | | | |
| GG | .90 (.73 1.11 .33) | | | | | |
| **p53 negative** | | | | | | |
| positive | 1.37 (.96 1.96 .08) | | | | | |
| missing | 1.63 (1.16 2.28 .005) | | | | | |
| **Stage 1** | | | | | | |
| 2 | 2.55 (1.88 3.46 <.001) | | | | | |
| 3 | 7.56 (5.18 11.02 <.001) | | | | | |
| missing | 2.55 (1.54 4.21 <.001) | | | | | |
| **Grade 1** | | | | | | |
| 2 | 1.12 (.77 1.62 .55) | | | | | |
| 3 | 2.39 (1.65 3.46 <.001) | | | | | |
| missing | 1.39 (0.87 2.22 .17) | | | | | |
| **ER negative** | | | | | | |
| positive | .61 (.44 .83 .002) | | | | | |
| missing | .65 (.46 .91 .01) | | | | | |
| **Age** | | | | | | |
| 1.02 | 1.01 | 1.03 | .001 | 1.00 | .99 | 1.02 | .54 | 1.01 | .98 | 1.04 | .70 |

Models have also been adjusted for study.
Central Hospital Research Fund, Academy of Finland (110663), Finnish Cancer Society, the Sigrid Juselius Foundation, and the Ida Montin Foundation. We highly appreciate the contributions of our Dutch colleagues at the NKI-AVL, among others Renate Udo, Linde Braaf and Hans Peterse; and at LUMC; Rob Tollenaar, Vincent Smits and Cees Cornelisse; the Dutch study was financed by the Dutch Cancer Society (NKI 2001-2423; 2007-8389) and the Cancer Genomics Center (Dutch Genomics Initiative). We are grateful to our German colleagues Michael Bremer, Andreas Meyer, Johann H. Karstens and Peter Hillemanns for their support of the study at Hannover Medical School. The SEARCH study is funded by Cancer Research UK, and DFE is a Principal Research Fellow and PDP is a Senior Clinical Research Fellow of CRUK.

References

1. Schmidt MK, Reincio S, Broeks A, Braat LM, Hogervorst FBL, Tollenaar RAEM, Johnson N, Fletcher O, Petro J, Tommiska J, Blomqvist C, Nevanlinna HA, Healey CS, Dunning AM, Pharoah PD, Easton DF, Dork T, Van’t Veer LJ, on behalf of the Breast Cancer Association Consortium: Do MDM2 SNP309 and TP53 R72P Interact in Breast Cancer Susceptibility? A Large Pooled Series from the Breast Cancer Association Consortium. Cancer Res 2007, 67:9584-9590.

2. Fagerholm R, Hofstetter B, Tommiska J, Aaltonen K, Vrtel R, Syrjanen S, Combarnous Y, Kallioniemi A, Kilpivaara O, Mannermaa A, Koivisto M, Vrtel T, Kallioniemi O, Heikkinen K, Vortela S, Tenhunen M, Arvas NC, Bartkova J, Blomqvist C, Bartke J, Nevanlinna H: NAPDH:quinone oxidoreductase 1 NQO1*2 genotype (P187S) is a strong predictive and prognostic factor in breast cancer. Nat Genet 2008, 40:844-853.

3. Bond GL, Hsu W, Levine AJ: MDM2 is a central node in the p53 pathway: 12 years and counting. Curr Cancer Drug Targets 2009, 9:487-500.

4. Walker KK, Levine AJ: Identification of a novel p53 functional domain that is necessary for efficient growth suppression. Proc Natl Acad Sci USA 1996, 93:15335-15340.

5. Sakamuro D, Sabbatini P, White E, Prendergast GC: The poly-proline region of p53 is required to activate apoptosis but not growth arrest. Oncogene 1997, 15:887-898.

6. Thomas M, Kaila A, Labrecque S, Pin D, Banks L, Matlaleshwari G: Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol 1999, 19:1092-1100.

7. Pim D, Banks L: p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. Int J Cancer 2004, 108:196-199.

8. Berganschgi D, Samuels Y, Sullivan A, Zvelebil M, Breyssens H, Bisso A, Del Sal G, Syed N, Smith P, Gasco M, Crook T, Lu X: iASPPI preferentially binds p53 proline-rich region and modulates apoptotic function of codon 72-polymeric p53. Nat Genet 2008, 36:1133-1141.

9. Sridique MM, Balram C, Fiszer-Maliszewska L, Aggarwal A, Tan A, Tan P, Soo KC, Sabapathy K: Evidence for selective expression of the p53 codon 72 polymorphs: implications in cancer development. Cancer Epidemiol Biomarkers Prev 2005, 14:2245-2252.

10. Tommiska J, Eerola H, Heinonen M, Salonen L, Kaare M, Talilla J, Ristimaki A, von Smitten K, Attomaki K, Heikkinen P, Blomqvist C, Nevanlinna H: Breast Cancer Patients with p53 Pro72 Homozygous Genotype Have a Poorer Survival. Clin Cancer Res 2005, 11:5989-5993.

11. The Breast Cancer Association Consortium: Commonly Studied Single-Nucleotide Polymorphisms and Breast Cancer: Results From the Breast Cancer Association Consortium. J Natl Cancer Inst 2006, 98:1382-1396.

12. Bowden E, Dunning A, Kuschel B, Healey C, Day N, Ponder B, Easton D, Pharoah P: Effect of Germ-Line Genetic Variation on Breast Cancer Survival in a Population-Based Study. Cancer Res 2002, 62:3052-3057.

13. Toyama T, Zhang Z, Nishio M, Hamaguchi M, Kondo N, Iwase H, Iwata H, Takahashi S, Yamashita H, Fuji Y: Association of TP53 codon 72 polymorphism and the outcome of adjuvant therapy in breast cancer patients. Breast Cancer Res 2007, 9:R34.

14. Xu Y, Yao L, Ouyang T, Li J, Wang T, Fan Z, Lin B, Lu Y, Xie Y: p53 Codon 72 Polymorphism Predicts the Pathologic Response to Neoadjuvant Chemotherapy in Patients with Breast Cancer. Clin Cancer Res 2005, 11:7328-7333.

15. Kyndi M, Alasar J, Hansen LL, Sorensen FB, Overgaard J: LOH rather than genotypes of TP53 codon 72 is associated with disease-free survival in primary breast cancer. Acta Oncol 2008, 47:602-609.

16. Xu Y, Yao L, Zhao A, Ouyang T, Li J, Wang T, Fan Z, Fan T, Lin B, Lu Y, Xie Y: Effect of p53 codon 72 genotype on breast cancer survival depends on p53 gene status. Int J Cancer 2008, 122:2761-2766.

17. Bond GL, Hu W, Bond EE, Robbins H, Lutzker SG, Arva NC, Baro- getti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G, Levine AJ: A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 2004, 119:591-602.

18. Bougeraud G, Baert-Desurmont T, Tournaire S, Vasseur S, Martin C, Brugieres L, Chompret A, Bressac-de Paillerets B, Stoppa-Lyonnet D, Bonali-Pellier C, Frebourg T: Impact of the MDM2 SNP309 and p53 Arg72Pro polymorphisms on breast cancer susceptibility. Breast Cancer Res Treat 2004, 86:305-309.

19. Aaltonen K, Vrtel T, Vortela S, Cinkova J, Egan D, Fagerholm R: p53 Arg72Pro polymorphism is associated with breast cancer susceptibility. Breast Cancer Res Treat 2006, 98:373-378.

20. Boughey CQ, Baert-Desurmont T, Tournaire S, Vasseur S, Martin C, Brugieres L, Chompret A, Bressac-de Paillerets B, Stoppa-Lyonnet D, Bonali-Pellier C, Frebourg T: Impact of the MDM2 SNP309 and p53 Arg72Pro polymorphisms on breast cancer susceptibility. Breast Cancer Res Treat 2006, 98:305-309.

21. Cox DG, Deer D, Guo Q, Tworoger SS, Hankinson SE, Hunter DJ, De Vivo I: The p53 Arg72Pro and MDM2 -309 polymorphisms and risk of breast cancer in the Nurses’ Health Studies. Cancer Causes Control 2007, 18:621-625.

22. Boesma B, Howe T, Goodman J, Yantis H, Lee D, Chanock S, Ambos S: Association of Breast Cancer Outcome With Status of p53 and MDM2 SNP309. J Natl Cancer Inst 2006, 98:911-919.

23. Gryschenko I, Hofbauer S, Stoecher M, Daniel P, Steurer M, Gage A, Eigenberger K, Arend R, Tinhoffer I: p53 SNP309 Is Associated With Poor Outcome in B-Cell Chronic Lymphocytic Leukemia. J Clin Oncol 2008, 26:2252-2257.

24. Nechushtan H, Hamburger T, Mendelson S, Kadouri L, Sharon N, Pikarsky E, Peretz T: Effects of the single nucleotide polymorphism at MDM2 309 on breast cancer patients with/without BRCA1/2 mutations. BMC Cancer 2009, 9:60.

25. Garcia-Closas M, Hall P, Nevanlinna H, Pooley K, Morrison J, Richesson DA, Bojesen SE, Nordestgaard BG, Axelsson CK, Arias JI, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Zamora P, Brauch H, Vistisen C, Hagenberg KH, Ko YQ, Brunnauer T, Haas S, Dork T, Schumann P, Hillelmanns P, Bogdanova N, Bremer M, Karstens JH, Fagerholm R, Aaltonen K, Attomaki K: Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. PLoS Genet 2008, 4:e1000054.

26. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM: Reporting recommendations for tumor marker prognostic studies. J Clin Oncol 2005, 23:9067-9072.

27. Dumont P, Leu JI, Della Pietra AC III, George DL, Murphy M: The codon 72 polymorphic variants of p53 are unlikely to be a clinically significant genetic polymorphism. Mol Cancer 2004, 3:32.

28. Nechushtan H, Hamburger T, Mendelson S, Kadouri L, Sharon N, Pikarsky E, Peretz T: Effects of the single nucleotide polymorphism at MDM2 309 on breast cancer patients with/without BRCA1/2 mutations. BMC Cancer 2009, 9:60.

29. Garcia-Closas M, Hall P, Nevanlinna H, Pooley K, Morrison J, Richesson DA, Bojesen SE, Nordestgaard BG, Axelsson CK, Arias JI, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Zamora P, Brauch H, Vistisen C, Hagenberg KH, Ko YQ, Brunnauer T, Haas S, Dork T, Schumann P, Hillelmanns P, Bogdanova N, Bremer M, Karstens JH, Fagerholm R, Aaltonen K, Attomaki K: Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. PLoS Genet 2008, 4:e1000054.
31. Bonafe M, Ceccarelli C, Farabegoli F, Santini D, Tafturelli M, Barbi C, Malmi E, Trapassi C, Storci G, Olivieri F, Franceschi C: 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, 9:4860-4864.

32. Olivier M, Langerod A, Carrieri P, Bergh J, Klaar S, Eifjord J, Theillet C, Rodriguez C, Lidereau R, Bischof I, Varley J, Bignon Y, Uhrhammer N, Winqvist R, Jukkola-Vuorinen A, Niederacher D, Kato S, Ishioka C, Hainaut P, Borresen-Dale A: The Clinical Value of Somatic TP53 Gene Mutations in 1,794 Patients with Breast Cancer. *Clin Cancer Res* 2006, 12:1157-1167.

33. Petitjean A, Achatz MI, Borresen-Dale AL, Hainaut P, Olivier M: TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. *Oncogene* 2007, 26:2157-2165.

34. Soussi T, Beroud C: Assessing TP53 status in human tumours to evaluate clinical outcome. *Nat Rev Cancer* 2001, 1:233-240.

35. Sjogren S, Inganas M, Norberg T, Lindgren A, Nordgren H, Holmberg L, Bergh J: The p53 gene in breast cancer: prognostic value of complementary DNA sequencing versus immunohistochemistry. *J Natl Cancer Inst* 1996, 88:173-182.

36. Bidard FC, Matthieu MC, Chollet P, Raefelis I, Abrial C, Domont J, Spielmann M, Delalage S, Andre F, Penault-Llorca F: p53 status and efficacy of primary anthracyclines/alkylating agent-based regimen according to breast cancer molecular classes. *Ann Oncol* 2008, 19:1261-1265.

37. Bertheau P, Espie M, Turpin E, Lehmann J, Plassa LF, Varna M, Janin A, de The H: TP53 status and response to chemotherapy in breast cancer. *Pathobiology* 2008, 75:132-139.

38. Kbiosciences, Cambridge, UK [http://www.kbioscience.co.uk](http://www.kbioscience.co.uk)

39. Taqman, Applied Biosystems [http://www2.appliedbiosystems.com](http://www2.appliedbiosystems.com)

40. Illumina [http://www.illumina.com](http://www.illumina.com)

41. Schmidt MK, Tollenaar RAEM, de Kemp SR, Broeks A, Cornelisse CJ, Smit VTHBM, Peterse JL, van Leeuwen FE, Van’t Veer LJ: Breast Cancer Survival and Tumor Characteristics in Premenopausal Women Carrying the CHEK2*1100delC Germline Mutation. *J Clin Oncol* 2007, 25:64-69.

42. Steinmann D, Bremer M, Rades D, Skawran B, Siebrands C, Karstens JH, Dork T: Mutations of the BRCA1 and BRCA2 genes in patients with bilateral breast cancer. *Br J Cancer* 2001, 85:850-858.

43. Dork T, Bendix R, Bremer M, Rades D, Klopfer K, Nicke M, Skawran B, Hector A, Yamini P, Steinmann D, Weise S, Stuhmann M, Karstens JH: Spectrum of ATM gene mutations in a hospital-based series of unselected breast cancer patients. *Cancer Res* 2001, 61:7608-7615.

44. Syrjakoski K, Valteristo P, Eerola H, Tamminen A, Kiviniemi K, Sarantaus L, Hovi K, Biogmisti C, Kallionemi OP, Kainu T, Nevanlinna H: Population-based study of BRCA1 and BRCA2 mutations in 1035 unselected Finnish breast cancer patients. *J Natl Cancer Inst* 2000, 92:1529-1531.

45. Cebrian A, Pharoah P, Ahmed S, Ropero S, Fraga M, Smith P, Conroy D, Luben R, Perkins B, Easton D, Dunning A, Esteller M, RIDDLE B: Genetic variants in epigenetic genes and breast cancer risk. *Carcinogenesis* 2006, 27:1661-1669.