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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′	

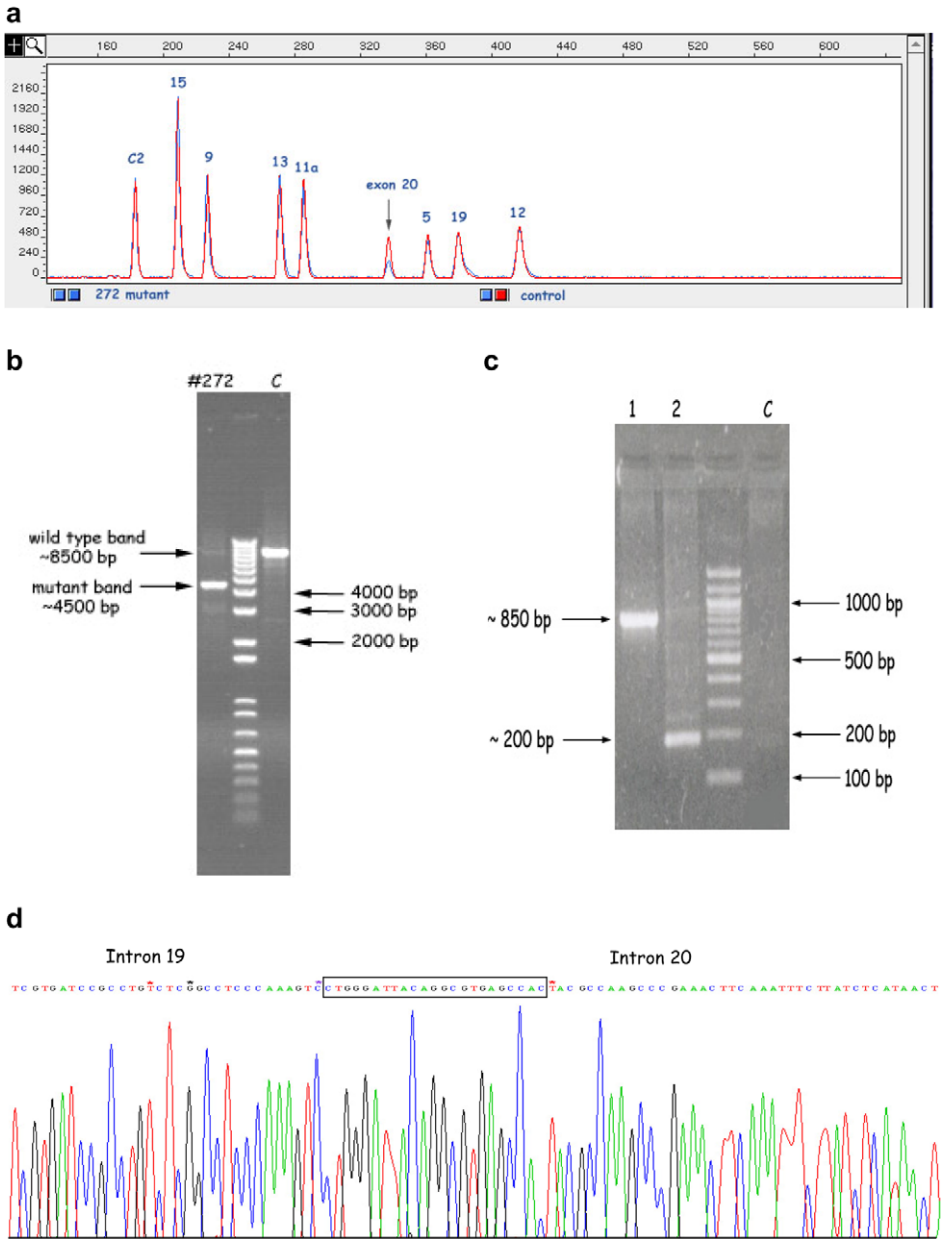


Fig. 1 – Detection and precise characterisation of exon 20 deletion. A. The QMPSF profile of patient #272 is shown in blue and is superposed to that of normal control DNA (red). The fluorescent profiles are normalised using GeneScan software to the level of the control peak C2 from exon 14 of *MLH1* gene. Blue arrow marks the deleted exon which shows a two-fold reduction of the fluorescent signal. B. Long-range PCR performed with primers int19F and int21R; only one band of the expected length is evident in control DNA (c), while the sample #272 shows a shorter band, corresponding to the deleted allele. C. PCR products of the mutant allele amplified with primers int19F3–int20R8 (lane 1) and int19F4–int20R8 (lane 2). In lane 1 the product size is 837 bp and in lane 2186 bp. The control DNA (c) could not be amplified with either of the above primer sets under the specific conditions because of its length (~ 4500 bp). D. Sequence electropherogram of the relevant portion of an 837 bp fragment containing the deletion breakpoint, obtained using primers int19F3 and int20R8 shown in C. The black box marks the 23 bp sequence of 100% homology which lies within both intron 19 and intron 20. The asterisks mark the nucleotides that differ among the repeat portions of *AluY* in intron 19 and in intron 20. E. Schematic representation of the deletion. i) The recombination site is located between nucleotides 71146–71168 in intron 19 (*AluY*) (orange) and nucleotides 75319–75341 in intron 20 (*AluY*) (green) of *BRCA1* gene (Accession number GenBank L78833). ii) Black vertical boxes indicate exons whereas the white vertical box indicates the deleted exon 20. Black vertical lines represent the *Alu* repeat positions within introns 19 and 20; orange vertical lines indicate the *AluY* positions within which the breakpoints of the specific deletion are located. Red arrows show the position of primers used for Long-range PCR and blue arrows show the position of primers used for the amplification of the 837 bp and 186 bp fragments shown in C.

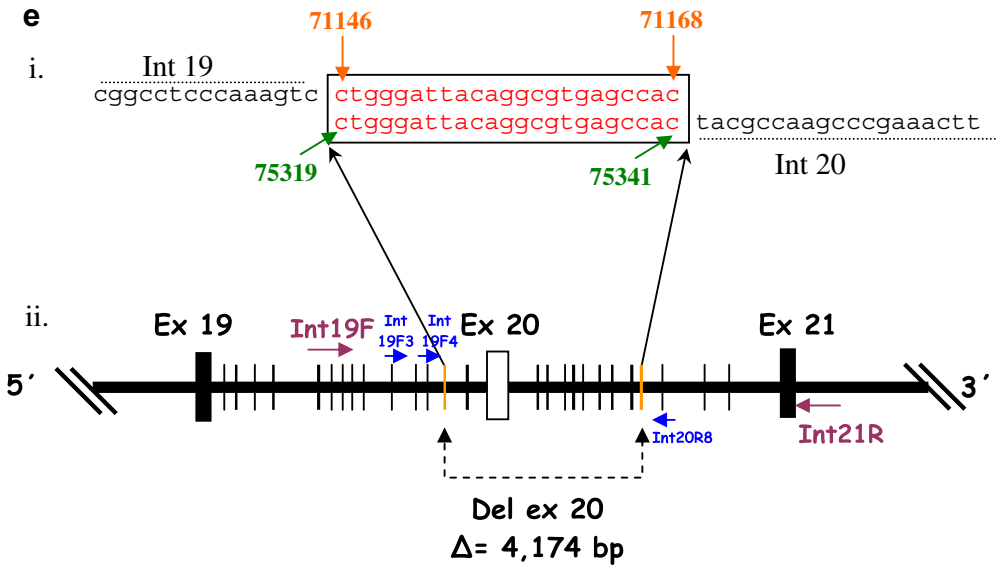


Fig. 1 - *continued*

2.2. *DNA extraction*

Genomic DNA was extracted from peripheral blood lymphocytes using the QIAamp® DNA blood Minikit (QIAGEN, Chatsworth, CA, USA) following the manufacturer's instructions. The quantity and the quality of the DNA samples were determined by UV absorbance and agarose electrophoresis.

2.3. *Quantitative multiplex PCR of short fluorescent fragments (QMPSF)*

Short exon fragments corresponding to the 22 protein-coding exons and one fragment containing the sequence of the 5′ untranslated region of the *BRCA1* gene were amplified by multiplex PCR from 100 to 150 ng of genomic DNA using chimeric and 5′-labelled with the 6-FAM fluorochrome primers. Primers and PCR conditions were performed as described elsewhere.[24]

DNA fragments were separated on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the GeneScan® TAMRA-500 size standard (Applied Biosystems). Data were analysed using the GeneScan™ 3.1 software (Applied Biosystems). Deletions were revealed by a two-fold reduction of the corresponding peak of the electropherogram.

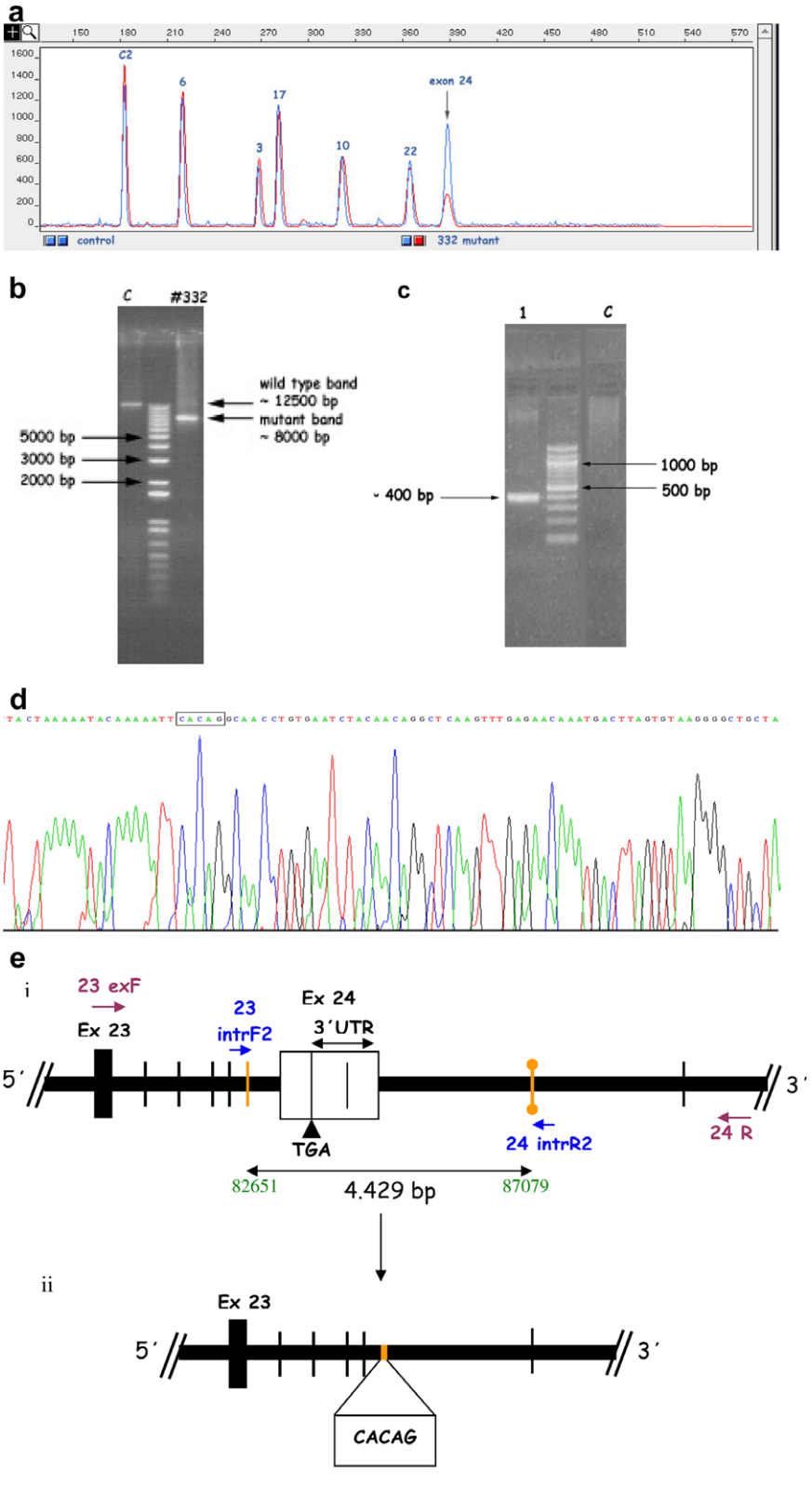
2.4. *Long-range PCR*

Genomic DNA was amplified in a reaction volume of 50 μl using 1.5 mM Mg^{2+}, 200 μM of each dNTP (dATP, dCTP, dGTP and dTTP), 10 units of Elongase® enzyme mix (Invitrogen, Carlsbad, CA, USA) and 100 ng of DNA.

Fig. 2 – Detection and precise characterisation of exon 24 deletion. A. **QMPSF analysis of the multiplex reaction in which exon 24 is amplified. Sample #332 which is superposed to that of normal control DNA (blue) and adjusted using the control amplicon C2 is shown in red. The peak which corresponds to exon 24 is indicated by the arrow above and shows a two-fold reduction of the fluorescent signal in relation to the normal one.** B. **Long-range PCR performed with primers 23exF and 24R; only one band of the expected length (~ 12.5 kb) is evident in control DNA (c), while sample 332 shows a shorter band (~ 8 kb), corresponding to the deleted allele.** C. **PCR amplification using primers 23intrF2 and 24intrR2. The fragment amplified in sample #332 (lane 1) corresponds to the mutant allele (~ 400bp). The control DNA (c) could not be amplified under the specific conditions because of its length (~ 5000 bp).** D. **Sequence electropherogram of the corresponding fragment of ~ 400 bp containing the deletion breakpoints, amplified with primers 23intrF2 and 24intrR2. The 5-nucleotide insertion is shown inside the box.** E. **Schematic representation of the exon 24 deletion. The recombination sites are located at nucleotide 82651 in intron 23 (*AluSx*) and nucleotide 87079 in the 3′ flanking region (*MER5A*) of the *BRCA1* gene (green) (Accession number GenBank L78833). Boxes represent exons. Black vertical lines correspond to *Alu* repeats found in intron 23 and the 3′ flanking region of exon 24. Orange vertical lines correspond to the repeats within which lie the breakpoints of the specific deletion; the orange line on the left indicates the *AluSx* position in intron 23 and the orange line on the right corresponds to the *MER5A* repeat sequence within which the 3′ boundary of the deletion in the 3′ flanking region of exon 24 was found. Primers used for Long-range PCR are shown with red arrows while blue arrows correspond to primers used for the PCR amplification of the deleted fragment, shown on C. The black horizontal arrow indicates the deleted fragment. Figure (ii) shows the position of the 5 bp insertion (CACAG).**

Primer design was performed using the 'Primer3' software http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi. The primers used for the detection of the exon 20 deletion were: int19F (forward) and int21R (reverse) (Table 2) at a concentration of 0.2 μM each. Cycling conditions were: 30 s of denaturation at 94 °C, then 35 cycles for 30 s at 94 °C, 30 s at 58 °C and 7 min at 68 °C, followed by extension at 68 °C for 20 min.

The primers used for the detection of the exon 24 deletion were: 23exF (forward) and 24 R (reverse) in the 3′-flanking region (Table 2) at a concentration of 0.2 μM each. Cycling conditions were: 4 min denaturation at 94 °C, followed by 5 cycles



at 94 °C for 15 s, 57 °C for 30 s and 68 °C for 15 min; the annealing temperature was then raised to 63 °C and the elongation time was increased by 20 s at each cycle for an additional 25 cycles, followed by a final extension at 68 °C for 7 min.

PCR products were electrophoresed through 0.6% agarose gel and junction fragments (resulting from the rearrangements) were visualised by ethidium bromide staining.

2.5. PCR amplification

For the detection of the boundaries of the two large genomic rearrangements, new primers were designed in order to reduce the size of the PCR products of the mutant alleles and facilitate the sequencing analysis.

For exon 20 deletion the primers used were: either the forward primer int19F3 or int19F4 and the reverse primer int20R8 (Table 2). The two alternative forward primers were used in order to determine the breakpoints of the deletion that were located on homologous Alu repeat sequences. For exon 24 deletion the primers used were: 23intF2 (forward) and 24intR2 (reverse) (Table 2). The same pairs of primers were also used as diagnostic primers for the screening of the additional 86 and 210 samples of breast/ovarian familial cancer and sporadic breast cancer respectively.

Genomic DNA was amplified in a reaction volume of 20 μl using 1.5 μM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer, and 1.25 units of enzyme (Taq DNA polymerase recombinant, Invitrogen, Carlsbad, CA, USA). PCR amplifications were performed in a Perkin-Elmer 2400 Thermocycler (Perkin Elmer, CA, USA) and in an Eppendorf 96 Master Cycler gradient Thermocycler under the following conditions: 94 °C for 45 s, 60 °C for 30 s and 72 °C for 30 s for 35 cycles. PCR products were electrophoresed through a 1% agarose gel and visualised by ethidium bromide staining under UV light.

2.6. mRNA analysis

RNA was extracted from peripheral blood leucocytes using the Macherey–Nagel Nucleospin RNA II Kit (Macherey–Nagel, Germany) according to the manufacturer's instructions. Total RNA (2 μg) was reverse transcribed using either an oligo$(dT)_{18}$-primer or random ninemers and 40 units of M-MuLV reverse transcriptase (Finnzymes, Finland) for 1 h at 37 °C.

2.7. Sequence analysis

PCR products were sequenced directly with the same forward and reverse primers used for PCR amplification. Sequencing was done using an ABI PRISM DyeDeoxy Terminator Cycle Sequencing Kit in ABI 310 and 3100 Genetic Analysers (Perkin Elmer, Applied Biosystems, CA, USA), according to manufacturer's instructions.

3. Results

Ninety-five probands from families with strong history of breast/ovarian cancer, previously found negative for point mutations or small insertions/deletions in the *BRCA1* and *BRCA2* genes[36–39 and unpublished data] were screened for genomic rearrangements in the *BRCA1* gene. Quantitative multiplex PCR of short fluorescent fragments (QMPSF) testing of those families showed two different rearrangements in two different families: a deletion of exon 20 (Family #272) and a deletion of exon 24 (Family #332). The intensity of the corresponding peaks was reduced two-fold (Figs. 1A and 2A).

In the case of the exon 20 deletion, all other peaks including those corresponding to exons 19 and 21 were normal (data not shown), indicating that the boundaries of the deletion were within introns 19 and 20. We therefore used various combinations of PCR primers to confirm the presence of the deletion and to obtain a junction fragment for sequence analysis. Starting from flanking exons, the primers that better amplified the deleted fragment were a forward primer within intron 19 (int19F) and a reverse primer containing the splice site of exon/intron 21 (int21R). Long-range PCR showed the presence of a 4.5 kb fragment (Fig. 1B) compared to the expected 8.5 kb wild type fragment, indicating that the deletion was approximately 4 kb.

In order to reduce the size of the breakpoint-containing segment to a minimum and thus facilitate sequencing, several sets of primers were designed and tested that gradually decreased the size of the amplified product. Eventually, a fragment of approximately 200 bp (Fig. 1C) was amplified by primer set int19F4 and int20R8. A larger ~800 bp segment also containing the deletion (Fig. 1C), amplified by primer set int19F3 and int20R8, was used in order to facilitate the understanding of the orientation of the sequenced product. Subsequent sequencing analysis revealed a deleted fragment of 4174 bp starting between nucleotides 71146–71168 in intron 19 and ending between nucleotides 75319–75341 in intron 20 (Accession number GenBank L78833). The exact breakpoints could not be defined with precision because of a 100% identical 23 nucleotide sequence between the two regions flanking the deletion (Fig. 1D and E).

In the case of family #332 a similar approach was followed. The 5′ boundary of the deletion was located within intron 23, since it was obvious from QMPSF testing that the pattern of the peak corresponding to exon 23 was normal (data not shown). However, since exon 24 is the terminal protein-coding exon of the *BRCA1* gene, the 3′ boundary of the deletion was at first unknown. Therefore, various combinations of primers were used in order to amplify both alleles using long-range PCR conditions. PCR showed that the deletion was lying downstream from the 3′ UTR.

Successful long-range PCR amplification between exon 23 and the 3′ flanking region with primers 23exF and 24 R indicated that the deletion included exon 24 and the 3′ UTR. The length of the amplified fragment was approximately 8 kb, in comparison to the 12 kb of the wild-type fragment showing that the deletion was approximately 4.5 kb (Fig. 2B). Narrowing of the breakpoint-containing region was achieved by using primer 23intrF2 within intron 23 and the reverse primer 24intrR2 located in the 3′ flanking region of the 3′ UTR. PCR amplification gave rise to a ~ 400 bp fragment (Fig. 2C) which was then sequenced with the same primers. The deleted fragment which was estimated to be 4429 bp had been replaced by a 5 bp fragment. The 3′ boundary of the deletion is at nucleotide 82651 and lies within an *AluSx*

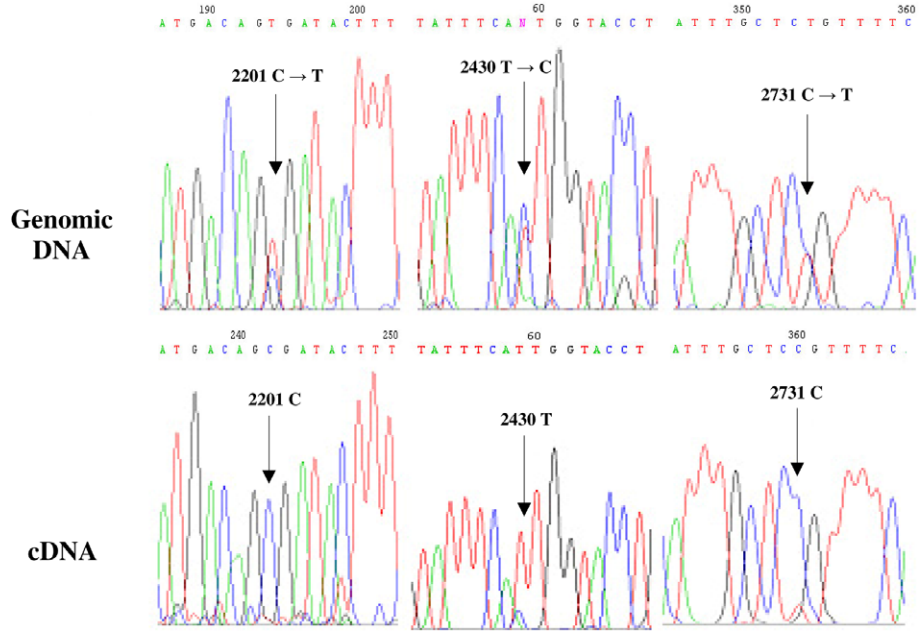


Fig. 3 – Sequence analysis of cDNA from a patient with exon 24 and 3′ UTR deletion of the BRCA1 gene. Detection of several common coding single nucleotide polymorphisms, e.g. 2201 C → T (S694S), 2430 T → C (L771L) and 2731 C → T (P871L), showed expression of only one allele of the BRCA1 gene (lower panel).

repeat sequence whereas the 5′ boundary is located at nucleotide 87079 and lies within a *MER5A* repeat (Fig. 2D and E).

Since the exon 24 deletion disrupted the polyA tail region as well as the 3′ untranslated region of *BRCA1*, we performed cDNA analysis in order to investigate whether this disruption could lead to a complete loss of mRNA transcripts of this allele. In order to distinguish between mRNA transcripts produced from each allele, the state of five common *BRCA1* exon 11 biallelic polymorphisms was examined. However, this approach was not informative for our index case, as DNA sequencing of genomic DNA revealed that patient #332 was homozygous for polymorphisms 2201 C → T, 2430 T → C, 2731 C → T, 3232 A → G and 3667 A → G. The patient's daughter however, also a carrier of the deletion, was heterozygous for the above polymorphisms; sequencing of RT-PCR products from this patient displayed *BRCA1* expression from only one allele (Fig. 3).

PCR-based screening for exon 20 and 24 genomic rearrangements using diagnostic primers was performed in an additional 86 patients from Greek families with breast/ovarian cancer and 210 patients with sporadic breast cancer. A second patient with breast and ovarian cancer was found to contain the same exon 24 deletion of 4429 bp replaced by the same 5 bp insertion detected by QMPSF analysis in family #332. A deletion of exon 20, 3246 bp in size, located between nucleotide 71617 within exon 20 and nucleotide 74863 within intron 20, was detected in a patient with sporadic cancer. The same rearrangement has already been reported in another Greek patient with breast cancer family history.[28]

4. Discussion

Germline genomic rearrangements have been found to be the cause of development of a variety of human genetic disorders, including several cancer-susceptibility syndromes.[40] Since 1997 when the first genomic rearrangement of *BRCA1* was described[5] it has been shown that genomic rearrangements make a significant contribution to the mutation spectrum of *BRCA1*. The proportion of *BRCA1* mutations due to genomic rearrangements ranges in different countries from 8% to 19% except when a founder effect results in a mutated allele with a high frequency, e.g. 27% in the Netherlands.[33]

Here we report the identification of three genomic rearrangements of the *BRCA1* gene in four Greek patients. One of the rearrangements found, involving exon 20, is novel

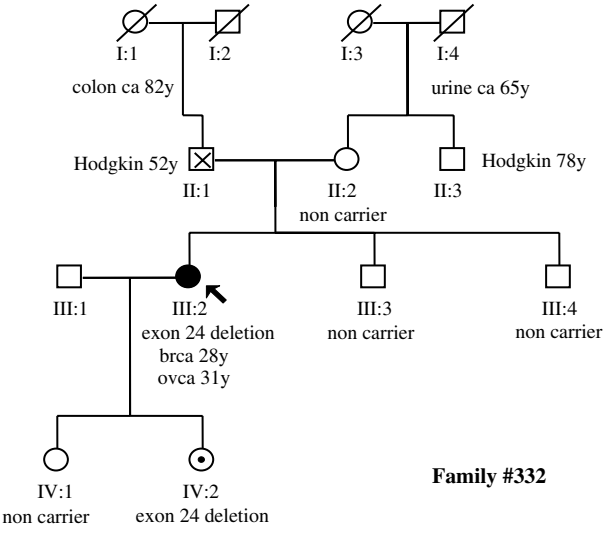


Fig. 4 – Pedigree of family #332 carrying the deletion of exon 24. y: indicates the age of onset of the disease. The arrow indicates the proband. The individual marked with X was not tested because no genetic material was available.

Table 3 – Characteristics of *BRCA1* genomic rearrangements found in four Greek breast/ovarian cancer families

Patient	*BRCA1* exons involved	Mutation name (GeneBank accession number)	Breakpoint coordinates		Notes	Family history	Detection method	Mechanism[a]	Effect on transcript
#272	20	g.71146–75319del (L78833)	Alu-Y Exon 20 start Exon 20 end Alu-Y	70878/71179 71598 71681 75052/352	4174 bp deletion	Proband had breast (40 year) and ovarian (52 year) cancer. No other family history reported.	QMPSF + LR-PCR	Alu-Alu HR	Unknown
#351	20	g.71618–74863del (L78833)	Exon 20 start Exon 20 end Alu-Sp	71598 71681 74745/75039	3246 bp deletion	Reported as sporadic.	PCR	Non Alu-Alu HR	Exon 20 skipping
#332	24	g.82651–87079del (L78833)	Alu-Sx Exon 24 start Exon 24 end 3′ UTR MER5A	82515/815 82936 84436 83061/84436 86964/87122	4429 bp deletion 5 bp insertion	Proband had breast (28 year), ovarian (31 year) cancer and adenocarcinoma of the endometrium (38 year). Many other cases of different cancers reported in the family as Hodgkin's lymphoma, colon, ureter cancer.	QMPSF + LR-PCR	Non Alu-Alu HR	No transcript
#284	24	g.82651–87079del (L78833)	Alu-Sx Exon 24 start Exon 24 end 3′ UTR MER5A	82515/815 82936 84436 83061/84436 86964/87122	4429 bp deletion 5 bp insertion	Proband had breast (53 year) and ovarian (52 year) cancer. Maternal aunt had breast cancer.	PCR	Non Alu-Alu HR	No transcript

a Mechanisms: HR, homologous recombination.

and was detected in a patient with breast and ovarian cancer. The other rearrangement also involving exon 20, previously reported in a Greek patient with breast cancer family history,[28] was detected in a Greek patient with sporadic breast cancer. The exon 24 genomic rearrangement is also novel and was detected in two apparently unrelated Greek patients with breast and ovarian cancer family history (Table 3).

The first rearrangement involves a 4.2 kb deletion of exon 20. Both breakpoints of the deletion occurred in a 23 bp region of perfect homology, within two *AluY* sequences located in introns 19 and 20 respectively and oriented in the reverse sense of the *BRCA1* transcript. This fact indicates that the deletion was most probably the result of mispairing slippage during replication and homologous recombination. Both *AluY* sequences involved in the specific rearrangement are conserved in non-human primates.[41] According to Pavlicek and collegues most Alu repeats involved in disease-associated genomic rearrangements are retained in non-human primates.[41]

This mutation was identified in a patient who developed breast cancer at the age of 40 and ovarian cancer at the age of 52. The patient deceased 6 years later. Despite our intention to study the effect of the rearrangement on the gene transcript, the first-degree relatives of the patient denied genetic testing thus the absence of genetic material did not allow us to perform mRNA analysis in order to investigate potential exon skipping.

The second rearrangement detected is a 3.2 kb deletion of exon 20, starting 20 bases away from the 5′ end inside exon 20 and extending for 3.2 kb into intron 20. Its end is lying in an *AluSp* repeated sequence and was detected in a patient reported as sporadic breast cancer. According to previously published results this deletion causes skipping of the entire exon 20 when transcribed.[28] Deletions within or involving exon 20 have been reported three times before[7,27,35] but were all different from the ones described here, as our breakpoint mapping revealed.

The 28 aminoacids encoded by exon 20 are part of the first BRCT domain and the linker domain suggesting a crucial role for the genomic rearrangements involving exon 20 of the *BRCA1* gene since they might affect the packing of the two BRCT domains and as a consequence the folding of the whole BRCA1 protein.[42]

Exon 24 genomic rearrangement involves a 4.4 kb deletion. The 5′ boundary of the deletion is located within intron 23 in an *AluSx* repeat region whereas the 3′ is localised within a *MER5A* repeat sequence and extends beyond the 3′ UTR. The results of cDNA analysis showed that the allele which carried the exon 24 and 3′ UTR deletion could not be expressed, demonstrating the significance of this genomic rearrangement in the expression of *BRCA1*. This is probably due to the fact that as the shortening of the poly-A tail (deadenylation) appears to be the first step in mRNA decay, a truncated mRNA devoid of the polyadenylation signal is degraded shortly after transcription.[43]

Deletions involving exon 24 have been reported before and concern deletions of exons 1–24, 8–24, 21–24 and 23–24.[29,31,35] In two of the above cases where breakpoints have been determined, transcript analysis has shown the existence of the mutated transcript[35] whereas in the other two cases breakpoints have not yet been determined.[29,31] However the deletion described here is a novel one since it only involves exon 24 and the 3′ UTR.

This deletion was identified in two patients: one who developed breast cancer at 28, ovarian cancer at 31 and adenocarcinoma of the endometrium at 38 (Fig. 4) and a second patient who developed ovarian cancer at 52 and breast cancer at 53.

Three out of four genomic rearrangements described in this study were detected in patients who had developed breast and ovarian cancer, thus suggesting a correlation between the specific phenotype and the high probability of detecting inherited rearrangements in *BRCA1*. These results are consistent with data published elsewhere.[34,35]

Genomic rearrangements as it was revealed by the present study account for approximately 10% (four out of 42) of the *BRCA1* mutations detected in Greek patients with breast/ovarian family history. These data are in agreement with the reported contribution of *BRCA1* rearrangements in the spectrum of *BRCA1* mutations.[33]

The identification of a specific *BRCA1* rearrangement in the set of 210 sporadic cases demonstrates that cancer predisposition is not always obvious based on family history especially in the cases of families with few female individuals and/or transmission through the paternal lineage. Thus there is a chance that family history does exist but individuals are not aware of it. The latter seems to be the case for patient #351 who was detected to carry an exon 20 deletion.

The use of diagnostic primers is very important in order to investigate the frequency of specific rearrangements in a rapid and cost-effective way. Considering the high cost of genetic testing in families with few affected members and patients with sporadic cancers, mutation- specific primers seem to be the most suitable tools in clinical trials.

Conflict of interest statement

None declared.

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