

Short communication

Low frequency of large genomic rearrangements of *BRCA1* and *BRCA2* in western Denmark

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Abstract

Germline mutations in *BRCA1* and *BRCA2* predispose female carriers to breast and ovarian cancer. The majority of mutations identified are small deletions or insertions or are nonsense mutations. Large genomic rearrangements in *BRCA1* are found with varying frequencies in different populations, but *BRCA2* rearrangements have not been investigated thoroughly. The objective in this study was to determine the frequency of large genomic rearrangements in *BRCA1* and *BRCA2* in a large group of Danish families with increased risk of breast and ovarian cancer. A total of 617 families previously tested negative for mutations involving few bases were screened with multiplex ligation-dependent probe amplification (MLPA). Two deletions in *BRCA1* were identified in three families; no large rearrangements were detected in *BRCA2*. The large deletions constitute 3.8% of the *BRCA1* mutations identified, which is low compared to several other populations. © 2006 Elsevier Inc. All rights reserved.

1. Introduction

Breast and ovarian cancers are among the most frequent fatal malignancies in women. Mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* are causative for different proportions of hereditary breast and ovarian cancer in different populations. The most frequent mutations in *BRCA1* and *BRCA2* involve deletions or insertions of a few bases or single-base substitutions resulting in premature stop codons [1].

Technical difficulties have delayed and limited screening for large genomic rearrangements. Early studies were performed with Southern blotting [2–8] and long-range polymerase chain reaction (PCR) [9]. Later, quantitative PCR methods [10,11] and color bar code on combed DNA [12–14] were developed. The introduction of multiplex ligation-dependent probe amplification (MLPA) has facilitated the screening for duplications and deletions and has been implemented in many laboratories for screening of *BRCA1* [15–18] and recently also *BRCA2* [18].

The frequency of large genomic rearrangements varies considerably among examined populations. In Holland,

for example, large genomic rearrangements in *BRCA1* constitute 36% of the mutations detected in *BRCA1* [2], but no rearrangements were detected among 82 Finnish families with moderate or high risk of hereditary breast and ovarian cancer [8]. Few studies have examined the incidence of *BRCA2* rearrangements in larger patient populations. In one recent report from Australia, large genomic rearrangements in *BRCA2* were identified in 2% of 149 high-risk families previously tested negative for *BRCA1* and *BRCA2* mutations [18].

Here we report the results from screening for large genomic rearrangements in *BRCA1* and *BRCA2* in a large cohort of Danish families with increased risk for hereditary breast and ovarian cancer.

2. Methods

Families with high or moderate risk of breast and ovarian cancer in western Denmark were referred to three regional departments of clinical genetics. Blood samples (or, in three cases, tumor tissue samples) were analyzed at Odense University Hospital. In all, 745 families were screened for mutations in *BRCA1* and *BRCA2* with the protein truncation test (TnT Quick Coupled Transcription/Translation systems, Promega) and denaturing high

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performance liquid chromatography (WAVE system; Transgenomics, Omaha, NE). Deleterious mutations were identified in 128 of the 745 families (617 families, where no mutations have previously been identified, were examined in this study).

Clinical data and pedigrees were available for 215 of the high-risk families (clinical data were not available for the remainder). Of these, 154 tested negative for mutations in *BRCA1* and *BRCA2* [19]. Risk assessment of these 215 families was based on evaluation of the pedigree manually and by use of tables and computer models (either or both) including families with $\geq 30\%$ life-time risk of developing breast cancer. A pedigree compatible with monogenic inheritance was classified as high risk, typically including early breast cancer, ovarian cancer, male breast cancer, or breast and ovarian cancer in the same individual. Families with isolated cases of early breast cancer were not classified as high risk. The study was approved by the scientific ethical committee for the counties of Funen and Vejle.

MLPA analysis was performed according to the manufacturer's instructions (MRC Holland, Amsterdam, the Netherlands). The kits contain in general one probe pair per exon, except that large exons are covered by two probe pairs and there are several control probe pairs specific for chromosomes other than 13 or 17. The probe oligonucleotides are ligated and then amplified with polymerase chain reaction using universal primers.

An ABI 377 automated sequencer (Applied Biosystems, Foster City, CA) was used to separate the amplified products. The relative peak area (RPA) for each probe pair was calculated as the ratio of each single peak to the total area of all peaks in the sample. Patient samples were assessed by comparing the RPA value with the expected value (calculated as the mean of the concerned probe pair in all samples in the run, including several control samples). The RPA value was decided to be abnormal if deviating by $> 25\%$ from the expected value. In these cases the analysis was repeated. If the result reappeared, the probe region was sequenced to exclude single-base mutations disturbing the annealing of the probes.

Long-range PCR (Expand Long Template PCR system; Roche Diagnostics, Mannheim, Germany) was used to examine all potential rearrangements. A *BRCA1* deletion of exons 3–16 was examined with the primers i2F: GAAAAAGTAAGAGACACCTATAG and i16R: CTTTA-TAAGCTGAGAGGTAAGTAG. As a preliminary test of the MLPA procedure, we included five samples with single-exon deletions or duplication in *BRCA1* received from other European laboratories. All mutations were identified promptly with RPA deviating $> 40\%$ from the expected values.

3. Results and discussion

The *BRCA1* MLPA analysis of 617 families resulted in the identification of two genomic rearrangements in three

families. One family had a deletion of exons 13–15, verified by long-range PCR and sequencing of the breakpoints (data not shown). The breakpoints appeared to be located at the same positions as a previously reported deletion in a French family [12]. The other rearrangement, a deletion of exons 3–16 not previously described, was detected in two families.

Long-range PCR was used to narrow down the breakpoint area, and sequencing revealed the breakpoints (Fig. 1). The breakpoints in both mutations were located in highly homologous Alu sequences, which has been reported for most genomic rearrangement identified in *BRCA1* [20]. The identification of this deletion in two families suggests that it may be a founder deletion in Denmark. *BRCA1* rearrangements have been reported as founder mutations in other populations (e.g., exon 13 and exon 22 deletions in Holland [2]). Hendrickson et al. [21] examined five *BRCA1* rearrangements in 20,000 families with hereditary breast and ovarian cancer and found exon 13 duplication to be the most common founder mutation (53 out of 20,000). The analysis of the *BRCA2* gene with MLPA resulted in no detection of rearrangements.

Three samples of DNA out of the 617 index samples were prepared from frozen tumor tissue. In one of these, a deletion of the entire *BRCA1* gene was detected, and a duplication of the entire gene was identified in another sample. However, MLPA examination of normal tissue (microdissected frozen or paraffin-embedded nonneoplastic tissue) from these patients revealed that the mutations were somatic (data not shown). Frozen tumor tissue can be useful for screening when no affected family members are alive; however, the results must be interpreted with caution. We also identified a missense mutation and a silent mutation in exon 18 in *BRCA1* and a missense mutation in exon 18 of *BRCA2* located at the MLPA probe binding sites, resulting in reduced RPA values (data not shown). For these reasons care must be taken when tumor tissue is examined or when only one MLPA fragment indicates an aberration.

In all three families with *BRCA1* deletions, at least one individual was affected with both breast and ovarian cancer. The earliest age of breast cancer was 32 years in two families and 42 years in the third family. The families were characterized by several cases of ovarian cancer, and one of the families also had a case of malignant melanoma in the woman with both bilateral breast cancer and ovarian cancer. All diagnoses were verified from hospital records.

Clinical data including pedigrees were available for 154 of the families in which mutations could not be detected by conventional methods. Large genomic rearrangements in *BRCA1* were detected in two (1.3%) of these 154 families and in *BRCA2* in none of the families (Table 1). Woodward et al. [18] detected *BRCA1* rearrangements in 2.2% of high-risk families in which no mutations were identified by conventional methods. The majority of rearrangements were also found in families with both breast and ovarian cancer.

Altogether, the large genomic rearrangements in *BRCA1* and *BRCA2* accounted for 2.3% (3 of 131) of the mutations

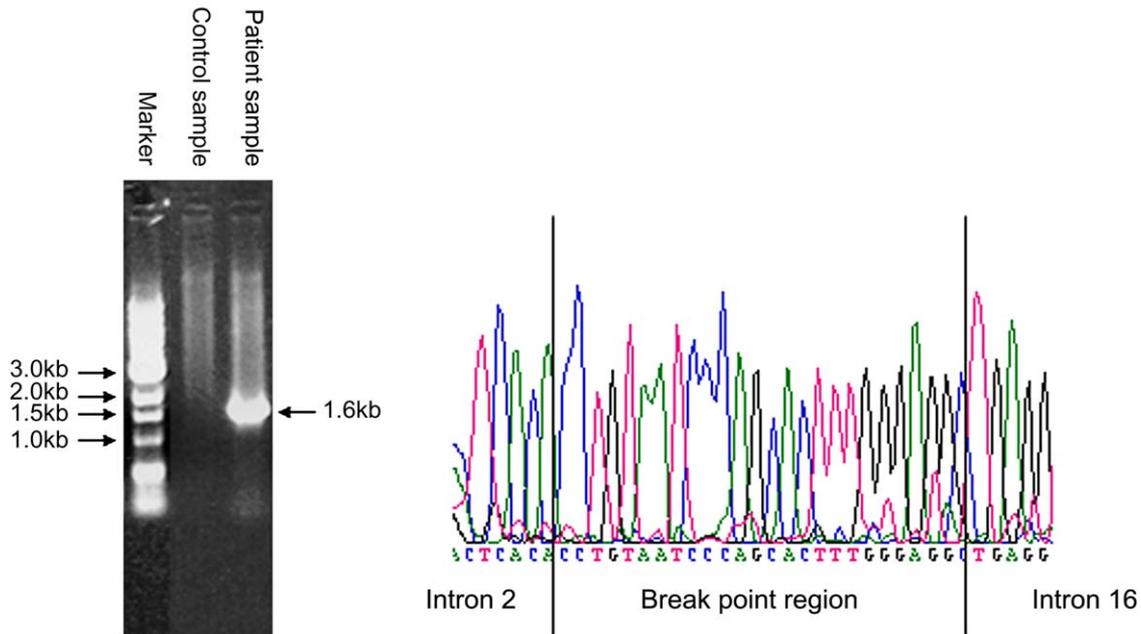


Fig. 1. Deletion of exons 3–16 in *BRCA1*. *Left*: Long-range PCR analysis with primers located in intron 2 and 17 resulted in a 1.6-kb amplicon in the patient sample, but no amplicons in a sample from a healthy donor. The wild-type fragment was not amplified due to large size of the deleted region. *Right*: Sequence analysis showed a deletion of 46,583 bp, denoted as IVS2+7220_IVS16+717del.

identified in the 745 Danish families with increased risk. Woodward et al. [18] reported this percentage to be 4.2.

When considering only *BRCA1* mutations, the proportion of large rearrangements was 3.8% (3 of 78). This proportion is considerably below the reported 27–36% in Holland [2,15], 9.5% in France [14], and 8% in Germany [17], but more comparable to a Finnish study [8], in which no mutations were detected in 82 families with high or moderate risk of breast and ovarian cancer.

The lack of *BRCA2* rearrangements is not surprising, considering previous reports of no rearrangements among 58 Dutch [5], 82 Finnish [8], and 26 French [13] high-risk families in which no mutations had been found previously. Woodward et al. [18], however, found three *BRCA2* rearrangements in 25 families with at least one male breast cancer but no *BRCA2* rearrangements in 114 families without

male breast cancer, and Tournier et al. [22] found three *BRCA2* rearrangements in 39 French families with at least one case of male cancer. This indicates that large genomic rearrangements in *BRCA2* are more frequent in families with male breast cancer. This could not be verified in the present study, which included only nine families with male cancer.

4. Conclusion

Two *BRCA1* deletions in three families, but no *BRCA2* rearrangements, were detected with MLPA in 617 families previously tested negative for *BRCA1/2* mutations. The large genomic rearrangements accounted for 2.3% of the mutations detected in *BRCA1* and *BRCA2* in western Danish families with hereditary breast and ovarian cancer. The *BRCA1* rearrangements constituted 3.8% of the *BRCA1* mutations, which was considerably lower than reported for several western European countries and could, together with the Finnish data [8], indicate a lower frequency of these rearrangements in the Nordic countries.

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Table 1
Frequency of large genomic rearrangements in *BRCA1* in 154 high-risk families

Family characteristics	<i>BRCA1</i>		
	No.	Total	%
Female breast cancer only	0	93	0
Female breast and ovarian cancer	2	52	3.8
Female and male cancer	0	9	0
Total	2	154	1.3

These high-risk families are those for whom clinical data and pedigrees were available and in which no mutations were detected with protein truncation testing and denaturing high performance liquid chromatography.

In none of the 154 families were any large genomic rearrangements found in *BRCA2*.

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