

Large genomic *BRCA2* rearrangements and male breast cancer

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Abstract

Background: Germ-line mutations of the *BRCA2* gene are the highest known risk factors for male breast cancer (MBC). Mutations in *BRCA2* are mainly point mutations in contrast to *BRCA1* in which large genomic rearrangements are quite common. In recent literature, however, genomic alterations of *BRCA2* have been linked especially to male breast cancer families. We wanted to screen large genomic deletions and duplications of *BRCA2* among Finnish male breast cancer patients. **Methods:** We used multiplex ligation-dependent probe amplification (MLPA) to detect large genomic rearrangements in the *BRCA2* gene among 36 unselected Finnish male breast cancer patients previously tested and found negative for Finnish *BRCA1* and *BRCA2* founder mutations. **Results:** No genomic mutations of *BRCA2* nor *CHEK2**1100delC point mutations, also included in the assay, were found in this study. **Conclusion:** Large genomic *BRCA2* rearrangements were not found among our 36 Finnish male breast cancer patients. Screening of large *BRCA2* rearrangements is not likely to be advantageous in Finland.

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1. Introduction

Male breast cancer (MBC) is a rare disease that accounts for less than one percent of all cancers in men. Risk factors for MBC include hormonal imbalance, increased estrogen to androgen level, and a family history of breast and ovarian cancer [1]. The highest risk for MBC is among the carriers of breast cancer susceptibility gene *BRCA2* mutations. Mutation carriers have a lifetime risk of 6.9% for developing MBC, which is approximately 80–100 times higher than in general population [2]. The frequency of *BRCA2* mutations in different MBC populations vary from 4 to 40% [3,4], being about 12% in Finnish MBC patients [5].

The characteristic feature among Finnish hereditary breast cancer is a strong founder effect caused by the earlier history of population isolation. Eleven founder mutations in *BRCA1* and *BRCA2* genes account for 84% of all the mutations in these genes [6,7]. In fact, the clustering of

mutations of *BRCA1* and *BRCA2* genes provides clinical advantages in the mutation screening of breast cancer families since exclusion of the founder mutations is often adequate. Furthermore, in contrast to most other countries, the *BRCA2* mutations are much more common than *BRCA1* mutations in Finland [8].

Most of the known germ-line mutations in the *BRCA2* gene are point mutations affecting one to three nucleotides at a time. However, the majority of the studies published so far have utilized conventional mutation analysis methods, which are unable to detect deletions and amplifications of complete exons. Multiplex ligation-dependent probe amplification (MLPA) is a method developed especially for detecting gross copy number changes in genomic sequences and it has been successfully applied for the analysis of *BRCA1* and *BRCA2* genes [9–11]. Genomic alterations are quite common mutation type in *BRCA1* gene [9,12,13], but they have been reported only in few studies in *BRCA2* (Table 1) [11,14–24]. In male breast cancer, however, large deletions and duplications of *BRCA2* gene seem to be more frequent [11,14]. Here, we applied MLPA to detect large

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Table 1
Summary of the publications on large genomic *BRCA2* rearrangements

Inclusion criteria	Index cases		<i>BRCA2</i> rearrangements			Country	Reference
	Total <i>n</i>	MBC <i>n</i>	Total <i>n</i> (%)	Non-MBC <i>n</i> (%)	MBC <i>n</i> (%)		
HBC/OC	149	25	3 (2)	0 (0)	3 (12)	Australia and New Zealand	Woodward et al. [11]
MBC	39	39	3 (8)	0 (0)	3 (8)	France	Tournier et al. [14]
HBC/OC	121	NA	3 (2)	2 (NA)	1 (NA)	Italy	Agata et al. [15]
HBC/OC	136	NA	1 (1)	1	NA	United Kingdom	Bunyan et al. [16]
HBC/OC	93	NA	1 (1)	1	NA	Sweden	Bergman et al. [17]
HBC/OC	47	NA	1 (2)	1	NA	Israel	Wang et al. [18]
BC	130	NA	0 (0)	NA	NA	United Kingdom	Chin et al. [21]
HBC/OC	69	NA	0 (0)	NA	NA	Finland	Lahti-Domenici et al. [22]
HBC/OC	58	NA	0 (0)	NA	NA	The Netherlands	Peelen et al. [23]
HBC	26	3	0 (0)	0 (0)	0 (0)	France	Gad et al. [24]
Total	969	67	14	7	7		

MBC, male breast cancer in the family; Non-MBC, no male breast cancer in the family; HBC/OC, patients with hereditary features of breast cancer/ovarian cancer, such as multiple breast/ovarian cancer patients in the family, young age at diagnosis, bilateral breast cancer, and breast and ovarian cancer in the same individual; BC/OC, breast and ovarian cancer in the family; BC, breast cancer patients; NA, data not available.

genomic deletions and duplications of *BRCA2* in Finnish MBC patients, previously tested negative for Finnish *BRCA1* and *BRCA2* founder mutations. The most common mutation of the *CHEK2* gene, namely 1100delC, was also tested by the MLPA method.

2. Patients and methods

2.1. Male breast cancer patients

Thirty-six male breast cancer patients, from whom DNA samples from whole blood were available, were included in the study. All of the patients had been found negative for Finnish *BRCA1* and *BRCA2* founder mutations by solid-phase minisequencing (single-base extension reaction). In addition, the entire *BRCA2* coding region of 30 MBC patients had been screened for mutations by applying protein truncation test and denaturing high-performance liquid chromatography [5].

The median age at diagnosis of the MBC patients was 62 years (range from 30 to 85 years). Five MBC patients had a first degree relative diagnosed with breast cancer and one with ovarian cancer. The research protocol was approved by the Ethical Committee of the Tampere University Hospital and the Ministry of Social Affairs and Health in Finland.

2.2. Control samples

For quality control, three samples with known alterations were used. As control #1 we used DNA from a known *BRCA2* gene mutation carrier. The mutation was a 6.2 kb deletion comprising whole exons 12 and 13 of *BRCA2* [18]. The control sample was a kind gift from Dr. Dvorah Abeliovich. DNA extracted from amniotic fluid cells from a pregnancy of a trisomy 13 fetus was used as control #2, and a DNA sample having the 1100delC mutation in *CHEK2* as control #3. Ninety unselected blood samples from healthy

blood donors obtained from the Blood Center of the Finnish Red Cross in Tampere were analyzed as reference. Reference samples were used to define the range of normal variation in the MLPA analysis.

2.3. Multiplex ligation-dependent probe amplification and fragment analysis

The SALSA P045 *BRCA2/CHEK2* MLPA test kit (MRC-Holland, Netherlands) was used according to manufacturer's instructions with minor modifications. Briefly, 100 ng of target DNA in total volume of 5 μ l TE was used for the ligation reaction. After ligation, multiplex PCR amplification (33 cycles) was performed by adding 5 μ l of the ligation mixture to 2 μ l 10 \times SALSA PCR buffer and 13 μ l aqua. Five microliter of Polymerase mix containing 1 μ l SALSA PCR-primers, 1 μ l SALSA Enzyme Dilution buffer, 0.25 μ l SALSA polymerase, and 2.8 μ l aqua, was added to the tubes on ice and put then into a PCR machine (PTC-200, DNA Engine, MJ Research, Bio-Rad Laboratories, CA) straight to the denaturation step at 95 $^{\circ}$ C. After PCR amplification, DNA fragments were analyzed on a DNA sequencer (ABI Prism 310, Applied Biosystems, Foster City, CA) using Genescan-ROX 500 size standards (Applied Biosystems). SALSA P045 *BRCA2*-MLPA kit contains probes for 23 of the 27 coding exons of the *BRCA2* gene. Two probes are present for the exon 1 and for the large exon 11. No probes are present for exons 5, 6, 23, and 26 in the assay. According to the manufacture's report, these four exons are missing because they are technically problematic for the MLPA method due to their AT rich DNA content and/or close adjacencies of the exons. Fourteen control probes for other human genes located on different chromosomes are included in the kit. Three of these control probes are specific for the *CHEK2* gene, one probe is for the 1100delC mutation, one for exon 2, and one for exon 12. Two of the control probes are located on chromosome 13, upstream and downstream of *BRCA2*.

The size and the peak area information of the PCR fragments were exported from the DNA sequencer and analyzed using Excel software. The data-analysis was then performed according to the quantification protocol provided with the SALSA P045 BRCA2-MLPA kit. Shortly, after removing the peaks from non-specific amplification products the peak areas of the expected MLPA products were normalized by dividing each peak area by the combined area of all peaks in the sample. The mean of the normalized peak areas of all 90 reference samples was calculated for all 39 probes of which 25 probes were for *BRCA2* gene and 14 represented control probes. All exons differing more than 20% from the mean of the references were considered as altered. In addition, to detect the possible whole gene duplications or deletions, the proportion of the peak areas of the probes for *BRCA2* gene and for other chromosomes were calculated.

3. Results

We aimed to evaluate the existence of large genomic rearrangements of *BRCA2* gene in 36 Finnish MBC patients. To this end, we applied a multiplex ligation-dependent probe amplification method, which is capable of detecting large deletions and duplications. No genomic *BRCA2* alterations were found. Our patients were unselected for family history of cancer, although six of the 36 MBC cases (16.7%) had a first-degree relative with breast or ovarian cancer.

All our three positive controls were reliably detected by MLPA. Control #1 with a 6.2 kb deletion in *BRCA2* gene showed a clear reduction in the peak heights and areas of exons 12 and 13 (Fig. 1). Also the control sample #2 with an extra copy of the entire chromosome 13 was detectable by the MLPA method. Twenty-seven of the probes located on chromosome 13, 25 on *BRCA2*, and two on both sides of the *BRCA2* gene, showed elevated peak areas compared to the peak areas of the probes from other chromosomes. The ratio of the total peak areas of chromosome 13 probes versus non-chromosome 13 probes was increased by 22%. Third control sample with the known *CHEK2* mutation 1100delC showed an additional peak of 490 bp in the electropherogram as expected (Fig. 1).

4. Discussion

Large genomic rearrangements of *BRCA2* have been reported only in eight studies in contrast to the quite high frequency of such mutations in *BRCA1* gene (Table 1) [9,11–24]. Notably, four European studies designed to detect rearrangements of *BRCA2* failed to find any mutations among 283 invasive breast cancer patients and/or patients selected for their high-risk to *BRCA2* mutations [21–24]. In male breast cancer, however, large deletions and duplications of *BRCA2* gene seem to be much more

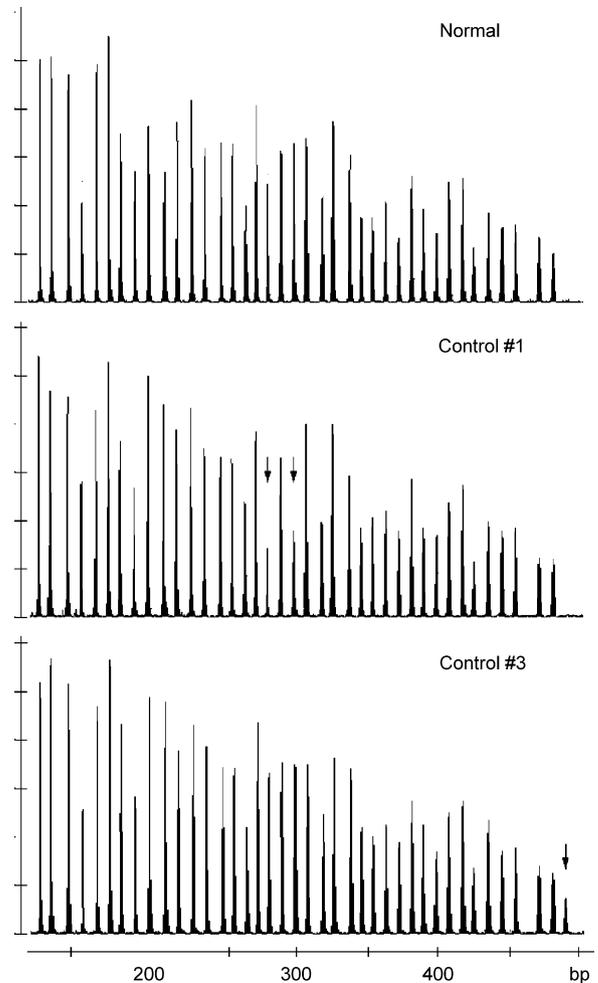


Fig. 1. MLPA fragment analysis of the *BRCA2* gene. Electropherograms of a normal reference sample and two positive controls are shown. Control #1 has a deletion of exons 12_13 (marked with arrows). Control #3, carrier of a *CHEK2**1100delC mutation, shows a peak of 490 bp (arrow).

frequent. Seven of the 14 (50%) reported *BRCA2* rearrangements have been found in 67 families with male breast cancer cases (10.4%) in contrast to 7 mutations found in 900 female breast cancer cases/families (0.8%) (Table 1). In a recent study from Australia and New Zealand, 12% (3/25) of MBC families carried *BRCA2* deletions [11]. In another study, three large *BRCA2* rearrangements were found among 39 French MBC families (8%) [14]. In Italy, three *BRCA2* deletions were detected among 121 selected breast cancer families. One of these three mutations was found in a family with a MBC case [15]. In summary, rearrangement mutations on *BRCA2* gene seem to be clustered to male breast cancer families.

In *BRCA2*, most of the reported fourteen rearrangement mutations have been found in only one patient or family. Swedish mutation c.504del15068insCCAT (numbers refer to Genbank sequence NM000059) is a recurrent mutation [17,19]. Deletion of exons 1_2 have been found in three families [11,16]. It is not possible to conclude from the published papers if the reported mutations are identical and,

if so, is deletion of exons 1_2 a founder mutation or a possible mutation hot spot.

The strongest known risk factor for the rare disease of male breast cancer is germ-line *BRCA2* mutations. *BRCA2* mutations are more frequent among MBC cases with a positive family history of breast/ovarian cancer [3–5]. However, high frequencies of *BRCA2* mutations have also been reported among family history negative MBC patients [25,26]. As many as 21% of Swedish and 43% of Hungarian family-negative MBC patients carried a *BRCA2* mutation [25,26] indicating that mutation screening is beneficial also among family negative MBC cases.

MLPA can detect several different kinds of genomic rearrangements from deletions, duplications, and amplifications of one or several exons to complete loss or duplication of a gene [9]. As many as half of the reported *BRCA2* rearrangement mutations have been found by MLPA method indicating the power of this method in the screening of large genomic alterations [11,15,16].

The frequencies of large genomic *BRCA1* rearrangements vary greatly in different populations (0–36%), just as frequencies of point mutations. For example, a great proportion (about 1/3) of detected *BRCA1* mutations is large genomic rearrangements in Dutch and Italian populations [9,12,27]. Recurrent *BRCA1* rearrangements account for a significant proportion of detected *BRCA1* mutations also in America and Germany [13,28]. However, no *BRCA1* rearrangements have been found in Finland [10,22]. Similarly in *BRCA2*, the mutation detection rate of genomic rearrangements seems to depend on population, although the clustering of mutations on MBC families is prominent (Table 1).

The *CHEK2* gene is a cell cycle checkpoint kinase. *CHEK2*1100delC* variant has been associated with an increased risk of breast cancer in non-*BRCA1/BRCA2* carriers, especially among patients with a positive family history of the disease [29]. *CHEK2*1100delC* has been shown to confer an approximately 2-fold increase of breast cancer risk in women and a 10-fold increase of risk in men lacking *BRCA1* and *BRCA2* mutations [30]. It was estimated to account for 1% of breast cancers in women and 9% of breast cancers in men [30]. We did not detect any *CHEK2*1100delC* mutations among our MBC patients. Three population-based studies of MBC from UK, USA and Israel support our results that *CHEK2*1100delC* is unlikely to account for a significant proportion of MBC cases [31–33].

In summary, large genomic *BRCA2* rearrangements were not found among our 36 Finnish male breast cancer patients. Screening of large *BRCA2* rearrangements is not likely to be advantageous in Finland.

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