

Research article

Open Access

BRIP1 (BACH1) variants and familial breast cancer risk: a case-control study

Bernd Frank*^{1,2}, Kari Hemminki³, Alfons Meindl⁴,
Barbara Wappenschmidt^{5,6}, Christian Sutter⁷, Marion Kiechle⁴, Peter Bugert⁸,
Rita K Schmutzler^{5,6}, Claus R Bartram⁷ and Barbara Burwinkel^{1,2}

Address: ¹Helmholtz-University Group Molecular Epidemiology, German Cancer Research Center, DKFZ, Heidelberg, Germany, ²Division of Molecular Genetic Epidemiology, German Cancer Research Center, DKFZ, Heidelberg, Germany, ³Center for Family Medicine, Karolinska Institute, Huddinge, Sweden, ⁴Department of Gynaecology and Obstetrics, Klinikum rechts der Isar at the Technical University, Munich, Germany, ⁵Division of Molecular Gynaeco-Oncology, Department of Gynaecology and Obstetrics, Clinical Center University of Cologne, Germany, ⁶Center of Molecular Medicine Cologne (CMMC), University Hospital of Cologne, Germany, ⁷Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany and ⁸Institute of Transfusion Medicine and Immunology, Red Cross Blood Service of Baden-Württemberg-Hessia, University of Heidelberg, Faculty of Clinical Medicine, Mannheim, Germany

Email: Bernd Frank* - b.frank@dkfz.de; Kari Hemminki - k.hemminki@dkfz.de; Alfons Meindl - alfons.meindl@lrz.tu-muenchen.de; Barbara Wappenschmidt - barbara.wappenschmidt@uk-koeln.de; Christian Sutter - Christian.Sutter@med.uni-heidelberg.de; Marion Kiechle - marion.kiechle@lrz.tum.de; Peter Bugert - p.bugert@blutspende.de; Rita K Schmutzler - rita.schmutzler@uk-koeln.de; Claus R Bartram - Cr.Bartram@med.uni-heidelberg.de; Barbara Burwinkel - b.burwinkel@dkfz.de

* Corresponding author

Published: 15 May 2007

Received: 31 January 2007

BMC Cancer 2007, 7:83 doi:10.1186/1471-2407-7-83

Accepted: 15 May 2007

This article is available from: <http://www.biomedcentral.com/1471-2407/7/83>

© 2007 Frank 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

Background: Inactivating and truncating mutations of the nuclear BRCA1-interacting protein 1 (BRIP1) have been shown to be the major cause of Fanconi anaemia and, due to subsequent alterations of BRCA1 function, predispose to breast cancer (BC).

Methods: We investigated the effect of BRIP1 -64G>A and Pro919Ser on familial BC risk by means of TaqMan allelic discrimination, analysing BRCA1/BRCA2 mutation-negative index patients of 571 German BC families and 712 control individuals.

Results: No significant differences in genotype frequencies between BC cases and controls for BRIP1 -64G>A and Pro919Ser were observed.

Conclusion: We found no effect of the putatively functional BRIP1 variants -64G>A and Pro919Ser on the risk of familial BC.

Background

Germline mutations in the high-penetrance genes *BRCA1* and *BRCA2* account for up to 25% of the hereditary forms of breast cancer (BC) [1,2]. The nuclear BRCA1-interacting protein 1 (BRIP1; also referred to as BRCA1-associated C-terminal helicase, BACH1) directly binds the BRCT-motif containing domain of BRCA1, thus likely contributing to its DNA repair and tumour suppressor functions

[3,4]. *BRIP1* deficiency has been described as the causation for cancer-predisposing Fanconi anaemia [5,6], and a recent study has identified constitutional truncating *BRIP1* mutations to confer susceptibility to BC [7].

Several genotyping studies have addressed the association between BRIP1 variants and BC risk, but the results have remained controversial [8-11]. The non-conservative

BRIP1 Pro919Ser substitution has been previously reported to be associated with an increased BC risk up to age 50 [12]. We evaluated the effects of BRIP -64G>A, which may affect gene regulation, and Pro919Ser, on a large German familial BC study cohort.

Methods

Study population

The familial breast cancer cohort comprised 571 unrelated German, female index cases (19 to 87 years of age; median 45) without deleterious *BRCA1* and *BRCA2* mutations. They were collected during the years 1996–2005 through the Institute of Human Genetics (Heidelberg, Germany), the Department of Gynaecology and Obstetrics (Cologne, Germany) and the Department of Medical Genetics (Munich, Germany). According to the German Consortium for Hereditary Breast and Ovarian Cancer, breast cancer cases are classified into six categories based on family history: (A1) families with two or more breast cancer cases including at least two cases with onset below the age of 50 years; (A2) families with at least one male breast cancer case; (B) families with at least one breast cancer and one ovarian cancer case; (C) families with at least two breast cancer cases including one case diagnosed before the age of 50 years; (D) families with at least two breast cancer cases diagnosed after the age of 50 years; (E) single cases of breast cancer with age of diagnosis before 35 years [2]. All women gave written consent to the molecular analysis of the *BRCA1* and *BRCA2* genes and potential new breast cancer susceptibility genes. Prior to DNA extraction, their blood (EDTA) was frozen at -20°C. The DNA was isolated by a conventional phenol-chloroform protocol. Mutations in the open reading frame of *BRCA1* and *BRCA2* were excluded by applying denaturing high performance liquid chromatography (DHPLC) on all exons, followed by direct sequencing of conspicuous exons. The control series consisted of 712 healthy, unrelated female blood donors (26 to 68 years of age, median 49). They were recruited in 2004 and 2005 by the Institute of Transfusion Medicine and Immunology (Mannheim, Germany) and share the ethnic background with the breast cancer patients. According to the German guidelines for blood donation, all blood donors were examined by a standard questionnaire. Buffy coat samples were taken from the anti-coagulated blood donations and were used for DNA isolation (FlexiGene® DNA Kit; Qiagen, Hilden Germany). All blood donors consented to the use of their samples for research studies. The study was approved by the Ethics Committee of the University of Heidelberg (Heidelberg, Germany).

Genotyping

BRIP1 genotyping was done by the TaqMan allelic discrimination method as previously described [13]. TaqMan primers and probes were provided by the assay-by-

design service (Applied Biosystems, Foster City, CA) and designed on the basis of the GenBank [NT_010783](#) sequence. Sequences of primers and probes are available upon request.

Statistical analysis

Genotype-specific odds ratios (ORs), 95% confidence intervals (95% CIs) and *P* values were computed by unconditional logistic regression using the Statistical Analysis System software (Version 9.1.; SAS Institute Inc., Cary, NC). Haplotypes were inferred using the SNPHAP programme created by D. Clayton [14]. Power calculation was carried out with the power and sample size calculation software PS version 2.1.31 [15].

Transcription factor search

The search for putative transcription factors was performed using TESS (Transcription Element Search Software, [16]) and TFSEARCH (Searching Transcription Factor Binding Sites, [17]).

Results and discussion

Mutations in the DNA helicase *BRIP1* have been implicated in the aetiology of Fanconi anaemia, a genetic disorder that is characterised by congenital abnormalities, progressive bone marrow failure, genomic instability and predisposition to cancer [5-7]. Our study assessed the relevance of the *BRIP1* variants -64G>A and Pro919Ser to familial BC. According to transcription factor binding site searches, the replacement of G by A at position -64 leads to the formation of a GATA or CCAAT motif, suggesting an modification of gene expression. *BRIP1* Pro919Ser is located in the *BRCA1*-interacting domain and may alter protein structure and function.

Genotype frequencies for the analysed polymorphisms were in agreement with Hardy-Weinberg expectations in controls. No significant differences in genotype frequencies between BC cases and controls for either *BRIP1* -64G>A or Pro919Ser were observed (see Table 1). Adjustment for age made no significant difference to the results, hence only unadjusted ORs are presented. Our findings are in accord with previously published data [8,11]. Though a recent kin-cohort study has shown a strong association with 4.5- to 6.9-fold familial BC risk for Pro919Ser in premenopausal women [12], our data do not support the observed effect when stratified according to age at diagnosis (see Table 1). Haplotype analysis with *BRIP1* -64G>A and Pro919Ser did not indicate any association with familial BC risk (data not shown).

The strengths of the present study are represented by a sound sample size and a homogeneous study cohort of a single ethnic group, comprising women selected for familial BC. Only *BRCA1* and *BRCA2* mutation-negative famil-

Table 1: Genotype distributions of the BRIP1 variants -64G>A (rs2048718§) and Pro919Ser (rs4986764§) among unrelated German BRCA1/2 mutation-negative familial breast cancer patients and healthy, unrelated female control subjects

BRIP -64G>A	CASES		CONTROLS		OR*	95% CI	P
	n	%	n	%			
All Σ	571		712				
GG	181	31.7	228	32.0	1		
GA	283	49.6	340	47.8	1.05	0.82–1.35	0.71
AA	107	18.7	144	20.2	0.94	0.68–1.29	0.68
GA+AA	390	68.3	484	68.0	1.02	0.80–1.29	0.90
< 50 years Σ	406		367				
GG	132	32.5	113	30.8	1		
GA	190	46.8	180	49.0	0.90	0.65–1.25	0.54
AA	84	20.7	74	20.2	0.97	0.65–1.45	0.89
GA+AA	274	67.5	254	69.2	0.92	0.68–1.25	0.61
50 years Σ	165		345				
GG	49	29.7	115	33.3	1		
GA	93	56.4	160	46.4	1.36	0.90–2.08	0.15
AA	23	13.9	70	20.3	0.77	0.43–1.37	0.38
GA+AA	116	70.3	230	66.7	1.18	0.79–1.77	0.41
BRIP Pro919Ser							
All Σ	571		712				
Pro/Pro	181	31.7	226	31.7	1		
Pro/Ser	295	51.7	365	51.3	1.01	0.79–1.29	0.94
Ser/Ser	95	16.6	121	17.0	0.98	0.70–1.37	0.91
Pro/Ser+Ser/Ser	390	68.3	486	68.3	1.00	0.79–1.27	0.97
< 50 years Σ	406		367				
Pro/Pro	121	29.8	118	32.2	1		
Pro/Ser	219	53.9	182	49.6	1.17	0.85–1.62	0.33
Ser/Ser	66	16.3	67	18.3	0.96	0.63–1.47	0.85
Pro/Ser+Ser/Ser	285	70.2	249	67.8	1.12	0.82–1.51	0.48
50 years Σ	165		345				
Pro/Pro	60	36.4	108	31.3	1		
Pro/Ser	76	46.1	183	53.0	0.75	0.49–1.13	0.17
Ser/Ser	29	17.6	54	15.7	0.97	0.56–1.68	0.90
Pro/Ser+Ser/Ser	105	63.6	237	68.7	0.80	0.54–1.18	0.26

*As compared to GG and Pro/Pro homozygotes, respectively; § dbSNP rs#; Hardy-Weinberg equilibrium test was undertaken using Pearson's goodness-of-fit chi-square test with one degree of freedom. Adjustment for age did not change the ORs, assuming that the distribution of the BRIP1 genotypes is age-independent.

ial BC cases were considered in order to avoid effects caused by these high-penetrance susceptibility genes. With the present sample size, we had a power of 80% at a significance level of 0.05 to detect an OR of ≥ 1.44 for both -64G>A and Pro919Ser. Moreover, the power of an association study based on cases with a family history of the disease is at least twice higher compared to a study using unselected cases [14]. As data of well-known risk factors (age of menarche, history of pregnancy etc.) were not available, we ignored to test for gene-environment interaction.

Conclusion

Both the BRIP1 -64G>A and Pro919Ser variants show no effect on familial BC risk in the German population.

Abbreviations

BC – breast cancer

BACH1 – BRCA1-associated C-terminal helicase

BRIP1 – BRCA1-interacting protein 1

DHPLC – denaturing high performance liquid chromatography

95% C.I. – 95% confidence interval

OR – odds ratio

SNP – single nucleotide polymorphism

Competing interests

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

Authors' contributions

BF conducted the experiments, performed data acquisition and interpretation, and drafted the manuscript. KH participated in the study coordination and revised the manuscript. AM, BW, CS, MK, PB, RKS and CRB collected DNA samples and were responsible for the BRCA1/BRCA2 mutation screening. BB designed and coordinated the study and reviewed the manuscript. All authors read and approved the final version of the submitted manuscript.

Acknowledgements

The German breast cancer samples were collected within a project funded by the Deutsche Krebshilfe. It was supported by the Center of Molecular Medicine Cologne (CMCC), and the EU, LSHC-CT-2004-503465.

References

1. Venkitaraman AR: **Cancer susceptibility and the functions of BRCA1 and BRCA2.** Cell 2002, **108**:171-182.
2. Meindl A, the German Consortium for Hereditary Breast and Ovarian Cancer: **Comprehensive analysis of 989 patients with breast or ovarian cancer provides BRCA1 and BRCA2 mutation**

- profiles and frequencies for the German population. *Int J Cancer* 2002, **97**:472-480.
3. Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DC, Sgroi DC, Lane WS, Haber DA, Livingston DM: **BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function.** *Cell* 2001, **105**:149-160.
 4. Cantor S, Drapkin R, Zhang F, Lin Y, Han J, Pamidi S, Livingston DM: **The BRCA1-associated protein BACH1 is a DNA helicase targeted by clinically relevant inactivating mutations.** *Proc Natl Acad Sci USA* 2004, **101**:2357-2362.
 5. Levran O, Attwooll C, Henry RT, Milton KL, Neveling K, Rio P, Batish SD, Kalb R, Velleuer E, Barral S, Ott J, Petrini J, Schindler D, Hanenberg H, Auerbach AD: **The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia.** *Nat Genet* 2005, **37**:931-933.
 6. Mathew CG: **Fanconi anaemia genes and susceptibility to cancer.** *Oncogene* 2006, **25**:5875-5884.
 7. Seal S, Thompson D, Renwick A, Elliott A, Kelly P, Barfoot R, Chagtai T, Jayatilake H, Ahmed M, Spanova K, North B, McGuffog L, Evans DG, Eccles D, Breast Cancer Susceptibility Collaboration (UK), Easton DF, Stratton MR, Rahman N: **Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles.** *Nat Genet* 2006, **38**:1239-1241.
 8. Vahteristo P, Yliannala K, Tamminen A, Eerola H, Blomqvist C, Nevanlinna H: **BACH1 Ser919Pro variant and breast cancer risk.** *BMC Cancer* 2006, **6**:19.
 9. Lewis AG, Flanagan J, Marsh A, Pupo GM, Mann G, Spurdle AB, Lindeman GJ, Visvader JE, Brown MA, Chenevix-Trench G, Kathleen Cunningham Foundation Consortium for Research into Familial Breast Cancer: **Mutation analysis of FANCD2, BRIP1/BACH1, LMO4 and SFN in familial breast cancer.** *Breast Cancer Res* 2005, **7**:R1005-1016.
 10. Karppinen SM, Vuosku J, Heikkinen K, Allinen M, Winqvist R: **No evidence of involvement of germline BACH1 mutations in Finnish breast and ovarian cancer families.** *Eur J Cancer* 2003, **39**:366-371.
 11. Rutter JL, Smith AM, Davila MR, Sigurdson AJ, Giusti RM, Pineda MA, Doody MM, Tucker MA, Greene MH, Zhang J, Struwing JP: **Mutational analysis of the BRCA1-interacting genes ZNF350/ZBRK1 and BRIP1/BACH1 among BRCA1 and BRCA2-negative probands from breast-ovarian cancer families and among early-onset breast cancer cases and reference individuals.** *Hum Mutat* 2003, **22**:121-128.
 12. Sigurdson AJ, Hauptmann M, Chatterjee N, Alexander BH, Doody MM, Rutter JL, Struwing JP: **Kin-cohort estimates for familial breast cancer risk in relation to variants in DNA base excision repair, BRCA1 interacting and growth factor genes.** *BMC Cancer* 2004, **4**:9.
 13. Frank B, Hemminki K, Wirtenberger M, Bermejo JL, Bugert P, Klaes R, Schmutzler RK, Wappenschmidt B, Bartram CR, Burwinkel B: **The rare ERBB2 variant Ile654Val is associated with an increased familial breast cancer risk.** *Carcinogenesis* 2005, **26**:643-647.
 14. David Clayton [<http://www.gene.cimr.cam.ac.uk/clayton/>]
 15. PowerSampleSize [<http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>]
 16. TESS : Transcription Element Search System [<http://www.cbil.upenn.edu/tess>]
 17. TFSEARCH [<http://www.cbrc.jp/research/db/TFSEARCH.html>]
 18. Antoniou AC, Easton DF: **Polygenic inheritance of breast cancer: Implications for design of association studies.** *Genet Epidemiol* 2003, **25**:190-202.

Pre-publication history

The pre-publication history for this paper can be accessed here:

<http://www.biomedcentral.com/1471-2407/7/83/prepub>

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

