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Novel genomic rearrangements in the *BRCA1* gene detected in greek breast/ovarian cancer patients

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ABSTRACT

The identification of genomic rearrangements in breast/ovarian cancer families has widened the mutational spectrum of the *BRCA1* gene, increasing the number of patients who can benefit from molecular screening. More than 60 different *BRCA1* genomic rearrangements with mapped breakpoints have been reported up to date, in all exons of the gene. The proportion of *BRCA1* mutations due to genomic rearrangements varies from 8 to 27% in different populations, probably due to both ethnic diversity and the technical approach employed. In order to estimate the contribution of *BRCA1* genomic rearrangements to hereditary breast/ovarian cancer (HBOC) predisposition in Greek families, probands from 95 families with breast/ovarian history but negative for point mutations or small insertions/deletions in *BRCA1* and *BRCA2* genes, were screened using Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF). Two large deletions of 4.2 and 4.4 kb were identified in exons 20 and 24 respectively. Additional screening, using diagnostic primers for the above deletions in exons 20 and 24, performed on another 86 probands from families with breast/ovarian cancer history and 210 cases of sporadic breast/ovarian cancer resulted in the identification of two more large genomic rearrangements. One, identified in a familial case, identical to the previous exon 24 deletion and a second, identified in a case reported as sporadic, 3.2 kb deletion involving exon 20 and reported elsewhere in another Greek patient. Three out of four genomic rearrangements described in this study were detected in patients who had developed both breast and ovarian cancer; thus suggesting a correlation between the specific phenotype and the high probability of detecting inherited rearrangements in *BRCA1*.

1. Introduction

The *BRCA1* gene was localised in chromosome 17 in 1990[1] and cloned in 1994.[2] The complete sequence and analysis of the gene was published in 1996[3] revealing that the 24 exons of *BRCA1* span an 81 kb region that has an unusually high density of *Alu* repetitive DNA (41.5%) and a relatively low density (4.8%) of other repetitive sequences. This high density of *Alu*

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sequences could provide hotspots for unequal homologous recombination.[4]

The first reported *BRCA1* rearrangement was in 1997,[5] and since then more than 60 different large genomic rearrangements involving one or more exons of the *BRCA1* gene have been described. Fifty four are deletions, eight are duplications, one is a triplication, and three combine both deletion and insertion events.[5–35] Most of them are caused by recombination between *Alu* repeats, whereas four rearrangements have been generated through unequal homologous recombination events that do not involve *Alu* repeats; instead, they are the result of recombination between the *BRCA1* gene and the *BRCA1* pseudogene.[3,6,30] Only six rearrangements have shown a founder effect while the majority of rearrangements are unique.[12–16,18–21,26] At least one genomic rearrangement has been detected in each of the *BRCA1* exons.

The proportion of *BRCA1* mutations due to genomic rearrangements varies in different countries from a highest of 27% in the Netherlands[12] to 19% in Italy,[31] 15% in American families,[14] 12% in French families,[15] 8.2% in Spain[29] and 8% in German families.[21]

In this study we report the screening for *BRCA1* genomic rearrangements, using Quantitative Multiplex PCR of Short Fluorescent Fragments, in 95 probands from breast-ovarian cancer families that were found negative for point mutations or small insertions/deletions in the *BRCA1* and *BRCA2* genes[36–39]. As two different genomic rearrangements were found in exons 20 and 24, additional screening, using diagnostic primers for these two specific exons, was performed in another 86 patients from families with breast/ovarian cancer history and 210 cases of sporadic breast/ovarian cancer.

2. Patients and methods

2.1. Patients

Screening for rearrangements in *BRCA1* was performed in 95 patients from Greek breast and/or ovarian cancer families from several Greek hospitals. 53 samples originated from the Northern Greece region (AHEPA, Papageorgiou, Theageneio Hospitals, Ormylia Monastery, University of Ioannina and University of Larissa) whereas 42 samples originated from the region of Athens (St. Savas, Alexandra, Hygeia, Iasso, Laiko, Mitera, Prolipsis and Hippokrateio Hospitals).

Table 1 – Probands selected according to family history

No. of cases of breast cancer within a family	No of cases of ovarian cancer within a family			Total cases
	0	1	2	
0		3	1	
1	17	12	1	
2	22	5		
3	10	5	1	
4	8	2		
5	4	1		
6	1			
7	1			
8	1			
Total cases	64	28	3	95

These families were selected because of a particularly high probability of *BRCA*-linked predisposition. The criteria used were: 1) one case of breast or ovarian cancer developed before the age of 35, or 2) two or more cases of breast or ovarian cancer within a family, or 3) one or more cases with breast and ovarian cancer. From the 95 families tested in our laboratory, 64 (67%) were breast cancer only families, 4 (4%) were ovarian cancer only cases and 27 (29%) were breast and ovarian cancer families (Table 1).

Probands were tested previously for point mutations or small insertions/deletions in the *BRCA1* and *BRCA2* coding regions or splice sites using protein truncation test (PTT), denaturing high performance liquid chromatography (dHPLC) and direct sequencing. All the subjects included in this study were negative for point mutations and small insertions and deletions in the *BRCA1/2* coding regions[36,37,39 and unpublished data].

An additional 86 Greek patients with breast/ovarian cancer family history and 210 Greek patients with sporadic breast cancer were evaluated only for the two genomic rearrangements identified in the cohort described above.

The study was approved by the hospitals' ethical committee. All participants provided written informed consent.

Table 2 – Primer sequences

Primer name	Primer sequence	Use
int19F	5′-AAT ATG GGG GAG TGG GAA AG-3′	Long-range PCR for exon 20 deletion
int21R	5′-GGG TTC TCC CAG GCT CTT AC-3′	
23exF	5′-GAC AGA GGA CAA TGG CTT CC-3′	Long-range PCR for exon 24 deletion
24R	5′-CTA GCT GCC TGG AAA CCA AG-3′	
int19F3	5′-TCC TCC AGC TTC AGC TTT TC-3′	PCR for exon 20 deletion
int19F4	5′-TCT CGA TCT CCT GAC CTC GT-3′	
int20R8	5′-GAG CCA AAT GCT GAC ATG AA-3′	
23intF2	5′-GGT CAG GAG TTC CAG AGC AG-3′	PCR for exon 24 deletion
24intR2	5′-TGA CTG GTT TCC GGA ATT TT-3′	