

German family study on hereditary breast-ovarian cancer

Ute Hamann, Heiko Becher, Thomas Zimmermann, Kristina Pella, Gunther Bastert, Jenny Chang-Claude

Abstract

An estimated 5% to 10% of all breast and ovarian cancers are attributed to dominant susceptibility genes. Two such genes, BRCA1 and BRCA2, were recently identified. The involvement of these genes was studied in 43 German breast only and breast-ovarian cancer families. All families contained three or more cases of breast or ovarian cancer, with at least two diagnosed under the age of 60 years. Multipoint linkage analysis gave a maximum lod score of 2.13 at the BRCA1 locus under the assumption of genetic heterogeneity, with an estimated 50% of families being linked. Among the 33 breast only cancer and 10 breast-ovarian cancer families, the estimated proportions of linked families were 35% and 75%, respectively. Sixteen families with at least four cases of female breast cancer diagnosed under the age of 60 years, or male breast cancer diagnosed at any age, were analysed for linkage to BRCA2. Positive lod scores at BRCA2 were obtained in six families.

(*J Med Genet* 1996;33:633-635)

Key words: breast/ovarian cancer; linkage analysis; BRCA1; BRCA2.

Breast cancer is the most common and ovarian cancer is the fourth leading malignancy among women in western countries. A small proportion of breast cancers and ovarian cancers, in particular those with an early age of onset, are the result of genetic predisposition by inheritance of dominant susceptibility genes.

Genetic epidemiological studies have provided evidence for at least two genes conferring inherited susceptibility to breast and ovarian cancer. These are the BRCA1 gene on chromosome 17q21¹⁻³ and the BRCA2 gene on chromosome 13q12.⁴⁻⁵ Germline mutations in both genes have been identified in breast and ovarian cancer patients from families linked to BRCA1 and BRCA2, respectively, by using the protein truncation test, single strand conformational polymorphism analysis, and direct sequencing.⁵⁻¹⁰

BRCA1 germline mutations are estimated to account for 40% to 50% of early onset female breast cancer families and most breast and ovarian cancer families.¹¹⁻¹² The cumulative breast cancer risk in BRCA1 gene carriers has been estimated to be 51% by the age of 50 and 85% by the age of 70, and the ovarian cancer risk to be 23% by the age of 50 and 63% by the age of 70.¹³ Mutations in BRCA2 confer a similar risk to female breast cancer as BRCA1, with an estimated risk of 87% by the age of 80. However, the risk of male breast cancer appears to be substantially higher and the risk of ovarian cancer lower.⁴

Genetic heterogeneity concerning BRCA1 involvement has been observed in seven Icelandic and 11 Japanese breast/ovarian cancer families with 30% and 0% of families being linked, respectively.¹⁴⁻¹⁵ However, these estimates may be influenced by a selection bias.

In order to analyse genetic heterogeneity of breast cancer resulting from BRCA1 and BRCA2, 43 German breast/ovarian cancer families were analysed by linkage analysis.

Material and methods

FAMILIES

Families were collected from all regions in Germany. Kindreds were ascertained on the basis of the presence of breast cancer or ovarian cancer in at least three family members. Forty-three families fulfilling a further criterion of including at least two cases diagnosed under the age of 60 years were included in this study. The disease status of all other family members was ascertained by questionnaire and further contact by telephone. Pathology records were requested in every case and were available for 117 of the 176 breast cancer cases including three male breast cancer cases and 10 of the 15 ovarian cancer patients.

DNA ANALYSIS

Genomic DNA was isolated by proteinase K digestion and standard phenol/chloroform extraction. Genotypes were analysed at three to six chromosome 17q loci and three 13q loci. The loci analysed were D17S250, THRA1, D17S800, D17S855, D17S579, and D17S588 on chromosome 17q and D13S289, D13S260, and D13S267 on chromosome 13q.¹¹⁻¹⁶⁻¹⁷ All

Division of Molecular
Genome Analysis 0842,
Deutsches
Krebsforschungszentrum,
Im Neuenheimer Feld
280, D-69120
Heidelberg, Germany
U Hamann

Division of
Epidemiology,
Deutsches
Krebsforschungszentrum,
D-69120 Heidelberg,
Germany
H Becher
T Zimmermann
J Chang-Claude

Division of Molecular
Biology of the Cell II,
Deutsches
Krebsforschungszentrum,
D-69120 Heidelberg,
Germany
K Pella

Women's Clinic,
University of
Heidelberg, D-69115
Heidelberg, Germany
G Bastert

Correspondence to:
Dr Hamann.

Received 27 February 1996
Revised version accepted for
publication 4 April 1996

Table 1 Characteristics of the 43 families studied for linkage

	No of families	No of breast cancer cases/family*				
		2	3	4	5	6 +
Breast-ovarian cancer families	10	1	2	4	1	2
No of ovarian cases/family:						
1	7		1	4	1	1
2	2		1			1
3 +	1	1				
Breast only cancer families	33		15	10	4	4
Total	43					
No of female breast cancer cases with age of diagnosis <50:						
0-2	24	1	13	8	2	
3-4	14		4	6	2	2
5 +	5				1	4

* Including three male breast cancer cases in three breast only cancer families.

markers represented CA repeat polymorphisms with a high frequency of heterozygosity. PCR conditions for amplification were according to Weber and May.¹⁸ Alleles were separated on denaturing sequencing gels and blotted onto a nylon membrane. One of the PCR primers was end labelled with ($\gamma^{32}\text{P}$)-ATP using T4 polynucleotide kinase and hybridised to the amplified alleles on a nylon filter. Alleles were detected by autoradiography.

STATISTICAL METHODS

Multipoint linkage analyses were carried out using the LINKAGE program,^{19, 20} under the assumption of an autosomal dominant disease gene with a gene frequency of 0.003, conferring breast cancer risks corresponding to the model of Claus *et al.*²¹ and specific penetrances as implemented by Easton *et al.*¹¹ For BRCA1, lod scores were generated by multipoint analysis of D17S250 and D17S588, or THRA1 and D17S588, or D17S579 and the disease locus, and for BRCA2, D13S289 and D13S260, or D13S260 and D13S267 and the disease locus. Analyses of heterogeneity of linkage for various subgroups of families were performed using the HOMOG program.²²

Results

CLINICAL RESULTS

The 43 cancer families studied for linkage included 10 breast-ovarian cancer families and 33 breast only cancer families. Each family contained at least three breast or ovarian cancer patients of whom at least two were diagnosed before the age of 60 years. The age of onset of the disease ranged between 19 and 81 years for breast cancer and between 39 and 67 years for ovarian cancer. The average age at diagnosis was below the age of 45 years in 13 families, between 45 and 55 years in 22 families, and above 55 years in eight families. There were 176 breast cancers including three male breast cancers and 15 ovarian cancers in these families. The distribution of breast and ovarian cancers in these families is shown in table 1.

MULTIPOINT LINKAGE ANALYSIS AND GENETIC HETEROGENEITY

A total of 419 subjects including 114 cases with breast cancer and four patients with ovarian cancer were genotyped at three to six 17q loci and 170 subjects from 16 families at three 13q loci. Results from multipoint linkage

Table 2 Multipoint lod scores at BRCA1 and BRCA2

No	Family	Lod score at		Family type*
		BRCA1	BRCA2	
1	GF06	0.00	—	1
2	GF17	0.02	—	1
3	GF57	0.04	-0.27	1
4	GF66	0.04	—	1
5	GF42	0.05	—	1
6	GF63†	0.06	0.08	1
7	GF09	0.08	—	1
8	GF05	0.09	—	1
9	GF44	0.10	-0.07	1
10	GF106	0.10	—	1
11	GF56	0.12	-0.20	1
12	GF54	0.21	—	1
13	GF34	0.25	—	1
14	GF60	0.45	—	1
15	GF08	0.54	-0.75	1
16	GF74	0.60	-0.90	1
17	GF13	0.68	0.31	1
18	GF24	0.68	—	1
19	GF52	-0.03	—	1
20	GF30	-0.04	—	1
21	GF45	-0.08	—	1
22	GF38	-0.10	—	1
23	GF53	-0.16	—	1
24	GF32	-0.17	—	1
25	GF59	-0.19	—	1
26	GF67	-0.31	—	1
27	GF12	-0.33	—	1
28	GF33	-0.44	0.51	1
29	GF69†	-0.56	-0.60	1
30	GF41	-0.61	0.09	1
31	GF26	-0.83	—	1
32	GF37	-1.11	—	1
33	GF39†	-1.16	-0.18	1
34	GF19	0.25	-0.71	2
35	GF22	0.25	—	2
36	GF72	0.29	—	2
37	GF02	0.41	-0.50	2
38	GF36	0.78	0.67	2
39	GF35	1.14	—	2
40	GF65	-0.13	—	2
41	GF11	-0.21	0.34	2
42	GF48	-0.33	—	2
43	GF76	-1.06	-0.92	2

* 1 = breast only cancer family; 2 = breast-ovarian cancer family.

† Including a male breast cancer.

analyses at BRCA1 and BRCA2 are shown in table 2. Six breast-ovarian (GF 02, GF 19, GF 22, GF 35, GF 36, GF 72) and six breast only cancer families (GF 08, GF 13, GF 24, GF 34, GF 60, GF 74) yielded positive lod scores between 0.25 and 1.14 at the BRCA1 locus. One breast-ovarian cancer family (GF 76) and five breast only cancer families (GF 26, GF 37, GF 39, GF 41, GF 69) gave negative lod scores between -0.56 and -1.16. Of the remaining 25 families, lod scores were between -0.44 and 0.21.

The results of the multipoint heterogeneity analysis for BRCA1 are given in table 3. When the 43 families are considered together there is some evidence for genetic heterogeneity with an estimated 50% being linked (lod-1 support interval 10%-85%) and a maximum lod score of 2.13 at the BRCA1 locus. Among the breast-ovarian cancer families an estimated 75% were linked with a maximum lod score of 1.67, compared with 35% of the breast only cancer families with a maximum lod score of 0.68. The corresponding lod-1 support regions for both groups are large and span almost the total range for $\alpha \in [0,1]$.

Linkage analysis with BRCA2 markers yielded lod scores between 0.31 and 0.67 in two breast-ovarian cancer (GF 11, GF 36) and two breast only cancer families (GF 13, GF

Table 3 Multipoint heterogeneity analysis for BRCA1

Group (No of families)	Lod score	Proportion linked
All families (43)	2.13	0.50
Breast-ovarian cancer families (10)	1.67	0.75
Breast only cancer families (33)	0.68	0.35

33), between -0.5 and -0.92 in three breast-ovarian cancer families (GF 02, GF 19, GF 76) and three breast only cancer families (GF 08, GF 69, GF 74), and between -0.27 and 0.09 in the remaining families (table 2).

In two families (GF 13, GF 36) lod scores were positive at both BRCA1 and BRCA2. In both families lod scores at BRCA1 (0.68, 0.78) were greater than at BRCA2 (0.31, 0.67). Three families including two families with male breast cancers (39, 69, 76) yielded negative lod scores at both loci.

Discussion

The genetic analysis reported here provides some evidence for linkage of at least a proportion of breast only cancer families and breast-ovarian cancer families to either BRCA1 or BRCA2. Under the heterogeneity model the multipoint lod score was 2.13 at BRCA1 in 43 families. Six of 16 families studied yielded positive lod scores at the BRCA2 locus. There was some evidence of genetic heterogeneity with 35% breast only cancer families and 75% breast-ovarian cancer families estimated to be linked to BRCA1.

For most families, the individual lod scores were too small to allow a conclusion as to the presence or absence of linkage to either BRCA1 or BRCA2. Linkage evidence obtained in these families was weak either because the families were small or because affected subjects had died and no blood samples were available for genotype analysis. Interpretation of genetic linkage results may be further complicated by the presence of sporadic breast or ovarian cancer cases occurring in these families.

We found three breast only cancer families with weak evidence against linkage with BRCA1 as well as BRCA2 suggesting the existence of an additional gene(s) conferring susceptibility to breast cancer. The male breast cancer risk in BRCA2 mutation carriers has been reported to be small but probably greater than in male BRCA1 mutation carriers.⁴ Two of our families with weak evidence against linkage to both genes contained individual cases of affected males. These data suggest that another gene besides BRCA1 and BRCA2 may be involved in the pathogenesis of male breast cancer. The ovarian cancer risk was reported to be higher in BRCA1 mutation carriers than in BRCA2 mutation carriers. Two of our breast-ovarian cancer families including one case of ovarian cancer showed evidence for linkage to 13q loci suggesting that BRCA2 may be involved in these families.

Risk estimates in families with small lod scores will rely more heavily on the a priori probability of linkage. For this reason it is important to know with what probability

different types of families are linked to BRCA1. Our results of 35% linked breast only cancer families and 75% linked breast-ovarian cancer families are in agreement with the estimations of The Breast Cancer Linkage Consortium with a 45% a priori probability of linkage for breast only cancer families and an 85% a priori probability for breast-ovarian cancer families.^{11 12} Since predictive testing is only appropriate in families where the linkage evidence is conclusive, disease carriers in our collection of breast only cancer and breast-ovarian cancer families have to be identified by detection of BRCA1 and BRCA2 germline mutations.

We thank the family members for their participation and the clinicians and pathologists for their help in collecting the material. We also thank Dr Nancy Eby for critically reading the manuscript, Ursula Eilber and Andreas Beckenbach for excellent documentation and data management, and Claudia Steger, Thomas Bruch, and Thorsten Kuiper for expert technical assistance. This work was supported by the Deutsche Krebs-hilfe e V / Mildred Scheel Foundation.

- Hall JM, Lee MK, Newman B, *et al.* Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 1990; 50:1684-9.
- Narod SA, Feunteun J, Lynch HT, *et al.* Familial breast-ovarian cancer locus on chromosome 17q12-q23. *Lancet* 1991;338:82-3.
- 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.
- Wooster R, Neuhausen SL, Mangion J, *et al.* Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science* 1994;265:2088-90.
- Wooster R, Bignell G, Lancaster J, *et al.* Identification of the breast cancer susceptibility gene BRCA2. *Nature* 1995; 378:789-92.
- Castilla LH, Couch FJ, Erdos MR, *et al.* Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer. *Nature Genet* 1994;8:387-91.
- Friedman LS, Ostermeyer EA, Szabo CI, *et al.* Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nature Genet* 1994;8:399-404.
- Simard J, Tonin P, Durocher F, *et al.* Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families. *Nature Genet* 1994;8:392-8.
- Shattuck-Eidens D, McClure M, Simard J, *et al.* A collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene. Implications for pre-symptomatic testing and screening. *JAMA* 1995;273:535-41.
- Hogervorst FBL, Cornelis RS, Bout M, *et al.* Rapid detection of BRCA1 mutations by the protein truncation test. *Nature Genet* 1995;10:208-12.
- Easton DF, Bishop DT, Ford D, Crockford GP, Breast Cancer Linkage Consortium. Genetic linkage analysis in familial breast and ovarian cancer: Results from 214 families. *Am J Hum Genet* 1993;52:678-701.
- Narod SA, Ford D, Devilee P, *et al.* An evaluation of genetic heterogeneity in 145 breast-ovarian cancer families. *Am J Hum Genet* 1995;56:254-64.
- Easton DF, Ford D, Bishop T, Breast Cancer Linkage Consortium. Breast and ovarian cancer incidence in BRCA1-mutation carriers. *Am J Hum Genet*, 1995;56:265-71.
- Arason A, Barkardóttir R, Egilsson V. Linkage analysis of chromosome 17q markers and breast-ovarian cancer in Icelandic families, and possible relationship to prostatic cancer. *Am J Hum Genet* 1993;52:711-17.
- Inoue R, Fukutomi T, Ushijima T, Matsumoto Y, Sugimura T, Nagao M. Linkage analysis of BRCA1 in Japanese breast cancer families. *Jpn J Cancer Res* 1994;85:1233-9.
- Gyapay G, Morissette J, Vignal A, *et al.* The 1992-94 Génethon human genetic linkage map. *Nature Genet* 1994; 7:246-339.
- Anderson LA, Friedman L, Osborne-Lawrence S, *et al.* High-density genetic map of the BRCA1 region of chromosome 17q12-q21. *Genomics* 1993;17:618-23.
- Weber JL, May PE. Abundant class of DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet* 1989;44:388-96.
- Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;81:3443-6.
- Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 1984;36:460-5.
- Claus EB, Risch N, Thompson WD. Genetic analysis of breast cancer in the Cancer and Steroid Hormone Study. *Am J Hum Genet* 1991;48:232-42.
- Ott J. *Analysis of human genetic linkage*. Baltimore: Johns Hopkins University Press, 1985.