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Low frequency of BRCA1 germline mutations in 45 German breast/ovarian cancer families

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Abstract

In this study we investigated 45 German breast/ovarian cancer families for germline mutations in the BRCA1 gene. We identified four germline mutations in three breast cancer families and in one breast-ovarian cancer family. Among these were one frameshift mutation, one nonsense mutation, one novel splice site mutation, and one missense mutation. The missense mutation was also found in 2.8% of the general population, suggesting that it is not disease associated. The average age of disease onset in those families harbouring causative mutations was between 32.3 and 37.4 years, whereas the family harbouring the missense mutation had an average age of onset of 51.2 years. These findings show that BRCA1 is implicated in a small fraction of breast/ovarian cancer families suggesting the involvement of another susceptibility gene(s).
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Keywords: BRCA1; germline mutations; breast/ovarian cancer

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Germline mutations in the BRCA1 gene have been identified in approximately half of all families that display an inherited susceptibility to breast/ovarian cancer.[1-11] Women harbouring a mutated BRCA1 allele have a lifetime risk of 80-90% of developing breast cancer and a 40-50% risk of developing ovarian cancer.[12,13] The wild type allele of BRCA1 is invariably lost in tumours derived from women harbouring germline BRCA1 mutations. This implies that BRCA1 functions as a tumour suppressor gene.[14,15] Unlike other susceptibility genes, BRCA1 does not appear to be directly involved in sporadic breast cancer. To date, no somatic BRCA1 mutations have been identified in sporadic breast tumours.[3] However, there have been a few reports indicating that BRCA1 mutations do occasionally occur in sporadic ovarian cancers.[3,16,17]

BRCA1 was originally described as being composed of 24 exons of which 22 were coding, giving rise to a transcript of approximately 7.8 kb in size.[2] Exon 11 is the largest exon of the gene contributing to more than 60% of the entire coding sequence. BRCA1 consists of a 1863 amino acid zinc finger protein which is inducible by oestrogens.[18-20] Recently, a RING protein (termed BARD1) has been identified that interacts in vivo with the BRCA1 protein. Missense mutations in BRCA1 disrupt the interaction between these two proteins implying that BARD1 may be involved in mediating tumour suppression by BRCA1.[21]

In addition to missense mutations, several different types of mutations have been described which segregate with an increased susceptibility to develop disease. These include frameshift and nonsense mutations that truncate the BRCA1 protein and are estimated to account for approximately 85% of all causative changes. Splice donor/acceptor mutations have been described which can also alter the reading frame of BRCA1.[6-8,22,23]

In this study, 45 German breast/ovarian cancer families have been systematically studied for germline mutations in the BRCA1 gene. The entire coding sequence of the BRCA1 gene was screened using single strand conformational polymorphism (SSCP) analysis, the protein truncation test (PTT), followed by sequencing analysis. The results confirm previous findings with respect to the paucity of mutations in exon 11 in the German population and that only a small proportion of families exhibiting an aggregation of early onset breast cancer appears to be the result of mutations in the BRCA1 gene.

Material and methods

FAMILIES

Forty-five families, 37 breast cancer families and eight breast-ovarian cancer families of German origin, were analysed in this study. All families contained three or more affected members with at least two diagnosed under the age of 60 years. Thirty-two of these families were previously included in a linkage study.[24] In the 32 families, 93 of the 133 breast and ovarian cancer cases were histologically verified and the remaining were ascertained by anamnestic questioning. Multipoint lod scores at BRCA1 varied from 0.45 to −1.16. The characteristics of the cancer families are given in table 1.

DNA ANALYSIS

Genomic DNA was isolated according to standard procedures. Genotypes were analysed at chromosome 17q microsatellite loci D17S250, D17S579, and D17S588.[25-27] DNA was amplified using PCR according to Weber and May[28] and visualised by autoradiography.

PCR ANALYSIS

The entire BRCA1 coding region (Genbank accession number U14680), the splice junctions, and the non-coding exon 4 were amplified from genomic DNA using 44 primer sets. PCR was carried out as described by us previously.[23] Reactions were performed in 25 µl

Table 1 Characteristics of 45 cancer families studied for BRCA1 germline mutations

Family	*Age at breast cancer diagnosis*	*No of ovarian cancers*	*Lod score at BRCA1*
5	26,38,53,62,69	0	0.09
6	41,59,71	0	0.00
12	47,48,51,71	0	−0.33
17	49,53,68	0	0.02
26	34,36,44	0	−0.83
29	57,62,66	0	—
30	34,49,71,71	0	−0.04
32	40,44,64,65	0	−0.17
33	28,34/57,38,39,52,55,58,59	0	−0.44
34	41,49,64	0	0.25
37	28,29,40	0	−1.11
38	43,57,58	0	−0.10
39*	46,56,61	0	−1.16
41	40,49,53,54,73	0	−0.61
42	41,45,60	0	0.05
44	37,45,45/62,56	0	0.10
45	28,34,50	0	−0.08
52	44,45,70	0	−0.03
53	50/?,50/51,52	0	−0.16
54	41,43,51	0	0.21
56	29,37,38,40,43/46	0	0.12
57	32,37,43,45,53,58,65	0	0.04
59	43,52,62,81	0	−0.19
60	38,48,49,62	0	0.45
63*	40,44,52,53,66	0	0.06
66	38,42,57	0	0.04
67	40/58,41,55	0	−0.31
69*	44,44,45,48,48,59,68	0	−0.56
77	32,38,38,63,63	0	—
84	47,47/53,88	0	—
90	50,60,61	0	—
94	44,50,51,57,77	0	—
101	42,48,67	0	—
106	44,52,74	0	0.10
116	44/59,45,52,58/67,74	0	—
132	46,54,58	0	—
B1	36/37,28/38,51,54	0	—
11	33,38/38,42,46	1	−0.21
65	40,45,65,68	1	−0.13
76	26,29,35/36,43	1	−1.06
102	33,49	1	—
125	47,47,49,53,53,58	1	—
87	52,56,61,63,?	2	—
83	60	3†	—
48	55,60	4	−0.33

*Including a male breast cancer.
†Including a borderline tumour.

Table 2 BRCA1 germline mutations in breast/ovarian cancer families

Family	*Phenotype*	*Exon*	*Nucleotide*	*Codon*	*Mutation*	*Type**
45	Breast cancer	11	1806	563	Gln-Stop (CAG→TAG)	NS†
37	Breast cancer	12	4282	1388	delAG→Stop1389	FS‡
56	Breast cancer	Intron 20	1§	-	G→T	SP
125	Breast-ovarian cancer	15	4654	1512	Ser-Ile (AGT→ATT)	MS ‖

*NS: nonsense mutation; MS: missense mutation; SP: splice mutation; FS: frameshift mutation.
†Described by Johannsson *et al.*[7]
‡Described by Jandrig *et al.*[29]
§Numbered from the 3' end of intron 20.
‖ Identified by Weber/Borresen and Mathew (BIC).

volumes containing 50 ng DNA, 1 × PCR buffer (AGS, Heidelberg), 2.5 μl 10 × gelatine (1 mg/ml), 0.1 μmol/l of each primer, 250 μmol/l dATP, dGTP, dTTP, 12.5 μmol/l dCTP (Promega, Boston), 0.25 μCi ^{32}P-dCTP (Amersham, Braunschweig), and 1 U *Taq* DNA polymerase (AGS, Heidelberg) in a Hybaid Omnigene thermal cycler. Primer pairs and cycling conditions were mainly according to Friedman *et al.*[6]

SSCP ANALYSIS

Amplified samples were diluted 1:10 in formamide buffer (98% formamide, 10 mmol/l EDTA, pH 8, 0.05% bromophenol blue, 0.05% xylene cyanol), heated to 95°C for five minutes, and chilled on ice for 10 minutes. Three μl of this mixture were loaded onto vertical non-denaturing 6% polyacrylamide gels and run at 6 W at constant power for 7-16 hours in 0.5 × TBE at 4°C. In addition, samples were run on non-denaturing 6% polyacrylamide gels containing 5% glycerol at room temperature. Variant bands were detected by autoradiography. All exons were analysed by SSCP analysis to rule out the presence of additional mutations.

PTT ANALYSIS

Mutation detection in exon 11 was performed using the protein truncation test (PTT) on genomic DNA. Exon 11 was subdivided into three segments of 925, 1460, and 1456 bp in size. PCR amplification of the three segments was as follows: 200 ng genomic DNA was used as template in a 25 μl reaction volume using standard PCR conditions except that the primer concentration was 200 nmol/l. Samples were amplified using the following cycling conditions: 94°C for 30 seconds, 55°C for 30 seconds, 72°C for two minutes, 35 times. Coupled transcription and translation was done using 1 μl of PCR product in a total volume of 5 μl which included 1.5 μl of TNT T7 reticulocyte lysate (Promega, Madison); 0.2 μl of ^{35}S-methionine (NEN, Boston) was included in each reaction. Labelled protein products were denatured and size fractionated on 12% SDS-PAGE, dried, and autoradiographed. Each reaction was performed three times in the presence of a positive control for each PTT segment.

DNA SEQUENCE ANALYSIS

PCR products were prepared in 100 μl volumes and purified by electrophoresis in a Mermaid gel (Dianova, Hamburg). Specific bands were excised and DNA was recovered with Qiaquick (Qiagen, Hilden). PCR fragments were sequenced using the dye terminator method on a 373 DNA sequencer (Sequiserve). Both strands of genomic DNA were sequenced to confirm a mutation.

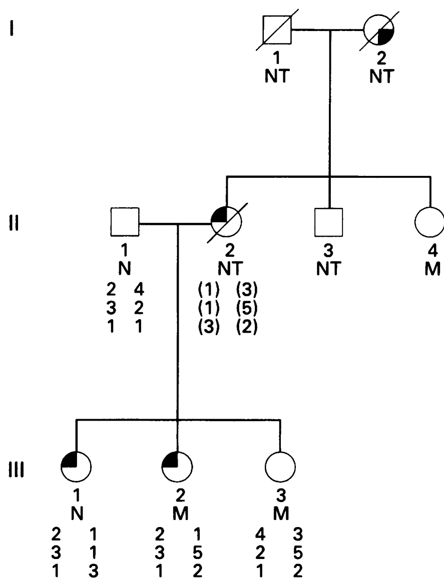
Results

BRCA1 GERMLINE MUTATIONS IN CANCER FAMILIES

Forty-five families including 37 breast cancer and eight breast-ovarian cancer families were studied for germline mutations in the BRCA1 gene. Four BRCA1 germline mutations were identified by using the combination of SSCP, PTT, and sequencing analysis. The mutations included one frameshift mutation, one nonsense mutation, one splice site mutation, and one missense mutation in three breast cancer families and in one breast-ovarian cancer family (table 2).

Three family members of kindred 37 carry the 4282delAG mutation in exon 12, which is predicted to terminate prematurely protein synthesis at codon 1389. One mutation carrier

A Family 37



B Family 56

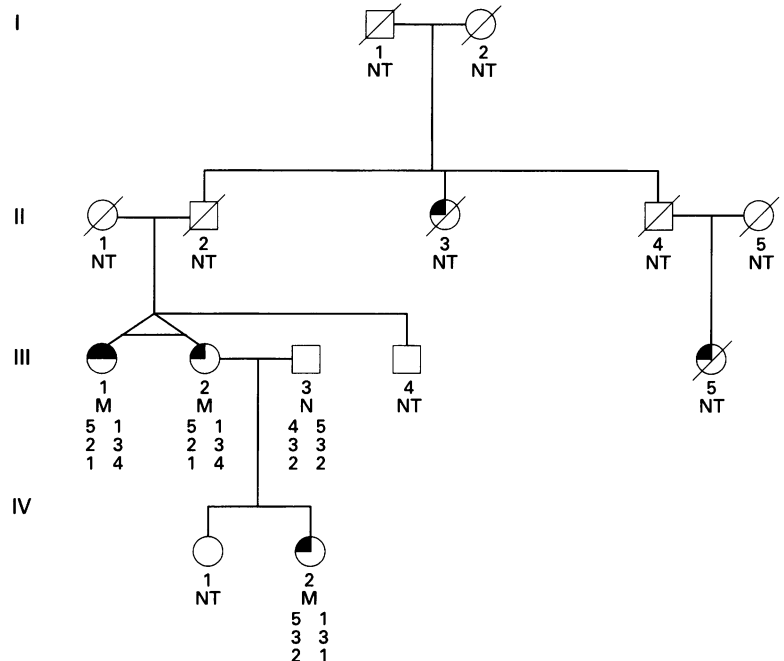


Figure 1 Pedigrees and haplotypes of breast cancer families 37 (A) and 56 (B) with BRCA1 germline mutations. Symbols with filled left upper quadrant: unilateral breast cancer; symbols with filled left and right upper quadrants: bilateral breast cancer; symbols with filled right lower quadrant: other cancer. The loci analysed from centromere to telomere are D17S250, D17S579, and D17S588. The numbers arranged vertically below the subjects indicate the marker alleles arranged into haplotypes; those in brackets are inferred. M=mutation carrier, N=negative for the mutation, NT=not tested.

(III.2) was diagnosed with early onset breast cancer at the age of 28 years. The BRCA1 mutation was probably inherited from her late mother, who was diagnosed with breast cancer at the age of 40 years. Interestingly, a sister (III.1) of the index patient (III.2) was diagnosed at 29 years of age with breast cancer, but was negative for this mutation which is confirmed by haplotype analysis (fig 1A). This result may explain the negative lod score of −1.11 at the BRCA1 locus as shown in table 1.

Family 45 harbours a C to T nonsense change at nucleotide 1806 in exon 11 causing a truncating mutation at codon 563. Two family members diagnosed with breast cancer at 28 and 34 years of age, respectively, were positive for this mutation.

A splice acceptor site mutation in intron 20 (G→T) was identified in family 56. This mutation probably leads to an altered transcript. In this family three females, of whom two were diagnosed with breast cancer at 29 and 37 years of age and one developed bilateral breast cancer at the ages of 43 and 46 years, harboured this mutation. Pedigree and haplotypes of kindred 56 are shown in fig 1B.

A missense mutation changing a Ser to an Ile was identified in family 125 which was inherited from the unaffected father. Two female gene carriers, 51 and 57 years of age, respectively, were asymptomatic. In this family one early onset breast cancer patient on the maternal side was negative for this mutation. It was impossible to determine if this mutation segregated with the disease in this family, since key persons in the pedigree were unavailable for testing. This missense mutation was also identified in three of 107 (2.8%) controls, suggesting that it is a relatively frequent polymorphism.

Discussion

In this study 45 breast/ovarian cancer families of German origin were studied for germline mutations in the BRCA1 gene, all of which had three or more affected family members. All but one of the 37 breast cancer families were characterised by early onset disease (≤50 years of age). As BRCA1 confers a high risk of ovarian cancer, eight breast-ovarian cancer families, of which three contained multiple cases of ovarian cancer at any age, were also included in this analysis.

Thirty-two families have previously been studied by linkage analysis.[24] Lod scores in all these families ranged between 0.45 and −1.16. The low lod scores are not necessarily an indication that these families are not linked to BRCA1. The absence of evidence of linkage is probably because of small family size, male carriers, and dead family members. Therefore, it was considered worthwhile to determine the percentage of families that could be accounted for by germline mutations in the BRCA1 gene.

Four BRCA1 germline mutations were identified in three breast cancer families and in one breast-ovarian cancer family. Three of these mutations have been previously identified in other families. The 4282delAG mutation was identified in breast cancer family 37 which had an average age of diagnosis of 32.3 years of age. This mutation has previously been detected in a breast-ovarian cancer family with an average age of diagnosis of 34.5 years.[29] A nonsense mutation in exon 11 was identified in breast cancer family 45. The average age of diagnosis

in this family was 37.3 years of age. This mutation has also been described in two breast cancer families with an average age of diagnosis of 35.7 and 43 years, respectively, and in one breast-ovarian cancer family with an average age of diagnosis of 30.3 years.[7] A missense mutation was identified in breast-ovarian cancer family 125 which has previously been suggested to be a polymorphism in the Breast cancer Information Core (BIC). Since this missense mutation occurred in a breast-ovarian cancer family where the a priori probability of linkage to BRCA1 is high it would on first examination appear to be associated with the disease. To clarify the role of this mutation in disease development, we analysed 107 healthy, unrelated subjects. Three of the 107 subjects harboured this nucleotide change suggesting that this is a common variant in the population. The variant is located in a region of low sequence conservation among species, thus supporting evidence that it is not associated with the disease.[30] It has recently been reported that some BRCA1 missense mutations disrupt the interaction between BRCA1 and BARD1, a protein that has been suggested to regulate the action of BRCA1.[21] The missense mutation described here does not occur in the region where this interaction takes place and is therefore unlikely to be causative. A novel splice acceptor site mutation was detected in breast cancer family 56 with an average age of diagnosis of 37.4 years. This mutation segregated with the disease in this family. Furthermore, the eight breast-ovarian cancer families included in this study were negative for BRCA1 mutations, which is surprising when taken in context with the high a priori probability of being linked to BRCA1.

Previously we have reported a paucity of BRCA1 mutations in exon 11 in the German population (0/7 mutations in exon 11 in 29 breast/ovarian cancer families), which has been shown to be statistically significant in comparison to other populations.[23] Furthermore, an additional report on the German population also confirms these findings (0/3 mutations in exon 11 in 20 breast/ovarian cancer families).[29] Taken together the results of these three studies support the notion that the mutation spectrum appears to be specific for this population.

The percentage of families with BRCA1 germline mutations is less than 10% of the total number of families. Given that the a priori probability of these families being linked to BRCA1 is 0.45 for breast cancer families and 0.85 for breast-ovarian cancer families, it is surprising that so few mutations have been identified. It could be argued that some of the families in this study had lower a priori probabilities of being linked to BRCA1 since the average age of disease onset was over 50 years of age. This could account for the low frequency of mutations. It is improbable that this would have a significant effect on the mutation rate since only three families with late age of disease onset were included. Furthermore, we believe that it is unlikely that we have missed a high percentage of mutations with our detection strategy. These results imply that there are additional susceptibility genes associated with an increased risk of developing early onset breast cancer which remain to be identified.

In summary, our results indicate that only a small percentage of familial aggregations of breast/ovarian cancer is the result of germline mutations in the BRCA1 gene. This brings into question the validity of screening for mutations. However, since approximately 10% of families harboured BRCA1 mutations, we would suggest that there is value in screening for mutations. Knowledge of the mutational status of BRCA1 in breast/ovarian cancer families remains controversial. Persons who are most likely to benefit from this information are those who do not harbour germline BRCA1 mutations, whereas asymptomatic gene carriers face more difficulties since little can be offered by way of prophylaxis.

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