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The Majority of 22 Dutch High-Risk Breast Cancer Families Are due to Either BRCA1 or BRCA2

Tamara Peelen[a]
Renée S. Cornelis[a]
Margreethe van Vliet[a]
Anne Petrij-Bosch[a]
Anne-Marie Cleton-Jansen[b]
Hanne Meijers-Heijboer[c]
Jan G.M. Klijn[d]
Hans F.A. Vasen[e]
Cees J. Cornelisse[b]
Peter Devilee[a,b]

[a] Department of Human Genetics, and
[b] Department of Pathology, University of Leiden,
[c] Department of Clinical Genetics, Erasmus University, Rotterdam,
[d] Daniël den Hoed Cancer Center, Rotterdam,
[e] Foundation for the Detection of Hereditary Tumors, Leiden, The Netherlands

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Abstract
We have analyzed, by a combination of mutation and linkage analysis, the genetic basis of 22 breast cancer families in which at least 4 cases of either breast cancer diagnosed under the age of 60 or ovarian cancer had occurred. Chain-terminating mutations in BRCA1 were evidenced in 6 families, and posterior probabilities of >0.90 of being linked to BRCA1 in 3. The breast versus ovarian cancer ratio in these 9 families was approximately 2:1. Among the remaining 13 families, significant linkage to markers flanking BRCA2 was established in the admixture test with a maximum multipoint lod score of 3.38, but there was no statistical evidence for genetic heterogeneity. The breast:ovarian cancer ratio in these families was 7:1, suggesting BRCA2 confers a much lower risk for ovarian cancer than does BRCA1. These results suggest that BRCA2 will explain a significant proportion of hereditary breast cancer in the Netherlands, and, together with BRCA1, account for the majority of all high-risk families.

Introduction

Breast cancer is the most frequent malignancy among women in Western industrialized countries. About 5% of all cases are thought to have a hereditary basis [1]. The first breast cancer susceptibility gene, BRCA1, was mapped by linkage to 17q12–21 [2]. Subsequently, the Breast Cancer Linkage Consortium (BCLC) established that BRCA1 also strongly predisposes to ovarian cancer [3, 4]. Cancer risk analyses indicate that BRCA1 is a highly penetrant gene, conferring a breast cancer risk of 85% before age 70 and an ovarian cancer risk of 63% before age 70 [5]. In addition, genetic heterogeneity was revealed, indicating that only 45% of the families with predominantly breast cancer, were estimated to be due to BRCA1 [4]. This observation has been extended to breast-ovarian cancer families, of which at least 82% were estimated to be due to BRCA1 [6]. The gene structure of BRCA1 was found to consist of 24 exons, scattered over a 100-kb of genomic DNA, which encode a protein of 1,863 amino acids [7]. The great majority of mutations in BRCA1 lead to premature chain-termination during protein translation [8], which are readily screened out by the protein truncation test [9].

One peculiarity of the families showing evidence against linkage to BRCA1 was the presence of male breast cancer [10]. Significant linkage evidence for the presence of a second breast cancer susceptibility gene was obtained with markers for chromosome region 13q12–13, among 15 early-onset breast-ovarian cancer families, 6 of which

KARGER
E-Mail karger@karger.ch
Fax + 41 61 306 12 34
http://www karger.ch

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Dr. P. Devilee
Department of Human Genetics
University of Leiden
Wassenaarseweg 72
NL-2333 AL Leiden (The Netherlands)

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Table 1. Cancer incidence, lod scores, BRCA1 mutations and posterior probabilities of linkage for 22 breast cancer families

Family	Reference	Number of cases[1]				Maximum lod score		BRCA1		Posterior probability	
		BrCa	OvCa	Br+Ov	typed	13q	17q	mutation[2]	class[3]	BRCA1	BRCA2
EUR017	9, 13	4 (4)	2	0	3	nd[7]	1.17	1410insT			
RUL019	3, 9, 13	2 (2)	2	0	3	nd	0.99	310del93			
RUL021	9	4 (4)	0	0	4	nd	1.18	2312del5			
RUL047	9, 13	4 (4)	0	1	2	−0.83	−0.59	2457C > T			
RUL049	6, 9, 11, 13	5[4] (5)	3	0	5	−0.21	−1.11	2457C > T			
RUL077	9	4 (4)	0	1	4	nd	1.56	3938insG			
EUR021	13	8 (8)	0	0	6	−0.99	3.01[5]	nf	2	0.99	0.00
RUL105		0 (2)[6]	8	2	4	−1.27	0.82	nf	4	0.99	0.00
RUL005	3, 12	5 (5)	0	0	4	−0.48	1.31	nf	2	0.92	0.01
RUL024		6 (6)	0	0	5	1.65	−0.03	nf	2	0.01	0.99
RUL023	13	6[4] (4)	1	0	2	1.16	0.09	nf	2	0.01	0.98
RUL009	6	8 (6)	0	1	3	1.13	−2.01	nf	3	0.00	0.93
RUL016	3, 12	5 (5)	1	1	4	1.22	0.15	nf	4	0.26	0.83
RUL010	3, 12	9 (8)	0	0	3	−0.30	−0.31	nf	2	0.22	0.56
RUL070		8 (5)	0	0	7	0.76	−1.70	nf	1	0.01	0.53
EUR019	13	3 (3)	6	3	2	−1.07	−2.01	nf	4	0.18	0.40
RUL003	3, 12	6 (6)	0	0	4	−0.54	0.37	nf	2	0.65	0.25
RUL037		6 (6)	0	0	2	−0.80	0.62	nf	2	0.81	0.11
RUL007		7 (4)	0	0	3	−0.19	−1.10	nf	1	0.04	0.11
RUL013	3	5 (4)	0	0	3	−0.28	−1.40	nf	1	0.02	0.09
RUL069		8 (4)	0	0	3	−0.40	−0.63	nf	1	0.11	0.07
RUL002	3, 12	6 (4)	0	0	3	−0.64	0.15	nf	1	0.44	0.03

[1] BrCa = breast cancer cases (under 60); OvCa = ovarian cancer cases; Bc + Ov = patients with both breast and ovarian cancer; typed = number of patients for which DNA was genotyped.
[2] nf = Not found by PTT; EUR021, RUL105 and RUL005 were also (partially) sequenced (see Results).
[3] Classification for prior probability of linkage to either BRCA1 or BRCA2 (see Materials and Methods).
[4] Includes 1 case of male breast cancer.
[5] Multipoint lod scores were calculated with the markers THRA1 and D17S855 (intragenic).
[6] Breast cancer cases under 60 were those from the 2 individuals with both breast and ovarian cancer.
[7] nd = Not done.

included at least 1 case of male breast cancer [11]. BRCA2 was placed in a 6-cM interval bracketed by the markers D13S289 and D13S267. Of note, significant evidence for heterogeneity was obtained, with 74% of the families being due to BRCA2. This suggests that additional high-risk breast cancer-predisposing genes may exist.

We have selected 22 high-risk Dutch breast cancer families, containing at least 4 cases of breast cancer diagnosed under the age of 60 and/or ovarian cancer at any age, and have estimated the proportions due to BRCA1 and BRCA2 by a combination of BRCA1 mutation screening and linkage analysis. Our results suggest that BRCA2 will explain a significant proportion of hereditary breast cancer in the Netherlands, and, together with BRCA1, perhaps account for all high-risk families.

Materials and Methods

Families Studied

Families were ascertained by the Foundation for the Detection of Hereditary Tumors in Leiden, and the Department of Clinical Genetics of the Erasmus University in Rotterdam. Cancer incidence was confirmed by either medical or pathology records. Of the 22 selected families (table 1), 9 have been enrolled in earlier BCLC studies [3, 6, 11], and 14 participated in either our earlier linkage studies [12, 13] or BRCA1 mutation screening studies [9].

Markers

The markers used in this study are polymorphic microsatellites, mainly dinucleotide repeats. From centromere to telomere, the assumed order for the used 17q12–q21 (BRCA1) markers is: D17S250, THRA1, D17S855 (intragenic), D17S579 and D17S588 distributed over a 13.2-cM interval. The predicted cen→tel order for the used 13q12–q13 (BRCA2) markers over a 6-cM interval is

D13S289/D13S290, D13S260, D13S171, D13S267. All polymorphisms are retrievable from the Genome Database (GDB). Allele numbering and frequencies were as listed in GDB.

DNA Isolation and Marker Analysis

DNA isolation from heparinized blood and marker analysis were performed as described by Cornelis et al. [13]. DNA was isolated from paraffin-embedded tissues as described [14] with minor modifications, and was used to genotype polymorphic markers in deceased individuals.

Protein Truncation Test

Screening for chain-terminating mutations in BRCA1 was done using the protein truncation test (PTT) as described [9]. Exon 11 of BRCA1 was screened in all families, using genomic DNA from at least two affected members of the family as template. RNA was available from an index case in only 3 families, so that in these cases exons 2–10 and 12–24 were also screened by the PTT.

Statistical Analysis

Lod scores were calculated using the LINKAGE program v5.1 [15]. The assumed penetrances for BRCA1 were as estimated by Narod et al. [6], while for BRCA2, the CASH model was applied [11]. A total of 21 liability classes were used, and the assignment of each individual to any of these has been described [6, 11]. Multipoint scores for BRCA1 were computed with the markers D17S250 and D17S579, and for BRCA2 with the markers D13S260 and D13S267, for all pedigrees, except EUR021 for which THRA1 and D17S855 were used instead of D17S250 and D17S579.

Testing for locus heterogeneity was done using the HOMOG algorithm v3.33 [16], and computed 3-point lod scores at 16 thetas from −0.30 to +0.30 as obtained with D13S260 and D13S267. Posterior probabilities of linkage to BRCA1 and BRCA2 were estimated using the prior probabilities of linkage to each locus for each given pedigree and their respective lod scores. These prior probabilities were derived from a large BCLC study of 228 hereditary breast cancer families [Easton and Ford, in preparation]. Five classes of α_1/α_2 combinations are defined by that study and used here, in which α_1 = proportion of families due to BRCA1 and α_2 = proportion of families due to BRCA2. These are: (1) $\alpha_1 = 0.33/\alpha_2 = 0.11$ for families with 4 or 5 cases of female breast cancer under the age of 60 only; (2) $\alpha_1 = 0.26/\alpha_2 = 0.58$ for families with at least 6 cases of female breast cancer under 60 only; (3) $\alpha_1 = 0.66/\alpha_2 = 0.17$ for breast-ovarian cancer families with 1 case of ovarian cancer and no male breast cancer; (4) $\alpha_1 = 0.79 / \alpha_2 = 0.19$ for breast-ovarian cancer families with at least 2 cases of ovarian cancer and no male breast cancer; (5) $\alpha_1 = 0.08 / \alpha_2 = 0.78$ for families with at least 1 case of male breast cancer. For the estimation of the posterior probability of linkage to BRCA2, a negative BRCA1 mutation screening result was also weighed, by assuming a detection rate of 45% if a mutation is present. This estimate was derived as follows: mostly exon 11 of BRCA1 was screened for chain-terminating mutations by the PTT, hence under the assumption of a random distribution of mutations over the gene, 85% of all mutations are screened out from 61% of the coding region; of this 61%, the first and last 5% were considered not to be reliably screened by the PTT.

Simulations were performed on a total of 61 pedigrees, selected on the basis of cancer incidence, using the SIMULATE program [16]. Individuals from which DNA was available from either white blood cells or paraffin-embedded tumor tissues were coded as such; a 4-allele marker with equal allele frequencies was simulated in 50 replicates of the pedigree. Pairwise lod scores were computed with LINKAGE using the 21 liability class model described above.

DNA Sequencing

Direct sequencing of PCR fragments was performed using M13-tailed and fluoresceinated primers on a Pharmacia ALF sequencer. Primer sequences used were essentially those published by Friedman et al. [17].

Results

Families were selected in which at least 4 cases of either breast cancer diagnosed under the age of 60 or ovarian cancer diagnosed at any age had occurred. A second criterion was the simulated maximum 2-point lod score, for which we applied a cut-off of 0.90 to select the more informative families for linkage analysis. Of the 61 which met the first criterion, 22 complied with the latter. Twelve of these were classified as 'breast-cancer-only', and 10 as 'breast-ovarian cancer' pedigrees (table 1). In 2 families, a single case of male breast cancer occurred. All families were analyzed for linkage to BRCA1, reported previously for 12 families [12, 13], and for the presence of a mutation in BRCA1 [9]. Linkage to BRCA2 was analyzed in all families in which mutation screening was negative, using D13S260 and D13S267 as flanking markers.

Six families were found to carry a chain-terminating mutation in BRCA1 [9]. The 3-point lod score with the flanking markers D17S250 and D17S579 had predicted the presence of a mutation in 4 of these (table 1). The families RUL047 and RUL049 had negative lod scores and were each found to contain a sporadic breast cancer case [6, 13, 18]. Of note, in both instances it concerned premenopausal breast cancer cases diagnosed at ages 43 and 35, respectively. Family RUL049 was furthermore peculiar in that it contained a case of male breast cancer.

In the remaining 16 families, 3-point linkage analyses were performed with the markers D13S260 and D13S267, which define a 3-cM interval known to contain BRCA2 [19]. The admixture test (HOMOG algorithm [16]) provided no evidence for genetic heterogeneity among these 16 families, and a maximum lod score of 2.60 was obtained for 13q under homogeneity at $\theta = 0.10$ distal to D13S267. However, there were 3 families (EUR021, RUL105, RUL005) with estimated posterior probabilities being due to BRCA1 or BRCA2 of greater than 90% and smaller than 2%, respectively (table 1). Among the remaining 13 families, a maximum lod score of 3.38 was obtained with HOMOG, again at $\theta = 0.10$

distal to D13S267. The family with the strongest evidence being linked to BRCA2 was RUL024.

Family EUR021 provided significant evidence of being linked to BRCA1 on its own, with a maximum 3-point score of 3.01, coincident with D17S855. The PTT for exon 11 was negative, as it was for RUL005 and RUL105. A patient carrying the disease haplotype was selected from each of these pedigrees and the gene regions reported earlier to contain either a missense mutation or a hotspot for mutation (exons 2, 5, 18, 20, 21, 24, and the first and last 400 bp of exon 11) were sequenced. This did not reveal a disease-associated sequence change relative to a healthy control individual. No RNA was available from families RUL105 and EUR021 to detect any splicing errors caused by intronic mutations. The entire coding region of BRCA1 was subsequently sequenced in the patient from family RUL005 and again no mutations were detected (data not shown). Analysis of cDNA from this patient did not reveal any gross alterations in the coding sequence. Unfortunately, several exonic polymorphisms were homozygous, precluding the detection of a possible loss of mutant transcript.

An interesting difference was observed in the breast:ovarian cancer ratios between the families classified as 'BRCA1' (n = 9) or 'non-BRCA1' (n = 13) (table 1). This ratio was 2:1 in the former (40:19) and 7:1 (88:13) in the latter group of families. The ratio in the 'non-BRCA1' group was determined greatly by family EUR019, which contains 3 cases of breast cancer and 6 cases of ovarian cancer. This family was furthermore interesting as it appeared to be linked to neither BRCA1 nor BRCA2. The breast:ovarian cancer ratio in the 6 families with a posterior probability of linkage to BRCA2 of greater than 0.50 was 11:1 (44:4), suggesting that BRCA2 confers a much lower risk for ovarian cancer than does BRCA1.

Discussion

In this study we have selected 22 breast cancer families with a high incidence of early-onset breast cancer and/or ovarian cancer at any age, to determine the proportion of families attributable to either BRCA1 or BRCA2, and to seek evidence for the existence of a third strong predisposing locus. Our results confirm a role for both BRCA1 and BRCA2 in significant proportions of Dutch high-risk breast cancer families, whereas no statistical evidence was found for an additional highly penetrant breast cancer gene (or genes) predisposing these families.

Among a set of 214 worldwide collected breast cancer kindreds, the BCLC estimated that 45% of the breast-cancer-only and at least 80% of the breast-ovarian cancer families are due to BRCA1 [3, 6]. We found a BRCA1 mutation or significant linkage evidence in 4 of the 14 breast-cancer-only families (29%), and in 5 of the 8 breast-ovarian cancer families (62%), which is in the predicted order of magnitude. In a previous study [13], we estimated the proportion of BRCA1-linked families to be smaller than predicted by the BCLC. Our current BRCA1 mutation screening method, consisting predominantly of the PTT for exon 11, was assumed to be 45% efficient. If the BRCA1 mutations were distributed randomly over the gene region, this would suggest that up to 60% of the breast-cancer-only families and all breast-ovarian cancer families studied here are in fact due to BRCA1. Yet family EUR019 appears not to be due to BRCA1. While mutation sites are indeed not clustered in any specific region of the gene [8], strong founder effects have been observed for a number of BRCA1 mutations in certain populations [20–23]. Hence it is very plausible that the efficacy of our mutation screening strategy is in fact higher than 45% because frame-shifting mutations in exon 11 constitute a greater proportion of BRCA1 mutations in the Dutch population than expected on the basis of the reported distribution [8]. Of note in this context, the mutations in RUL047 and RUL049 are identical [9] and occur on the same haplotype [unpubl. obs.], while none of the mutations reported in table 1 have been published to date.

There were 3 families in which we were unable to detect a BRCA1 mutation, yet linkage analysis suggested the presence of such a mutation. Neither the PTT nor random sequence analysis has revealed any disease-associated sequence change to date. Loss of transcript from the mutant allele has been reported in several families [7, 24, 25], but we were unable to investigate whether this might be involved in any of these 3 families due to either unavailability of RNA or constitutional homozygosity at several exonic polymorphisms. It is nonetheless possible that disease in these families is due to intronic mutations or very large deletions in the gene region; this hypothesis is the subject of current investigations.

Among the 13 families thus classified as 'non-BRCA1' we were able to obtain significant linkage evidence for BRCA2, indicating that this gene is involved in a significant proportion of Dutch hereditary breast cancer families. There was no evidence for genetic heterogeneity among this group of families, despite the fact that 8 families had negative lod scores for 13q, ranging from −0.19 to −1.07. All of these are easily explained by the presence of

a single sporadic case of breast cancer, similar to those ascertained in the 'BRCA1' families RUL047 and RUL049. Alternatively, in some of the families, even though they were selected for their informativity in linkage analysis, the markers may not have attained full statistical power. Hence upon direct mutation analysis an unknown proportion of these families may eventually turn out to be due to either BRCA1 or BRCA2, while our results do not exclude the presence of a third susceptibility gene.

One remarkable feature of BRCA2 is that it causes predisposition to breast cancer in males [11]. Indeed, we confirmed a case of male breast cancer in one of the clearly linked families (RUL023). Nonetheless, we also observed a male breast cancer in family RUL049, in which a nonsense mutation in BRCA1was detected. In this particular family, we were unable to demonstrate that this patient, who died in the late fifties, was carrying this mutation, but in another male breast cancer family reported by Struewing et al. [21] such evidence was provided. This indicates that male breast cancer is not an exclusive feature of BRCA2. Similarly, ovarian cancer is a clear hallmark of BRCA1, with a cumulative risk of 63% before age 70 in mutation carriers [5], the great majority of breast-ovarian cancer families being due to BRCA1 [3, 6]. Yet, our data suggest that the ovarian cancer risks conferred by BRCA2 are much lower than those associated with BRCA1, which supports the findings by Wooster et al. [11], although ovarian cancer might still be conspicuous in some BRCA2 families (e.g., RUL016). More accurate estimates of the BRCA2-associated ovarian cancer risks will need to come from larger studies.

In conclusion, our results show that BRCA2 explains a significant proportion of high-risk hereditary breast cancer families in the Netherlands. The size of this proportion is difficult to estimate due to the statistical limitations of linkage analysis, but no statistical evidence was obtained for the presence of a third highly penetrant breast cancer susceptibility locus. The current data suggest that BRCA1 and BRCA2 are about equally important factors in causing hereditary breast cancer in the Netherlands, although further work will be required to validate this prediction.

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