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Low proportion of *BRCA1* and *BRCA2* mutations in Finnish breast cancer families: evidence for additional susceptibility genes

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One hundred breast and breast-ovarian cancer families identified at the Helsinki University Central Hospital in southern Finland and previously screened for mutations in the *BRCA2* gene were now analyzed for mutations in the *BRCA1* gene. The coding region and splice boundaries of *BRCA1* were analyzed by protein truncation test (PTT) and heteroduplex analysis (HA)/SSCP in all 100 families, and 70 were also screened by direct sequencing. Contrary to expectations based on Finnish population history and strong founder effects in several monogenic diseases in Finland, a wide spectrum of *BRCA1* and *BRCA2* mutations was found. In the *BRCA1* gene, 10 different protein truncating mutations were found each in one family. Six of these are novel Finnish mutations and four have been previously found in other European populations. Six different *BRCA2* mutations were found in 11 families. Altogether only 21% of the breast cancer families were accounted for by mutations in these two genes. Linkage to both chromosome 17q21 (*BRCA1*) and 13q12 (*BRCA2*) was also excluded in a subset of seven mutation-negative families with four or more cases of breast or ovarian cancer. These data indicate that additional breast and breast-ovarian cancer susceptibility genes are likely to be important in Finland.

INTRODUCTION

The most prominent risk factor for breast cancer is a family history. It is estimated that 5–10% of all breast cancers may arise from hereditary predisposition (1,2). The two currently known major breast cancer predisposing genes, *BRCA1* (3,4) and *BRCA2* (5–7) were originally thought to account for the vast majority of breast cancer families. *BRCA1* was reported to account for ~45% of hereditary breast cancer families (especially those with ovarian cancer), and *BRCA2* for an additional 35%, (including those with male breast cancer) (5,8). These estimates were largely based on studies of the same large families that were initially used to assign linkage to these two genes.

Very recently, large scale mutation analyses of these two genes indicate that in many populations only ~30–60% of breast cancer families are attributable to *BRCA1* and *BRCA2* mutations. The proportion of large breast cancer pedigrees that are attributable to *BRCA1* and *BRCA2* is 21 and 9% in Britain, 24 and 18% in France, 40 and 16% in Canada, and 39 and 25% in the USA, respectively (9). In Sweden and in Hungary, ~35% of families carry mutations in these two genes (9). In isolated populations with a strong founder effect, the situation may be different. In Iceland, a single *BRCA2* founder mutation accounts for the majority of hereditary breast cancers (10,11), and in the Ashkenazi Jewish population, a very high carrier frequency has been reported for the three founder mutations, 185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2* (12–14).

The Finnish population is also considered an isolate. There are strong founder effects for several characteristic Finnish genetic diseases, while several genetic diseases that are common elsewhere in the world, are rare or even unknown in Finland (15). Therefore, substantial interest in the *BRCA1* and *BRCA2* mutation spectrum and its significance in this country exists. We recently reported the analysis of the *BRCA2* mutation spectrum in 100 Finnish breast cancer families with three or more breast or ovarian cancers (16). We have now analyzed mutations of the *BRCA1* gene in the same families, to complete the survey of the spectrum of mutations predisposing to breast cancer in the Finnish families. Additional data on recurrent *BRCA2* mutations were also obtained. The mutation

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spectrum was surprisingly wide. Ten different *BRCA1* mutations and six *BRCA2* mutations were discovered. Although several of these mutations were recurrent, there was no evidence of a major founding mutation in either gene. Only 21% of breast cancer families could be attributed to the effects of these two genes, suggesting that additional breast cancer predisposition genes are likely to be important in Finland.

RESULTS AND DISCUSSION

BRCA1 mutations

Mutation analysis of the *BRCA1* gene coding region and splice boundaries in 100 index cases by protein truncation test (PTT) and heteroduplex analysis (HA)/SSCP analysis, as well as by direct sequencing in 70 of these, revealed 10 different mutations each in one family (10%) (95% CI = 5–18) (Table 1). All of the mutations segregated with cancer in the families. Six of the 10 mutations were frameshift mutations including five deletions and one insertion, and three were base substitutions leading to immediate termination codon. One mutation was an exon 12 splice acceptor site nt –2 A→G substitution (Table 1). This mutation destroys the nearly invariant AG of the splice acceptor site (17) and is predicted to cause aberrant splicing and truncation of the protein product. This splice substitution was not seen in 186 normal control chromosomes as determined by ASO hybridization, and we therefore classified it as a mutation. The mutations were unevenly located over the *BRCA1* gene, with five mutations found in exon 11 and five mutations in exons 12–20.

Table 1. *BRCA1* mutations and phenotypes in the families[a]

Family	Mutation	Status	Breast cancer (ages of diagnosis, years)	Ovarian cancer	Breast and ovary	Other cancers
254	Exon 11 1924 delA	Finnish	2 (44,58)	1 (16)		skin (47)
160	Exon 11 2803 delAA	Dutch founder	2 (51,72)	2 (52,70)		unknown
112	Exon 11 3604 delA	Dutch/German	0	2 (43,48)	1 (41,44)	lung cancer (76)
188	Exon 11 3744 delT	Finnish Recurrent (3)	1 (bil) (30/42)	1 (48)		stomach (2 cases) skin (55)
167	Exon 11 3904 C→A	Finnish	2 (39,55)	2 (66, na)	1 (52,50)	peritoneal metastasis (female), primary unknown (58) skin (43), corpus carcinoma (61), pancreas (74), lung (63), salivary gland (87)
136	Exon 12 nt –2 A→G	Finnish Recurrent (2)	3 (1 bil) (28,34,43/51)	0		
286	Exon 13 4446 C→T	Hot spot? Recurrent (2)	9 (25,32,36,41,44,55,55,71,na)	0		lung (na)
141	Exon 17 5145 del11	Finnish	3 (32,35,35)	0		peritoneal metastasis (female), primary unknown (46), gall bladder (56)
263	Exon 20 5370 C→T	Finnish Recurrent (2)	2 (55,59)		1 (50,57)	peritoneal metastasis (female), primary unknown (d61),
280	Exon 20 5385 insC	N-E Europe/ Jewish founder	1 (49)	1 (na)		skin (63,74) lymphoma (69), multiple myeloma (80) skin (78)

Total: 28 patients with breast cancer (two bilateral), median age of onset 43.9 years, (25 patients with breast cancer only, three with breast and ovarian cancer). Twelve patients with ovarian cancer, median age of onset 49 years (nine patients with ovarian cancer only, three with breast and ovarian cancer).
[a]Carrier status of all family members is not known.
na, not available.

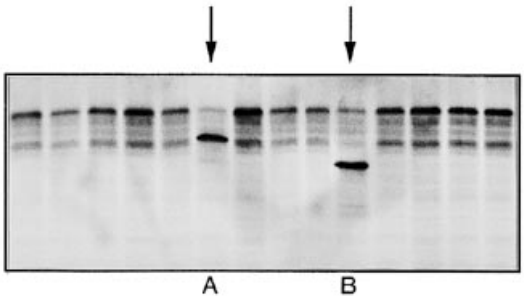

Figure 1. PTT analysis, revealing the 3904 C→A mutation in family 167 (**A**) and the 3604delA mutation in family 112 (**B**).

Missense variants of unknown consequence and infrequent polymorphisms were also found and are deposited in the BIC data base (18).

Recurrent *BRCA1* and *BRCA2* mutations

In this population of 100 families, each of the ten *BRCA1* mutations were found in one family only. However, outside this cohort we have identified four of the mutations in additional Finnish families by ASO hybridization (Table 1). The exon 11 nt 4446 C→T substitution, the exon 12 splice acceptor mutation at nucleotide –2 A→G and the exon 20 nt 5370 C→T substitution were identified each in one additional family. The exon 11 mutation 3744delT was additionally identified in a breast-ovarian cancer family and in a third family with three sisters affected with ovarian cancer, from a set of ovarian cancer families described elsewhere (19). Thus four of the mutations found here were recurrent, three were found in two families and one was found in three families.

This cohort of 100 breast cancer families was previously found to carry five different *BRCA2* mutations in eight families (8%) by PTT and HA/SSCP analysis (16). In the present study, linkage analysis of 12 families (see below) revealed haplotype sharing in chromosome 13q12 in one breast-ovarian cancer family. Direct sequencing of the *BRCA2* gene in the index patient DNA sample revealed a protein truncating mutation, 7708 C→T substitution in exon 15. This mutation was not detected by HA/SSCP analysis in the previous study, and has not been reported previously. ASO-hybridization analysis of the 100 index case samples identified two other families with this mutation in this cohort. In summary, a total of six different *BRCA2* mutations has now been detected in 11 families (11%) (95% CI = 6–19) from the cohort of 100 families.

Outside this cohort, the 7708 C→T mutation was also found in one additional family by ASO hybridization. Thus, four unrelated families carry the 7708 C→T mutation. The ancestry of all four families originates from the south-eastern part of the country, as dated back to the 1800s, suggesting a regional founder mutation. Previously, we described three recurrent *BRCA2* mutations as possible founding mutations each in two Finnish families (16). One of these was the Icelandic founder mutation 999del5. In follow-up studies of other families, we have now identified this mutation in seven unrelated Finnish families (including two from a set of breast cancer families described elsewhere; Ralf Krahe and Juha Kere, personal communication). The other recurrent mutations, the exon 18 nonsense mutation (8555T→G) and the exon 24 splice acceptor mutation (9346 nt –2 A→G) have now each been found in five unrelated families.

Accumulation of *BRCA1* and *BRCA2* mutation data from larger sets of families as well as unselected breast and ovarian cancer patients will reveal the prevalence of the different mutations and the significance of the recurrent, putative founder mutations in Finland. The large number of different *BRCA1* and *BRCA2* mutations identified in this study, and low frequency of any recurrent mutation so far, suggest that there may not be a strong *BRCA1* or *BRCA2* founder in Finland.

Finnish–European mutations

Of the 10 *BRCA1* mutations identified here, six are so far novel Finnish mutations while four have been described previously in other European populations or in the USA (Table 1). Of these, 2803delAA (also called 2804delAA) is a prominent founder mutation in The Netherlands and Belgium, but has not been found previously in other populations (20). Another mutation, 3604delA (Fig. 1), has been described in Dutch and German families (18,20). The 5385insC (also named as 5382insC) mutation found in one Finnish family has been described as a founder mutation in Russia (21) and in Hungary (22), in families of Jewish and non-Jewish ancestry. Finally, the 4446C→T mutation in exon 13 has been found multiple times on different haplotypes and may represent a mutational hotspot (23).

Of the six different *BRCA2* mutations, five were novel mutations so far unique to Finland, while one recurrent, and a proposed ancient founder mutation (999del5) (16) has been previously described as a strong founder in Iceland (10,11). It is of interest that the 999del5 mutation is also the most frequently found mutation in Finland so far.

While some of the mutations described in other European populations may have arrived in Finland during the past centuries, or have an independent origin, others, especially the 999del5 mutation, may reflect ancient genetic relationships between European populations (16). Additional haplotype analysis of the Finnish families carrying the recurrent mutations, including both the country-specific and Finnish–European mutations, will be required to establish the nature of these putative founder mutations. Haplotypes around shared mutations between the Finnish and other European families may help to estimate the age of these mutations as well as explain their emergence in Finland.

Phenotype in *BRCA1* mutation families

The ages at diagnosis, and the number and types of cancer in each family are shown in Table 1. An early age of breast cancer onset is clearly an indicator of *BRCA1* as well as *BRCA2* mutation carrier status in these Finnish breast cancer families. The age of onset in the *BRCA1* (median 43.9 years) (P <0.0001, unpaired t-test) and in the *BRCA2* families (median 49.2 years) (P = 0.0001) was significantly lower than in the mutation negative families (median 57.4 years). An older age of onset in the *BRCA2* families compared with *BRCA1* families has also been found in other studies (24), and is reflected as a smaller contribution of *BRCA2* to early onset breast cancer (25).

Seven of the 10 *BRCA1* families included ovarian cancer, and three of these multiple cases (Table 1). The median age of ovarian cancer onset was 49 years. A statistically significant correlation (P <0.005, Fisher's exact test) between the location of the mutation and prevalence of breast or ovarian cancer in the *BRCA1*

families was found. In the five families with exon 11 mutations, nine breast cancers and 10 ovarian cancers were seen whereas in families with mutations 3′ of exon 11, 19 breast cancers but only two ovarian cancers were seen. The proposed variation in breast and ovarian cancer risk for mutations in different halves of the gene (26) is supported by these data.

All families in this cohort that included patients diagnosed with both breast and ovarian cancer were found to carry a mutation in either the *BRCA1* or in the *BRCA2* gene, as well as four out of six families with multiple cases of ovarian cancer.

Frequency of *BRCA1* and *BRCA2* mutations

The types of families analyzed, and the frequencies of *BRCA1* and *BRCA2* mutations found are shown in Table 2. The *BRCA1* mutations were found more frequently in families with both breast and ovarian cancer than in families with breast cancer only. *BRCA2* mutations were most common in the largest families with four or more cases of breast cancer or both breast and ovarian cancer. Combined, mutations in the two genes were found in 12/27 (44%; 95% CI = 25–65) of the breast-ovarian cancer families and in 4/23 (17%; 95% CI = 5–39) of site specific breast cancer families with four or more cases of breast cancer. Only 4/50 (8%; 95% CI = 2–19) of breast cancer families with three affected members were mutation-positive.

The mutation detection methods available and in general use are not completely sensitive, and the HA and SSCP methods are estimated to have ~80% sensitivity (27). For the *BRCA1* gene, the sensitivity of mutation screening was improved by the use of two different mutation detection methods, PTT combined with HA/SSCP and direct sequencing in 70 of the families. Additional sequencing of *BRCA2* was also done in two larger breast cancer families with no identifiable mutations by HA/SSCP and PTT analysis and with other cancers that may be characteristic of *BRCA2* mutations (including ovarian, pancreatic and gastric cancer). No mutations were found by direct sequencing. The low proportion of the *BRCA1* and *BRCA2* mutations found in this study may thus closely reflect the role of the *BRCA1* and *BRCA2* genes in the Finnish breast cancer families.

The 21% (95% CI = 13–30) frequency of *BRCA1/2* involvement in Finland is lower than in any other country so far surveyed. This low proportion of families with mutations is consistent with the recent studies of breast and breast-ovarian cancer families. Thirty-four percent of families in Sweden, and 33% in Hungary had mutations in either the *BRCA1* or the *BRCA2* gene (22,28). In both populations, *BRCA1* mutations, including prominent founder mutations, accounted for ~23% of the families, while mutations in the *BRCA2* gene accounted for ~10% of the families.

A high proportion of site-specific breast cancer families unexplainable by *BRCA1* or *BRCA2* mutations in this and other studies suggests the existence of additional breast cancer susceptibility genes (29–31). In this Finnish population, *BRCA1* or *BRCA2* mutations were not detected in 83% (19/23) of families with four or more cases of breast cancer. In Sweden, 81% of families with only breast cancer had no identifiable mutations (28). However, a high proportion, 56% (15/27), of Finnish breast-ovarian cancer families also remained unexplained by *BRCA1* or *BRCA2* mutations.

Linkage analysis: exclusion of *BRCA1* and *BRCA2*

In order to confirm the low involvement of these genes, we also performed linkage exclusion analysis in the *BRCA1* and *BRCA2* regions of chromosome 17q21 and 13q12 in 12 of the larger families where mutations had not been found. These included six families with 4–7 cases of breast cancer only and six families with 4–5 cases of breast or ovarian cancer. Not enough samples were available for formal linkage analysis in the families, thus affected family members were studied for sharing alleles, or haplotypes where possible, of three microsatellite markers on each chromosome. The results of the linkage analysis are shown in Table 3. In seven of these families, linkage to both chromosome 17q21 (*BRCA1*) and 13q12 (*BRCA2*) was definitely excluded. Analysis of one breast-ovarian cancer family suggested linkage to *BRCA2*. Subsequently the 7708C→T nonsense mutation was found in this family by direct sequencing as described above. In four families the linkage analysis was not informative for either the *BRCA1* or *BRCA2* gene. However, no mutations were found by direct sequencing or by PTT and HA/SSCP analysis. There was no evidence of a common haplotype between families for which exclusion was not obtained. Mutation analysis combined with linkage exclusion analysis suggests that additional susceptibility genes may account for a large proportion of breast and also breast-ovarian cancer families in Finland.

Table 2. Families analyzed with *BRCA1* and *BRCA2* mutations

Types of families	Total analyzed	Families with *BRCA1* mutations	%	Families with *BRCA2* mutations	%	Total %
Families with four or more breast cancers only	23	1	4	3	13	17
Families with four or more breast and ovarian cancers	14	2	14	4	29	43
Families with three cancers, breast only	50	2	4	2	4	8
Families with three cancers, breast and ovarian	11	3	27	1	9	36
Families with two cancers, breast and ovarian	2	2		1		
Total	100	10	10	11	11	21

Table 3. Exclusion of linkage to *BRCA1* and *BRCA2*

Family	Linkage exclusion of *BRCA1*	*BRCA2*	Method of mutation exclusion	Phenotype	Ages of diagnosis
Breast cancer families					
7	yes	yes	PTT/HA/SSCP, seq. *BRCA1*[b]	7 bc	48,63,64,na
96	yes	yes	PTT/HA/SSCP, seq. *BRCA1*	4 bc	35,41,49,49
277	yes	yes	PTT/HA/SSCP	5 bc	40,49,58,63,67
204	yes	0[a]	PTT/HA/SSCP	4 bc	35,47,53,70
381	yes	0	PTT/HA/SSCP	5 bc	38,39,42,60,80
155	0	0	PTT/HA/SSCP	6 bc	37,61,64,65,66,68
			seq. BRAC1/*BRCA2*[c]		
Breast-ovarian cancer families					
122	yes	yes	PTT/HA/SSCP	4 bc, 1 oc	43,52,57,57,67
			seq. *BRCA1*/*BRCA2*		
135	yes	yes	PTT/HA/SSCP, seq. *BRCA1*	4 bc, 1 oc	39,42,50,71,36
153	yes	yes	PTT/HA/SSCP, seq. *BRCA1*	4 bc, 1 oc	41,47,49,55,60
437	yes	yes	PTT/HA/SSCP	3 bc, 1 oc	54,63,74,59
125	0	yes	PTT/HA/SSCP, seq. *BRCA1*	4 bc, 1 oc	45,46,50,na

[a]0 = not informative.
[b]seq. *BRCA1* = sequenced *BRCA1* all coding regions.
[c]seq. *BRCA2* = sequenced *BRCA2* exons 2–9 and 12–27.
na, not available.

In summary, a wide spectrum of *BRCA1* and *BRCA2* mutations was found in 100 Finnish breast cancer families. While some of these mutations were recurrent, no major founding mutation was identified in either gene. Instead, the recurrent mutations seemed to represent smaller, regional founder mutations. The mutation spectrum was wider in the *BRCA1* gene, while fewer mutations in the *BRCA2* gene accounted for a larger total number of families. In other populations, *BRCA2* mutations are more prevalent than those in *BRCA1* only in Iceland, where a strong *BRCA2* founder effect was found (10,11). Due to the low involvement of *BRCA1* and *BRCA2* genes discovered, it appears particularly promising to search for a *BRCA3* gene in this set of Finnish breast cancer families. It will be of interest to find out whether the molecular basis of the non-*BRCA1*/non-*BRCA2* hereditary breast cancer is equally diverse as discovered for these two genes, or whether there is a major founder effect in the possible *BRCA3* gene in the isolated Finnish population.

MATERIALS AND METHODS

Subjects

One hundred breast or breast-ovarian cancer families (98 with three or more cases of breast or ovarian cancer in first or second degree relatives and two with one case of breast and one ovarian cancer) were identified predominantly from a population based cohort of breast cancer patients at the Helsinki University Central Hospital (HUCH) in southern Finland as described previously (16). The genealogy of these patients was confirmed through church parish registries, and the cancer diagnoses of the patients and relatives (including those reported as healthy) were confirmed through hospital records of the Department of Oncology, HUCH, and the Finnish Cancer Registry.

Blood samples were obtained from index patients and families willing to participate in the genetic analysis and written informed consent was obtained at the time of sample donation. This study was approved by the Ministry of Social Affairs and Health in Finland and by the ethical committees of the Department of Oncology and Department of Obstetrics and Gynecology, HUCH.

The phenotypes of the families analyzed are shown in Table 2. The index case DNA samples of these families have been previously screened for mutations in the *BRCA2* gene (16). Leukocyte DNA samples from 93 unrelated healthy individuals that had been stripped of all identification were used as controls for polymorphic status of the changes found but not for mutation search. The controls, as well as the study population, were of Finnish (Caucasian) origin.

Mutation detection

The 100 index patient DNA samples were screened for germ-line mutations in the coding regions and splice boundaries of the *BRCA1* gene by the HA/SSCP technique for exons 2–10 and 12–24 and PTT for the exon 11 as described (32,33).

Seventy of the DNA samples were also screened for *BRCA1* mutations by direct sequencing through the coding regions and splice sites. Sequence analysis was performed at Myriad Genetic Laboratories, Salt Lake City, UT. Briefly, exons 2–24 of the *BRCA1* gene were amplified using 35 pairs of PCR primers designed to avoid common polymorphisms that might inhibit equal amplification of both alleles. Dye primer sequencing was performed using fluorescent energy transfer primers (Amersham Life Science Inc. Cleveland OH, USA), the mutant *Taq* polymerase F667Y and a thermal stable pyrophosphatase (both from Perkin Elmer, Norwalk, CT, USA). Sequencing reaction products were electrophoresed and detected using a Perkin Elmer Applied Biosystems 377 sequencing gel. Analysis of sequence data was performed using software developed by Myriad Genetic

Laboratories, Inc. All analyses demonstrating mutations were repeated for verification.

Index patient DNA samples from three large families with no identifiable *BRCA2* mutations as analyzed by HA/SSCP and PTT in the previous study were further screened for *BRCA2* mutations by sequencing through exons 2–9 and 12–27 of the gene on an ABI 310 Genetic Analyzer using the dye terminator chemistry (Perkin Elmer) according to the manufacturer's instructions.

Sequencing of the variants

When aberrant mobility was detected on HA/SSCP or PTT gels, the variants were reamplified from genomic DNA and directly sequenced as described previously (16). Alternatively, sequencing was carried out using the ABI 310 Genetic Analyzer as above.

Allele Specific Oligonucleotide hybridization

Allele specific oligonucleotides (ASO) detecting the variant sequence and the corresponding normal sequence were designed for the mutations and used for analyzing the mutation/polymorphism status in normal control DNA samples (186 chromosomes) and for segregation analysis of the mutations in the families. The mutations found by sequencing and not detectable by HA/SSCP analysis were analyzed in all samples by ASO hybridization. The ASO hybridization technique has been described previously (34) and the ASO sequences used are available upon request.

Linkage analysis of *BRCA1* and *BRCA2* regions

Haplotype analysis in 12 families in which *BRCA1* or *BRCA2* mutations had not been detected (six families with four or more cases of breast cancer and six with four or more cases of breast and ovarian cancer) was carried out to test for linkage to chromosome regions 17q21 (*BRCA1*) and 13q12 (*BRCA2*). Polymorphic microsatellite repeat markers for 17q21 linkage analysis were *D17S1185*, *D17S855* and *D17S579* (23,35), and markers for 13q12 linkage were *D13S260*, *D13S1701* and *D13S267* (5,36). Haplotyping for linkage analysis was performed as described (16).

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