European Journal of Human Genetics (2003) 11, 464–467

www.nature.com/ejhg

ARTICLE

Similar contributions of *BRCA1* and *BRCA2* germline mutations to early-onset breast cancer in Germany

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This study was undertaken to investigate the prevalence of *BRCA1* and *BRCA2* germline mutations in 91 German patients unselected for family history, who were diagnosed with breast cancer before the age of 41 years. Clinical information and blood samples were obtained from all patients. A comprehensive *BRCA1* and *BRCA2* mutational analysis was performed using the protein truncation assay and single-strand conformational polymorphism analysis followed by DNA sequencing of variant signals detected by these assays. Five different deleterious germline mutations including four frameshift mutations and one missense mutation were identified, three in *BRCA1* (3.3%) and two mutations (2.2%) in *BRCA2*. Both *BRCA2* mutations are novel and might be specific for the German population. An additional *BRCA1* missense mutation previously described and classified as an unknown variant was found. This mutation was also detected in two breast cancer patients of family P 328 and not in 140 healthy controls suggesting that it is disease associated. In addition, one common polymorphism and five novel intronic sequence variants with unknown significance were found. Our findings show that mutations in *BRCA1* and *BRCA2* may contribute similarly to early-onset breast cancer in Germany. Given current constraints on health-care resources, these results support the notion that *BRCA1* and *BRCA2* mutation screening may have the strongest impact on health-care when targeted to high-risk populations.
European Journal of Clinical Nutrition (2003) **11,** 464–467. doi:10.1038/sj.ejhg.5200988

Keywords: *BRCA1/2*; germline mutations; early-onset breast cancer; German population

Approximately 5% of breast and ovarian cancers are because of highly penetrant germline mutations in cancer predisposing genes. Two genes, *BRCA1*[1] and *BRCA2*,[2,3] account for at least half of these cases.[4] Individuals with mutations in these genes show increased lifetime risks of female breast cancer and ovarian cancer, and also prostate cancer and cancers at various other sites.[5] In Germany, most information on the prevalence of *BRCA1* and *BRCA2* mutations has come from research on high-risk families selected for multiple occurrences of breast and ovarian cancer in multiple generations.[6–10] Little is known about the prevalence of these mutations in other patient populations. Two previous studies analysing the prevalence of *BRCA1* and *BRCA2* mutations in German women with early-onset breast cancer (aged younger or equal to 35 and 40 years of age, respectively) have estimated a mutation frequency of 8% for *BRCA1* and somewhere between 4 and 12.5% for *BRCA2*.[10,11] Both studies,

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Received 6 September 2002; revised 24 January 2003; accepted 11 February 2003

Table 1 Clinical and histopathological characteristics of the 91 German early-onset breast cancer patients

Characteristics	*Number of patients (%)*
Age of onset (years)	
21–30	16 (18)
31–35	28 (31)
36–40	47 (52)
Family history of breast cancer	
None	50 (55)
At least one first-degree relative	15 (16)
At least one second-degree relative	8 (9)
Unknown	18 (20)
Tumour histology	
Ductal carcinoma in situ	4 (4)
Infiltrating ductal	69 (76)
Medullary	9 (10)
Other	9 (10)
Pathologic stage	
0	4 (4)
I	27 (30)
II	49 (54)
III	9 (10)
IV	2 (2)
Histological grade	
Non-high grade (1, 2)	32 (35)
High grade (3, 4)	59 (65)
Lymph node status	
0	42 (46)
1–3	25 (27)
4–9	6 (7)
>10	13 (14)
Unknown	5 (5)
Oestrogen/progesterone receptor status[a]	
Positive	52 (57)
Negative	31 (34)
Unknown	8 (9)
Laterality	
Unilateral	90 (99)
Bilateral	1 (1)

[a]Positive: $\geqslant 20$ fmol/mg or IRS$\geqslant 1$; negative: < 20 fmol/mg or IRS=0.

however, have been limited by a small number of cases (40 and 45, respectively), and only one focused on *BRCA2*.

In this study, we searched for germline mutations in *BRCA1* and *BRCA2* in 91 women unselected for family history who were diagnosed with primary invasive or *in situ* breast cancer before the age of 41 years at the Städtisches Klinikum Karlsruhe between August 1993 and November 2001 and at the Women's Clinic, University of Heidelberg between July 1992 and April 1994 and September 1997 to August 1999, and with at least one parent with German ancestry. The mean age at onset of the disease of the study participants was 35 years (range 21–40 years). Written informed consent was obtained from all study participants. Of these patients, 53 were previously included in a screen for the 6 kb exon 13 duplication of the *BRCA1* gene.[12] Cancer diagnosis for each patient was confirmed by pathology reports in all cases. Family histories obtained from medical records and/or self-reported questionnaires were available from 73 cases. A family history was defined by at least one first- or second-degree female relative with breast or ovarian cancer. In all, 50 patients had no family history, 23 patients had a family history and no information was available from the remaining 18 patients. The clinical and pathological features of the study participants are shown in Table 1.

The complete coding regions of the *BRCA1* and *BRCA2* genes were screened as described previously.[7–9] Genomic DNA from peripheral blood lymphocytes was used as the source of DNA for gene analysis. *BRCA1* exon 11 and *BRCA2* exons 10 and 11 were screened using the protein truncation test (PTT), and the remaining exons were screened by single-strand conformational polymorphism analysis (SSCP). Samples revealing variant bands were analysed using direct DNA sequencing. Two by two contingency tables were tested using Fisher's exact test. Two-sided *P*-values of 0.05 or less were considered significant.

Five different deleterious germline mutations were identified (5/91, 5.5%; 95% CI 1.8–12.4): three in *BRCA1* (3/91, 3.3%; 95% CI 0.7–9.3) and two in *BRCA2* (2/91, 2.2%; 95% CI 0.3-7.7) (Table 2a, b). Both *BRCA2* mutations were novel and have not been previously described in other populations. They were not detected in 60 controls. The mean ages at diagnosis of breast cancer in *BRCA1* mutation carriers ($n=3$) and *BRCA2* mutation carriers ($n=2$) were 36 and 38.5 years, respectively. The mutations included four frameshift mutations predicted to cause premature termination codons at positions 1163 and 1829 of *BRCA1* and at codons 1200 and 1229 of *BRCA2* and one *BRCA1* missense mutation predicted to destroy the protein RING-finger.

Five additional rare *BRCA1* sequence variants were also identified: one missense mutation in exon 8 and four novel sequence variants in introns 13, 20, 22 and 24 (Table 2c). One sequence variant may be of functional importance. The missense mutation, Y179C, previously reported to the Breast Cancer Information Core database (BIC)[13], affects the tyrosine residue at codon 179 that is conserved among mouse, dog and rat homologues.[14,15] This mutation was also identified in the German breast cancer family P 328 (U Hamann, unpublished data). In this family, two females diagnosed with breast cancer at the ages of 51 and 67 years harboured the mutation, whereas one female diagnosed with breast cancer at the age of 34 years did not. It was not detected in 140 healthy controls. In *BRCA2*, a common K3326X nonsense mutation in exon 27 with uncertain significance and a novel nucleotide change in intron 26 were identified (Table 2d). All intronic *BRCA1* and *BRCA2* changes were assessed for their potential to affect RNA

Table 2 *BRCA1* and *BRCA2* germline mutations in German early-onset breast cancer patients

Patient	*Position*	*Nucleotide change*	*Effect*	*Type*[a]	BIC[b] 2002	*Age at diagnosis (years)*	*Family history of breast or ovarian cancer (age at diagnosis)*	*Other family history of cancer (age at diagnosis)*
(a) *BRCA1* mutations detected in women with breast cancer diagnosed before 41 years								
P 465	Exon 5	300T>G	C61G	MS	67	28/28	Mother, breast (33); paternal grandmother, breast (41/68)	None
R 1018	Exon 11	3600delGAAGATACTAG	1163X	FS	20	40	Mother, breast (25); maternal aunt, breast (54)	None
R 1198	Exon 20	5382insC	1829X	FS	268	40	Mother, breast (39); maternal aunt, breast (40); maternal grandmother, breast (55)	Maternal uncle, stomach (49)
(b) *BRCA2* mutations detected in women with breast cancer diagnosed before 41 years								
R 701	Exon 11	3827delGT	1200X	FS	0	38	None	Maternal grandmother, gynaecological (65)
R 423	Exon 11	3873delGTinsTAAAAAG	1229X	FS	0	39	None	Father, larynx (53); maternal grandmother, pancreas (70)
(c) Sequence variants in *BRCA1*								
P 476	Exon 8	655A>G	Y179C	MS	9	39	None	None
R 177	Intron 13	IVS13 −37G>A	—	UV	0	38	None	None
P 479	Intron 20	IVS20 +59GTATTCCACTCC	—	UV	0	34	Paternal grandmother, ovarian or cervix (71), breast (77)	Father, colon; paternal grandfather, lung (79)
BKS 275	Intron 22	IVS22 –25T>A	—	UV	0	36	Unknown	Unknown
R 751	Intron 24	IVS24 +38A>G	—	UV	0	22	None	None
(d) Sequence variants in *BRCA2*								
P 409	Intron 26	IVS26 +79delA	—	UV	0	29	None	Other cancers
R 177	Exon 27	10204A>T	3326X	UV	156	38	None	None

[a]FS: frameshift mutation; MS: missense mutation; NS: nonsense mutation; UV: unclassified variant.
[b]BIC: Breast Cancer Information Core database.[13]

splicing using the algorithm available at the University of California, Berkley web site (http://www.fruitfly.org/seq_tools/splice.html). None was deemed to affect splicing efficiency.

Among the five women with deleterious *BRCA* mutations, all three women harbouring a *BRCA1* mutation had a strong family history of breast cancer with at least two breast cancer cases among first- and second-degree relatives (Table 2a). The mother of patient P 465 was diagnosed with breast cancer at the age of 33 years and the paternal grandmother with bilateral disease at the ages of 41 and 68 years. As parental blood samples were available from the parents, the mutation was shown to be transmitted from the father. In the family of patient R 1018, the mother and a maternal aunt were diagnosed with breast cancer at the ages of 25 and 54 years, respectively. In this family the mutation most likely was inherited from the deceased mother, as the father did not carry the mutation. The mutation was also not detected in the affected aunt, indicating that she is a sporadic case. In the family of patient R 1198, the mother, a maternal aunt and the maternal grandmother were diagnosed with breast cancer at the ages of 39, 40 and 55 years, respectively. Patient R 423 carrying a *BRCA2* mutation had no family history of breast/ovarian cancer (Table 2b). She was diagnosed with ovarian cancer at the age of 40 years, 1 year after the diagnosis of breast cancer.

In this study, similar mutation frequencies of 3.3% for *BRCA1* and 2.2% for *BRCA2* ($p=1$) were found in German breast cancer patients diagnosed before the age of 41 years and unselected for family history of the disease. Our mutation frequencies are comparable with those previously reported in other populations without strong founder effects. The mutation frequencies in various population-based studies in Britain, Australia, and North America were 3.5% in 254 cases under 36[16], 4.1% in 57 cases under 35[17], and 3.8% in 91 cases under 40[18] for *BRCA1* and 2.7% in 73 cases under 33[19], and 2.4% in 254 cases under 36 for *BRCA2*.[16] Some other studies estimated higher mutation frequencies ranging from 5.9 to 7.5% in

234 cases under 41 from Sweden[20] and in 80, 193 and 203 cases under 35 from North America[21–23] for *BRCA1* and from 8.3% to 12.5% in 57 cases under 35 from Britain[17] and in 40 cases under 40 from Germany[11] for *BRCA2*. The different mutation frequencies within and between various populations may be explained by differences in the selection criteria, the sensitivity of the mutation detection or both. Further, estimates may be imprecise because of the small number of mutation carriers. However, it is also possible that these different frequencies may reflect real differences in the contribution of *BRCA1* and *BRCA2* mutations to early-onset breast cancer in populations that vary from one another in their racial and ethnic backgrounds.

The mutation frequencies observed in this study are likely to represent underestimates. Some of the *BRCA1* sequence variants may be classified incorrectly as not disease-causing. In addition, we cannot exclude the possibility that some mutations have been missed by the mutation detection strategy adopted. Furthermore, the mutation frequencies may be higher as we did not screen for large genomic deletions and mutations in the promoter region.

Our findings show that mutations in the *BRCA1* and *BRCA2* genes may contribute similarly to early-onset breast cancer in Germany. Only a small proportion of early-onset breast cancer patients carry mutations in one or the other gene. Given current constraints on health-care resources, these results support the notion that *BRCA1* and *BRCA2* mutation screening may have the strongest impact on health-care when targeted to high-risk populations.

Acknowledgements

We are grateful to all patients for their participation in this study. This study was in part supported by the Tumorzentrum Heidelberg/ Mannheim D.10029190. We thank Rodney J Scott for a critical reading of the manuscript and Antje Seidel-Renkert, Michaela Schleicher and Michael Gilbert for expert technical assistance.

References

1 Miki Y, Swensen J, Shattuck-Eidens D *et al*: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994; **266**: 66–71.
2 Wooster R, Bignell G, Lancaster J *et al*: Identification of the breast cancer susceptibility gene BRCA2 [published erratum appears in *Nature* 1996; **379:** 749]. *Nature* 1995; **378:** 789–792.
3 Tavtigian SV, Simard J, Rommens J *et al*: The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds [see comments]. *Nat Genet* 1996; **12**: 333–337.
4 Ford D, Easton DF, Stratton M *et al*: Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. *Am J Hum Genet* 1998; **62**: 676–689.
5 The Breast Cancer Linkage Consortium: Cancer risks in BRCA2 mutation carriers. *J Natl Cancer Inst* 1999; **91:** 1310–1316.
6 Jandrig B, Grade K, Seitz S *et al*: BRCA1 mutations in German breast-cancer families. *Int J Cancer* 1996; **68**: 188–192.
7 Hamann U, Brauch H, Garvin AM, Bastert G, Scott RJ: German family study on hereditary breast and/or ovarian cancer: germline mutation analysis of the *BRCA1* gene. *Genes Chrom Cancer* 1997; **18**: 126–132.
8 Hamann U, Haner M, Stosiek U, Bastert G, Scott RJ: Low frequency of *BRCA1* germline mutations in 45 German breast/ ovarian cancer families. *J Med Genet* 1997; **34**: 884–888.
9 Hamann U, Liu X, Lange S, Ulmer HU, Benner A, Scott RJ: Contribution of *BRCA2* germline mutations to hereditary breast/ ovarian cancer in Germany. *J Med Genet* 2002; **39**: e12.
10 Meindl A: 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.
11 Plaschke J, Commer T, Jacobi C, Schackert HK, Chang-Claude J: BRCA2 germline mutations among early onset breast cancer patients unselected for family history of the disease. *J Med Genet* 2000; **37**: e17.
12 The BRCA1 Exon 13 Duplication Screening Group: The exon 13 duplication in the BRCA1 gene is a founder mutation present in geographically diverse populations. *Am J Hum Genet* 2000; **67**: 207–212.
13 Breast Cancer Information Core (BIC) database [http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/]. 2002.
14 Chen KS, Shepel LA, Haag JD, Heil GM, Gould MN: Cloning, genetic mapping and expression studies of the rat Brca1 gene. *Carcinogenesis* 1996; **17**: 1561–1566.
15 Szabo CI, Wagner LA, Francisco LV *et al*: Human, canine and murine BRCA1 genes: sequence comparison among species. *Hum Mol Genet* 1996; **5**: 1289–1298.
16 Peto J, Collins N, Barfoot R *et al*: Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. *J Natl Cancer Inst* 1999; **91**: 943–949.
17 Anglian Breast Cancer Study Group: Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. *Br. J. Cancer* 2000; **83**: 1301–1308.
18 Southey MC, Tesoriero AA, Andersen CR *et al*: BRCA1 mutations and other sequence variants in a population-based sample of Australian women with breast cancer. *Br J Cancer* 1999; **79**: 34–39.
19 Krainer M, Silva-Arrieta S, FitzGerald MG *et al*: Differential contributions of BRCA1 and BRCA2 to early-onset breast cancer. *N Engl J Med* 1997; **336**: 1416–1421.
20 Loman N, Johannsson O, Kristoffersson U, Olsson H, Borg A: Family history of breast and ovarian cancers and BRCA1 and BRCA2 mutations in a population-based series of early-onset breast cancer. *J Natl Cancer Inst* 2001; **93**: 1215–1223.
21 Langston AA, Malone KE, Thompson JD, Daling JR, Ostrander EA: BRCA1 mutations in a population-based sample of young women with breast cancer. *N Engl J Med* 1996; **334**: 137–142.
22 Malone KE, Daling JR, Thompson JD, O'Brien CA, Francisco LV, Ostrander EA: BRCA1 mutations and breast cancer in the general population: analyses in women before age 35 years and in women before age 45 years with first-degree family history. *JAMA* 1998; **279**: 922–929.
23 Malone KE, Daling JR, *et al*: Frequency of BRCA1/BRCA2 mutations in a population-based sample of young breast carcinoma cases. *Cancer* 2000; **88**: 1393–1402.