TP53-binding protein variants and breast cancer risk: a case-control study

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Abstract

Introduction The TP53-binding protein (53BP1) has been shown to influence TP53-mediated transcriptional activation, thus playing a pivotal role in DNA damage signalling. Genetic aberrations in TP53 and in ATM and CHEK2 predispose to cancer. We have therefore examined the effects of 53BP1 single nucleotide polymorphisms (D353E, G412S, and K1136Q) and the novel 53BP1 6bp deletion (1347_1352delTATCCC) on breast cancer risk.

Methods Allelic discrimination was performed to investigate the frequencies of 53BP1 D353E, G412S, or K1136Q and of 1347_1352delTATCCC in 353 patients with breast cancer and 960 control individuals.

Results No significant association of 53BP1 D353E, G412S, or K1136Q with breast cancer risk was detected. 53BP1 1347_1352delTATCCC, leading to the loss of an isoleucine and a proline residue, showed a nonsignificant inverse association with breast cancer risk (odds ratio = 0.61, 95% confidence interval = 0.22 to 1.68, \( P = 0.34 \)).

Conclusion The lack of association casts doubt on the putative effects of D353E, G412S, and K1136Q on breast cancer risk. Investigating a larger study cohort might elucidate the influence of the 6bp deletion 1347_1352delTATCCC. Studying the functional effect and the impact of this variant on the risk of other cancers may be revealing.

Introduction

The TP53-binding protein (53BP1), a conserved nuclear protein, was initially identified to interact with the DNA-binding domain of TP53, thus enhancing TP53-mediated transcriptional activation [1,2]. In response to exogenous exposure to ionising radiation, 53BP1 becomes hyperphosphorylated and rapidly localises to sites of DNA double-strand breaks, demonstrating its determining role in DNA damage signalling [3,4]. 53BP1-deficient mice exhibit growth retardation, high radiation sensitivity, and tumour development — features that are indicative of a defective DNA damage response [5]. 53BP1 is involved in the phosphorylation of various ataxia telangiectasia mutated protein (ATM) substrates such as cell cycle checkpoint kinase 2 (CHEK2) [3,6]. Mutations in ATM, CKEK2, and its substrate, TP53, have been shown to predispose to cancer [6-9]. Therefore, we selected 53BP1 as an attractive candidate gene for breast cancer susceptibility.

This is the first study to investigate the effects of the 53BP1 single nucleotide polymorphisms (SNPs) D353E (1059C>G), G412S (1234G>A), and K1136Q (3406A>C) on breast cancer risk, analysing 353 German patients with breast cancer and 960 controls. 53BP1 D353E, G412S, and K1136Q showed no association with breast cancer risk. In addition, we detected a novel, very rare 53BP1 6bp deletion (1347_1352delTATCCC) showing an inverse association...
with breast cancer risk (age-adjusted odds ratio (OR) = 0.61, 95% confidence interval (CI) = 0.22 to 1.68), lacking significance (P = 0.34).

Materials and methods
SNP verification
A randomly chosen set of 23 German patients with familial breast cancer was initially screened for annotated 53BP1 SNPs (dbSNP database; NCBI (National Center for Biotechnology Information)) by DNA sequencing. Sequencing primers are available upon request. The initial analysis included 53BP1 exons 9, 11, and 17, harbouring three reported nonsynonymous polymorphisms (D353E: rs560191; G412S: rs689647; and K1136Q: rs2602141). When sequencing exon 11, we additionally detected the 6bp deletion 1347_1352delTATCCC. All validated variants were chosen for further analyses using a large cohort of breast cancer patients.

Subjects
The breast cancer patients were 353 unrelated German women (mean age 44.8 years, range 21 to 80 years) who were negative for BRCA1 and BRCA2 mutations. In accordance with the German Consortium for Hereditary Breast and Ovarian Cancer, they were classified into six categories based on family history: (A1) families with two or more breast cancer cases including two cases with onset below the age of 50 (39.3% of analysed cases); (A2) families with at least one male breast cancer case (0.9%); (B) families with at least one breast cancer and one ovarian cancer case (16.2%); (C) families with at least two breast cancer cases including one case diagnosed before the age of 50 (33.5%); (D) families with at least two breast cancer cases comprising two cases diagnosed after the age of 50 (5.5%); (E) single cases of breast cancer diagnosed after the age of 50 (4.6%) [10]. They were collected during the years 1997 to 2004 through the Institute of Human Genetics, Technical University Munich, Munich, Germany [12]. Age-adjusted ORs and corresponding 95% CIs were computed by means of unconditional logistic regression using SAS (Version 8.2; SAS Institute Inc, Cary, NC, USA). Haplotypes were assigned to subjects using the SNPHAP software (see [13]), which also reports the posterior probability of the most likely assignment [14,15].

Statistical methods
Calculations of Hardy–Weinberg equilibrium, genotype-specific OR, and 95% CI were carried out using a tool offered by the Institute of Human Genetics, Technical University Munich, Munich, Germany [12]. Age-adjusted ORs and corresponding 95% CIs were computed by means of unconditional logistic regression using SAS (Version 8.2; SAS Institute Inc, Cary, NC, USA). haplotypes were assigned to subjects using the SNPHAP software (see [13]), which also reports the posterior probability of the most likely assignment [14,15].

Results and discussion
Inactivation of ATM and ATM substrates such as CHEK2 have been shown to predispose to cancer in humans [7]. Along with ATM and CHEK2, 53BP1 is involved in DNA damage response and tumour suppression. Recent studies have shown that 53BP1 and ATM interact in irradiated cells, suggesting that ATM activation is the consequence of the recruitment of ATM to sites of DNA double-strand breaks by 53BP1 [7,9]. Thus, polymorphic variants in 53BP1 are excellent candidates for cancer susceptibility. We investigated the impact of three nonsynonymous amino acid exchanges in 53BP1 on breast cancer risk. 53BP1 G413S and K1136Q represented promising candidate SNPs, resulting in the replacement of a nonpolar by a polar amino acid. Genotype frequencies of the three 53BP1 polymorphisms between breast cancer cases and control samples were similar, showing no significant association with breast cancer risk (D353E: age-adjusted OR = 1.07, 95% CI = 0.81 to 1.34, P = 0.62; G412S: age-adjusted OR = 1.22, 95% CI = 0.86 to 1.74, P = 0.26; K1136Q: age-adjusted OR = 1.10, 95% CI = 0.82 to 1.47, P = 0.53; Table 1). Additionally, we detected a novel 53BP1 6 bp deletion, 1347_1352delTATCCC, leading to the loss of an isoleucine
Table 1
Genotype frequencies of 53BP1 polymorphisms in breast cancer patients and controls

| Polymorphism                  | No. (%) of cases | No. (%) of controls | AOR (95% CI)  | P    |
|-------------------------------|------------------|---------------------|---------------|------|
| **D353E (1059C>G)**          |                  |                     |               |      |
| CC                            | 165 (48.1)       | 453 (47.6)          |               |      |
| GC                            | 148 (43.1)       | 405 (42.5)          |               |      |
| GG                            | 30 (8.7)         | 94 (9.9)            | 1.07 (0.81–1.43) | 0.62 |
| Σ                             | 343              | 952                 |               |      |
| **G412S (1234G>A)**          |                  |                     |               |      |
| GG                            | 269 (78.7)       | 760 (80.1)          |               |      |
| AG                            | 67 (19.6)        | 174 (18.3)          |               |      |
| AA                            | 6 (1.8)          | 15 (1.6)            | 1.22 (0.86–1.74) | 0.26 |
| Σ                             | 342              | 949                 |               |      |
| **1347_1352delTATCCC**        |                  |                     |               |      |
| wt/wt                         | 334 (98.2)       | 931 (97.4)          |               |      |
| delTATCCC/wt                  | 6 (1.8)          | 25 (2.6)            |               |      |
| delTATCCC/delTATCCC           | 0 (0.0)          | 0 (0.0)             |               |      |
| Σ                             | 340              | 956                 |               |      |
| **K1136Q (3406A>C)**         |                  |                     |               |      |
| AA                            | 158 (47.4)       | 448 (47.8)          |               |      |
| CA                            | 144 (43.2)       | 396 (42.2)          |               |      |
| CC                            | 31 (9.3)         | 94 (10.0)           |               |      |
| Σ                             | 333              | 938                 |               |      |
| **CC+CA vs AA**               |                  |                     |               |      |

AOR, age-adjusted odds ratio; CI, confidence interval; wt, wild type.

Table 2
Haplotype distribution of 53BP1 polymorphisms in breast cancer patients and control individuals

| Haplotype 1059C>G-1234G>A-1347_1352+/+3406A>C | Cases        | Controls | OR \(^h\) | 95 % CI | P   |
|------------------------------------------------|--------------|----------|------------|--------|-----|
| No. (%)                                       | Mean posterior probability | No. (%) | Mean posterior probability |
| CG+A                                          | 444 (68.1)   | 1        | 1242 (67.5) | 1      | 1   | -   |
| CG+C                                          | 2 (0.3)      | 1        | 1 (0.1)     | 1      | n.a.| n.a.| n.a.|
| CG-A                                          | 6 (0.9)      | 0.90     | 25 (1.4)    | 0.99   | 0.63| 0.23–1.75| 0.38 |
| GG+A                                          | 1 (0.2)      | 1        | 1 (0.1)     | 1      | n.a.| n.a.| n.a.|
| GG+C                                          | 125 (19.2)   | 1        | 376 (20.4)  | 1      | 1.02| 0.79–1.33| 0.86 |
| GA+C                                          | 74 (11.3)    | 1        | 195 (10.6)  | 1      | 1.15| 0.83–1.61| 0.41 |

\(^h\)Polymorphisms D353E (1059C>G), G412S (1234G>A), 1347_1352delTATCCC, and K1136Q (3406A>C). \(^{1059C-1234G-1347_1352+}\)-3406A as reference. CI, confidence interval; n.a., not available; OR, odds ratio.
and a proline residue at positions 450 and 451, which has not been described previously. Comparing the occurrence of this rare, 6 bp deletion between cases and controls revealed an inverse association with breast cancer risk (OR = 0.61, 95% CI = 0.22 to 1.68, P = 0.34; Table 1), but lacking statistical significance.

The haplotype distribution and corresponding posterior probabilities are shown in Table 2. Since every mean posterior probability was higher than 0.9, only the most likely haplotypes were used to evaluate the association with breast cancer risk. Haplotype analysis showed a nonsignificant inverse association of the haplotype 1059C-1234G-1347_1352-3406A with breast cancer risk (age-adjusted OR = 0.63, 95% CI = 0.23 to 1.75, P = 0.38; Table 2). The distribution of the remaining haplotypes between breast cancer patients and controls was similar, indicating no significant effect with regard to breast cancer risk. Given our sample size, we had a 90% power to detect an odds ratio of 1.65 (D353E), 1.76 (G412S), and 1.66 (K1136Q), respectively [16]. Contrary to standard case-control association studies, this study comprised predominantly cases selected for family history of breast cancer. The use of unselected cases would have required at least twice the sample size to achieve the same power as in the present study [17,18]. The numbers of cases within the risk groups A1 to E were too low to be studied separately, as the power in these subgroups would have been limited. In addition to the results of this study, one cannot exclude the possibility that common 53BP1 SNPs may affect breast cancer risk. Regulatory polymorphisms, for example polymorphisms that reside in promoter or noncoding regions, have been shown to modify gene transcription, mRNA stability, and processing efficiency, as well as DNA methylation [19,20].

Conclusion
The three known 53BP1 SNPs – D353E, G412S, and K1136Q – lacked association with breast cancer risk. However, we detected a novel, very rare 6 bp deletion, 1347_1352delTATCCC, that showed a statistically nonsignificant inverse association with breast cancer risk. Concerning the latter, a much larger study cohort is required to verify any putative significant effect. Additionally, it would be valuable to investigate a possible functional effect of this 53BP1 deletion and its impact on other cancers.

Competing interests
The author(s) declare that they have no competing interests.

Authors’ contributions
All authors listed contributed to the production of this manuscript: RK, PB, BW, and RKS provided genomic DNAs of cases studied and helped to draft the manuscript. BB and KH participated in the design and coordination of the study and critically revised the manuscript. BF and JLB performed statistical analyses. BF carried out the SNP genotyping and wrote the manuscript. All authors read and approved the final manuscript.

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