

Germ line *BRCA1* & *BRCA2* mutations in Greek breast/ovarian cancer families: 5382insC is the most frequent mutation observed[☆]

A. Ladopoulou^{a,1}, C. Kroupis^{b,1}, I. Konstantopoulou^a, L. Ioannidou-Mouzaka^c,
A.C. Schofield^d, A. Pantazidis^a, S. Armaou^a, I. Tsiagas^b, E. Lianidou^b,
E. Efstathiou^e, C. Tsionou^f, C. Panopoulos^g, M. Mihalatos^h,
G. Nasioulas^h, D. Skarlosⁱ, N.E. Haites^d, G. Fountzilas^{j,k},
N. Pandis^l, D. Yannoukakos^{a,k,*}

^aMolecular Diagnostics Laboratory, I/R-RP, National Center for Scientific Research 'Demokritos', Ag. Paraskevi Attikis, 15310 Athens, Greece

^bDepartment of Chemistry, Laboratory of Analytical Chemistry, University of Athens, Athens, Greece

^cUniversity of Athens, Medical School, Athens, Greece

^dMedical Genetics, University of Aberdeen, Aberdeen, Scotland, UK

^eAlexandra Hospital, Athens, Greece

^fBreast Cancer Unit, MITERA Maternity and Surgical Center, Athens, Greece

^gAghioi Anargyroi Hospital, Athens, Greece

^hMolecular Biology Research Center HYGEIA, 'Antonis Papayiannis', Athens, Greece

ⁱAthens Medical Center, Athens, Greece

^jAHEPA Hospital, Aristotle University of Thessaloniki Medical School, Thessaloniki, Greece

^kHellenic Cooperative Oncology Group, Athens, Greece

^lDepartment of Genetics, Saint Savas Anticancer Hospital, Athens, Greece

Received 31 August 2001; received in revised form 13 November 2001; accepted 17 November 2001

Abstract

BRCA1 and *BRCA2* genes were screened for loss-of-function mutations in a series of 85 patients having at least one first- or second-degree relative affected by breast and/or ovarian cancer. All *BRCA1* exons and *BRCA2* exons 10 and 11 were screened with a combination of methods including SSCP, PTT and direct sequencing. We have found disease-associated mutations in 14 families (16.5%), eleven in *BRCA1* and three in *BRCA2*. The known founder mutation 5382insC of *BRCA1* was identified in seven unrelated families. The other mutations identified include the non-sense R1751X, the splice junction variant 5586G > A of *BRCA1* and three frameshifts, 2024del5, 3034del4, and 6631del5, of *BRCA2*. Nine out of these 14 families had a family history of three or more breast/ovarian cancer cases. A large number of polymorphic or unclassified variants is also reported. Combined with our previously published data 5382insC was found in nine out of 20 families (45%), suggesting that this mutation may represent a common founder mutation in the Greek population. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: *BRCA1*; *BRCA2*; Greece; Familial; Breast ovarian cancer

[☆] Supported by the Greek General Secretary for Research and Technology (97EKBAN2-1.2-112), the Greek Ministry of Health (2a/3608/99), and by a grant to Molecular Biology Research Center HYGEIA 'Antonis Papayiannis' from the Stavros Niarchos Foundation for Charity.

* Corresponding author. Tel.: +30-1-650-3936; fax: +30-1-654-3526.

E-mail address: yannouka@mail.demokritos.gr (D. Yannoukakos).

¹ A.L. and C.K. contributed equally to this study.

1. Introduction

Hereditary breast and ovarian cancer syndromes can be caused by loss-of-function germline mutations in one of the tumour suppressor genes *BRCA1* and *BRCA2* [1–3]. Carriers of germline mutations in these genes have a lifetime risk of breast cancer of 50–85% and a lifetime risk of ovarian cancer of 15–45%. These mutations account for a substantial proportion of inherited breast and ovarian cancer [4], but it is likely that additional susceptibility genes will be discovered [5]. Approximately 10% of ovarian and 7% of breast cancer cases in the general population are estimated to be carriers of a breast/ovarian cancer susceptibility gene; these women are found primarily in families characterized by multiple cases of early onset breast cancer [6].

The frequency of *BRCA1* and *BRCA2* mutation carriers in women with breast or ovarian cancer (or both) depends on the study population [4,7].

While the range of pathological alterations in some countries or ethnic groups is mainly limited to a few (three mutations for 90% of Ashkenazim, three mutations for 50% of Norwegians) or even one (Iceland) mutations, in other countries the broad mutational spectrum poses difficulties in detection efforts and high-risk population management [4,7,8].

The most common disease-predisposing allele in Russia, 5382insC, is also the most common among Europeans as a whole and has migrated far from the Baltic area where it probably originated [4]. This mutation has been the only one found in a relatively high frequency in southern countries neighboring to Greece, such as Italy [9], Yugoslavia [10], and Turkey [11] (see also Ref. [12]).

Breast cancer is among the most common malignancies in Greek women [13]. In our effort to determine the contribution of *BRCA1* and *BRCA2* deleterious mutations to the development of breast and/or ovarian cancer in the Greek population, we screened all exons and intron-exon boundaries of *BRCA1* and exons 10 and 11 of *BRCA2* gene in 85 patients with breast/ovarian family history.

2. Patients and methods

2.1. Patients and their families

The selection criteria used in this study are at least

two first or second-degree relatives from the same ancestral lineage affected with breast cancer under age 50. In the cases where ovarian cancer was also present in the family, inclusion was made independently of the age of onset. This study group (85 patients) contains also seven patients without family history but with very early-onset breast cancer (under age 35) or bilateral breast cancer. In the majority of cases, two or more generations are included, with the exception of family #85.

Patients were selected ad hoc, under informed consent, from several Greek hospitals mainly located in the Athens area including Saint Savas, Aghioi Anargyroi, Mitera, Hygeia, Athens Medical Center, and Alexandra, as well as AHEPA hospital of Thessaloniki in collaboration with the Hellenic Cooperative Oncology Group (HECOG). Some samples were also collected from the private practices of collaborating doctors. When available, proband relatives were also tested for mutations identified to determine carrier status; however, in all cases probands were patients and not healthy individuals. Number of affected members ranged from 2 to 7. This study group contained 85 families, comprising (i) seven patients with no family history but development of breast cancer at age lower than 35 years; (ii) 41 families with two cases; (iii) 25 families with three cases; and (iv) 12 families with more than three cases of breast or ovarian cancer. Characteristics of the families where loss-of-function mutations were identified, in terms of number of cancer cases and age of onset as well as mutations found, are given in Table 1.

2.2. Mutation screening

Mutation analysis was performed in two different centers in Greece and in Scotland with slightly different protocols. Genomic DNA from 60 patients was analyzed at the Molecular Diagnostics laboratory (Greece) and from 25 other patients in the Department of Medical Genetics, University of Aberdeen, Scotland.

In the first case (60 patients) our screening method employed PTT (see below) for exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2* and SSCP or direct sequencing for the rest of *BRCA1* exons. In 20 patients, exon 11 of *BRCA1* was also directly sequenced, in order to determine frequent point muta-

Table 1
Phenotypes and mutations identified in Greek breast/ovarian cancer patients

	Patient no.	Total no. of BrCa cases (age of onset)	No. of OvCa cases (age of onset)	Gene/exon	Mutation	Effect	Comments/other cancers
Families with >3 cases	177,187	5 (41, 36, 52, xx, xx years)		<i>BRCA1</i> /exon20	5382insC	ter 1829	
	180, 181	4 (47, 47, 30, xx years)		<i>BRCA1</i> /exon20	5382insC	ter 1829	
	198, 199, 200	4 (35, 50, 50, xx)		<i>BRCA1</i> /exon20	5382insC	ter 1829	Vitiligo
	178	4		<i>BRCA1</i> /exon23	5586G > A	Splice site	Leukemia, testicular, gastric, head, neck cancer
	144	4 (66, 33, 34, 39 years)		<i>BRCA2</i> /exon10	2024del5	ter 599	One case of male BrCa and one of prostate cancer
Families with three cases	172	3 (59, 39, 30 years)	4 (75, 74, xx, xx years)	<i>BRCA1</i> /exon20	5382insC	ter 1829	One case of bilateral BrCa
	AB07	3 (39, 45, 51)		<i>BRCA1</i> /exon20	5382insC	ter 1829	
	85	3 (36, 38, xx years)		<i>BRCA2</i> /exon11	6631del5	ter 2137	
Families with two cases	AB15	2 (32, 48 years)		<i>BRCA2</i> /exon11	3034del4	ter 958	One case of prostate cancer
	189	2 (41, 42, 32 years)	1	<i>BRCA1</i> /exon20	R1751X	Arg to stop	Br & OvCa in the same patient
	AB21	2 (47, 47)		<i>BRCA1</i> /exon20	5382insC	ter 1829	
	AB23	2 (47, 47)		<i>BRCA1</i> /exon20	5382insC	ter 1829	
	155	2 (40, 50 years)	1	<i>BRCA1</i> /exon23	5586G > A	Splice site	Br & OvCa in the same patient
No of family history	175	1 (45 years)		<i>BRCA1</i> /exon20	R1751X	Arg to stop	Bilateral BrCa

tions, missed by PTT. In the second case (25 patients) pre-screening was essentially performed with SSCP analysis concerning all exons of *BRCA1* and exons 10 and 11 of *BRCA2*.

Primer pairs were used to amplify exons and intron-exon boundaries from genomic DNA extracted from patient samples with routine techniques. Primer selection was made either from BIC database at http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/Member/BRCA1_mutation_database.html, or by using the 'Primer3' software at http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi. Genomic DNA was amplified by the polymerase chain reaction (PCR) in a Perkin-Elmer 2400 Thermocycler (Perkin Elmer, CA, USA) and in an Eppendorf 96 Master Cycler gradient Thermocycler, following the thermal cycling protocol as described [14]. Accession numbers used for cDNA sequences are for *BRCA1* U14680 and for *BRCA2* U43746.1.

2.3. SSCP analysis

Two sets of gel conditions were used for SSCP analyses: one 5% polyacrylamide gel containing 5% glycerol, and one 5% polyacrylamide gel without glycerol. Gel electrophoresis was performed under non-denaturing conditions at 5°C at 300 V for 3 h using the Protean II Xi cell electrophoresis system (Biorad). Following gel electrophoresis, gels were stained by silver staining to visualise the banding patterns.

2.4. PTT analysis

PTT analysis was performed on PCR-amplified fragments of the large exons of the two genes: three overlapping fragments A–C for exon 11 of *BRCA1*, one fragment (D) for exon 10 of *BRCA2* and four fragments (E–H) for exon 11 of *BRCA2*. Specially designed primers containing T7 promoter and Kozak translation initiation sequence were used [15–17] with the modification of altering the reverse primer for fragment A to 5'-CTCTTGGAAGGCTAGGATTGAC'-3 (residues 2283–2304, Dr F.B. Hogervorst, personal communication). PCR reactions were performed in 25- μ l total reaction volumes using 12.5 μ l Master Mix (QIAGEN), 20 pmoles of each primer and 2.5 μ l QIAGEN purified DNA. PCR program was as follows: initial step at 93°C 3 min, then 32 cycles of 93°C 1 min/

58°C 1 min/72°C 2.30 min for fragments A–D, and the same program with annealing at 55°C for fragments E–H. PCR product sizes ranged from 1.1 to 1.7 Kb. A total of 1 μ l of each product was subjected to single tube transcription/translation at 30°C for 90 min with 5 μ l of the TnT T7 Quick Coupled rabbit reticulocyte lysate (Promega) with 0.25 μ l magnesium acetate 25 mM and 0.25 μ l Easy Tag [³⁵S]-Methionine (NEN). A total of 0.5 μ l of the translation products were run on 12% SDS PAGE gels, fixed and dried. Gels were exposed to Kodak Biomax MS film in the corresponding Trans-Screen HE intensifying screen for 2 days at –70°C.

2.5. DNA sequencing

PCR products were sequenced directly with the same forward and, when needed, reverse primers used for PCR amplification. Sequencing was done using an ABI PRISM DyeDeoxy Terminator Cycle Sequencing Kit and an ABI 310 Genetic Analyzer (Perkin Elmer, Applied Biosystems, CA, USA), according to manufacturer's instructions. Any mutation found was confirmed on a second DNA sample isolated from a duplicate tube of blood followed by sequencing in both forward and reverse directions.

3. Results

Pedigrees of the most representative high-risk families where carriers were identified are shown in Fig. 1.

3.1. *BRCA1* mutation analysis

Screening of *BRCA1* exon 11 by PTT revealed no truncated protein products. Two deleterious mutations were identified by direct sequencing in exon 20, 5382insC and R1751X. The known founder mutation 5382insC was observed in seven families; four of these had a very strong family history with at least four members affected (Fig. 1). In three families (#177, 180, 198) where blood samples were available from an affected relative, the presence of 5382insC was documented in all of them. In one family (180) a healthy carrier was also identified (Table 1). The non-sense mutation R1751X was found in a patient (175) with bilateral breast cancer, but without any family history. The same mutation (R1751X) was

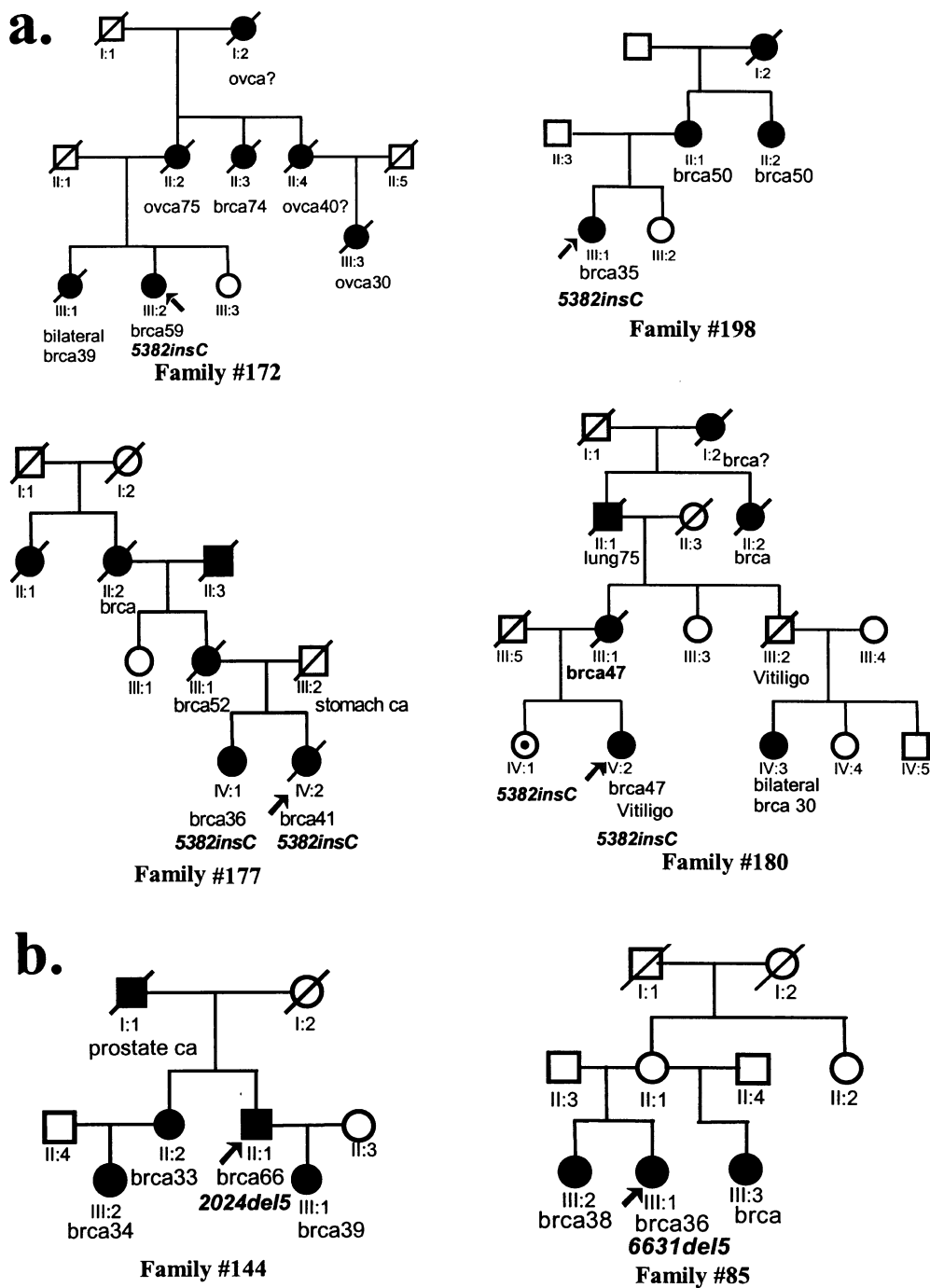


Fig. 1. Pedigrees of families carrying *BRCA1* and *BRCA2* mutations. Brca_{xx} indicates the age at diagnosis for the affected individuals and the arrow indicates the proband. For confidentiality and clarity of the figure, only the information useful for the evaluation of the genetic predisposition is shown. Available samples tested are marked with the mutation found.

found in another patient who developed breast and ovarian cancer at age 41 and 42 years old, respectively, with a family history of only one other member affected.

The splice-junction mutation 5586G > A, corresponding to an alteration of the last base in exon 23, was identified in two unrelated patients. This mutation may result in an alternatively spliced form of *BRCA1* and therefore be a disease-associated mutation.

3.2. *BRCA2* mutation analysis

Preliminary search for mutations on the *BRCA2* gene was performed by applying the PTT analysis to exons 10 and 11. PTT analysis led to the identification of truncated protein products, in families 144 and 85, respectively (see Fig. 2). Sequence analysis of the regions likely to contain the protein-terminating alterations revealed two frameshift mutations (Table 1, Fig. 3). Mutation 2024del5 causes premature protein termination at codon 599. This mutation was found in a male breast cancer patient having three female relatives affected by breast cancer only. Mutation 6631del5 causes premature protein termination at codon 2137 of *BRCA2* and was found in a family with three breast cancer cases.

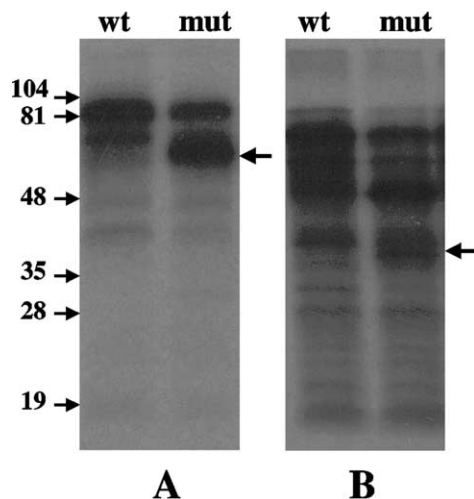


Fig. 2. PTT analysis applied to exons 10 and 11 of *BRCA2* gene. In vitro synthesized polypeptides correspond to exon 10 and to fragment H of exon 11. Abnormal peptides caused by frameshift mutations occurring in families 144 (panel A), and 85 (panel B) are indicated by arrows.

Another mutation (3034del4) was identified using SSCP in patient AB15 with a family history of one affected relative.

3.3. Unclassified variants

Interestingly, a rare missense mutation (G1738R) was identified in exon 20 of *BRCA1* in four unrelated patients. This mutation is reported only once in BIC by Myriad Genetics. It has been shown that a different alteration in the same residue, G1738E, results in loss of *BRCA1* protein function [18,19]; this residue is located inside the linker of the two BRCT domains in the C-terminal region of *BRCA1* [20]. We are still not able to do segregation analysis because of difficulties in obtaining samples from affected and healthy relatives.

Some other rare missense mutations were identified only once inside the BRCT domains of *BRCA1*, including A1823T (exon 23), V1833M (exon 24), P1856S (exon 24) which need further investigation in order to classify them.

Alterations identified in exonic or intervening sequences of *BRCA1* and *BRCA2* corresponding to polymorphisms or unclassified variants in this study of the Greek population are described in Table 2. Mutation IVS10 + 12delT in *BRCA2* was found surprisingly in 30 patients and 30 controls tested. This may be due to the polyT tract present in exon/intron ten boundaries.

3.4. Correlation with immunohistochemical data

Immunohistochemistry data were available in 5 *BRCA1* mutation carriers and 2 *BRCA2* mutation carriers. Four out of five tumors in *BRCA1* mutation carriers were negative for estrogen receptors (ER) and both *BRCA2* mutation carriers were estrogen receptor positive.

4. Discussion

In our previous study we had identified 6 patients carrying loss-of-function mutations from a series of 30 patients (20%) [12]. In this study 14 patients were identified carrying a deleterious mutation out of 85 screened (16.5%).

The only common mutation present in both groups

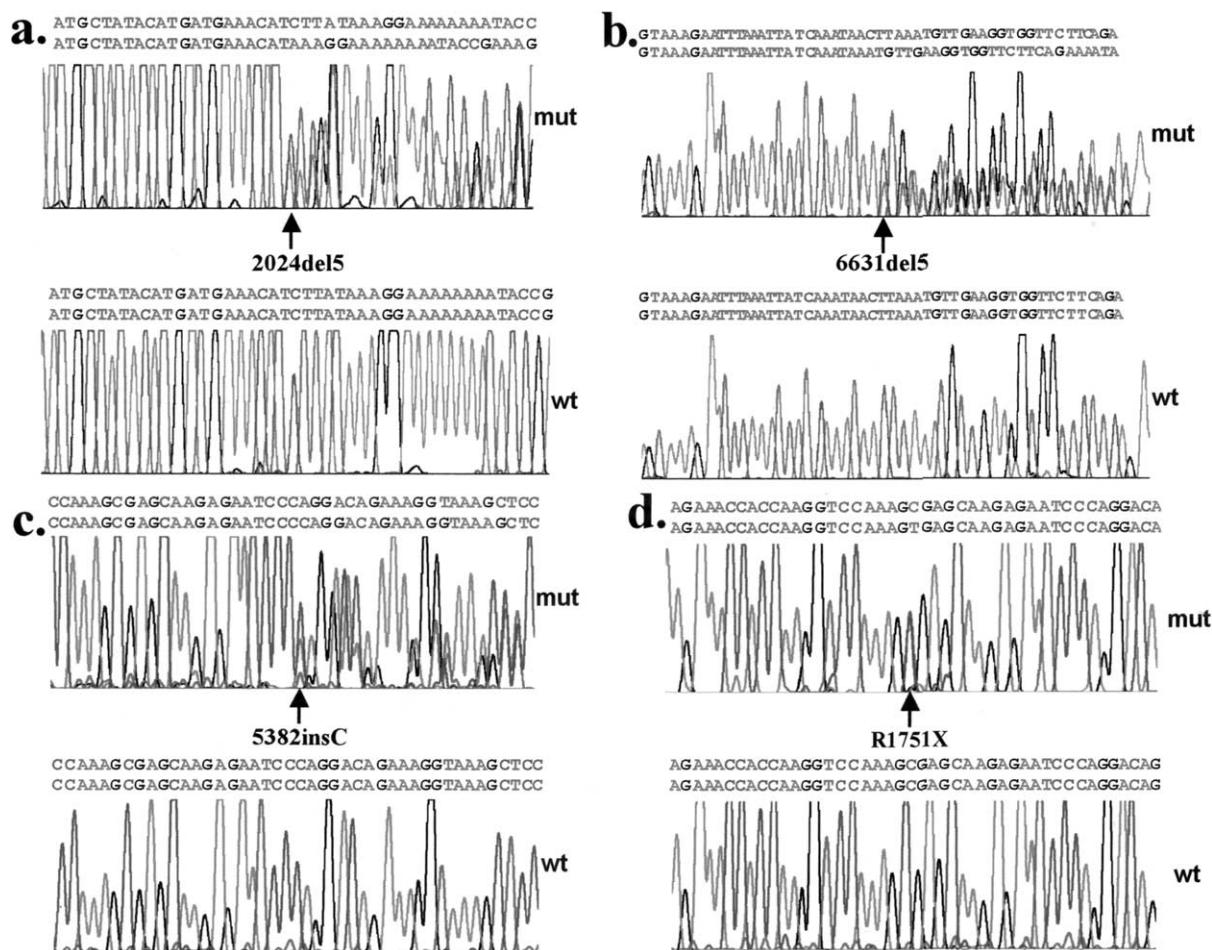


Fig. 3. DNA sequencing analysis of mutation-affected probands. In the lower panel of each set is the sequence from control individuals. (a) 2024del5; (b) 6631del5; (c) 5382insC; and (d) R1751X. Mutation sites are indicated by arrows.

studied is 5382insC, found in nine out of 20 families with a deleterious *BRCA1* or *BRCA2* mutation. Therefore the founder mutation 5382insC represents approximately 45% of the cases identified. Because of this result, 65 additional patients were screened only for 5382insC (making a total of 150 patients screened for this mutation), but no other carriers were found. It has to be highlighted at this point that our study group contains patients from families with no (<35 years old), medium (two members affected) and strong (three or more members affected) family history, while families carriers of 5382insC usually have a strong family history. In a multinational study, the single Greek patient reported was a carrier

of 5382insC [21]. This is not surprising as this mutation has been observed in high frequency by us, as well as in very high frequencies in North Eastern European countries (mainly Hungary and Russia) with geographic and historical proximity to Greece [4,7]. Mutation R1751X has been reported in Austrian patients [22] and four times in BIC.

Mutation 5586G > A probably affects splicing of *BRCA1* gene as it alters the last base of exon 23. However, further investigation will be needed in order to clarify this hypothesis. It has been reported only once in BIC.

The three *BRCA2* deleterious mutations have been reported in other countries and are therefore not

Table 2
Polymorphisms or unclassified variants identified in *BRCA1* and *BRCA2* genes

Gene/exon	Nucleotide	Codon	Base change	AA change	Designation	Mutation type	Mutation effect	Times in BIC
<i>BRCA1/1</i>	1802 ^a	5'UTR	C to G		1802C > G	P	P	–
<i>BRCA1/5</i>	IVS4	–	C to A		IVS4-19 C > A	P	P	–
<i>BRCA1/8</i>	IVS7	–	C to T		IVS7-34 C > T	P	P	6
<i>BRCA1/9</i>	IVS8	–	delT		IVS8-57delT	P	P	10
<i>BRCA1/9</i>	686	189	T to C	Asp to Asp	D189D ^b	P	P	–
<i>BRCA1/9</i>	710	197	C to T	Cys to Cys	C197C	P	P	28
<i>BRCA1/11</i>	1186	356	A to G	Gln to Arg	Q356R	M	P	29
<i>BRCA1/11</i>	2196	693	G to A	Asp to Asn	D693N	P	P	6
<i>BRCA1/11</i>	2201	694	C to T	Ser to Ser	S694S	P	P	11
<i>BRCA1/11</i>	2430	771	T to C	Leu to Leu	L771L ^b	P	P	22
<i>BRCA1/11</i>	2731	871	C to T	Pro to Leu	P871L ^b	M	P	20
<i>BRCA1/11</i>	3232	1038	C to T	Glu to Gly	E1038G ^b	M	P	10
<i>BRCA1/11</i>	3667	1183	A to G	Lys to Arg	K1183R ^b	M	P	30
<i>BRCA1/11</i>	3878	1253	T to G	Ser to Ser	S1253S	P	P	–
<i>BRCA1/13</i>	4427	1436	T to C	Ser to Ser	S1436S ^b	P	P	25
<i>BRCA1/16</i>	4956	1613	A to G	Ser to Gly	S1613G ^b	M	P	32
<i>BRCA1/16</i>	4962	1615	G to A	Ala to Thr	A1615T	M	UV	–
<i>BRCA1/16</i>	5012	1631	T to C	Ser to Ser	S1631S ^b	P	P	–
<i>BRCA1/16</i>	5075	1652	G to A	Met to Ile	M1652I ^b	M	UV	20
<i>BRCA1/16</i>	IVS16	–	G to A		IVS16-68G > A ^b	P	P	3
<i>BRCA1/16</i>	IVS16	–	G to A		IVS16-92G > A ^b	P	P	3
<i>BRCA1/18</i>	IVS17	–	C to T		IVS17-53 C > T	UV	UV	–
<i>BRCA1/18</i>	IVS18	–	G to A		IVS18 + 65G > A ^b	P	P	1
<i>BRCA1/20</i>	5331	1738	G to A	Gly to Arg	G1738R	M	UV	1
<i>BRCA1/20</i>	5375	1752	A to C	Ala to Ala	A1752A ^b	P	P	–
<i>BRCA1/23</i>	5586	1823	G to A	Ala to Thr	A1823T	UV	UV	–
<i>BRCA1/24</i>	5616	1833	G to A	Val to Met	V1833M	M	UV	1
<i>BRCA1/24</i>	5685	1856	C to T	Pro to Ser	P1856S	UV	UV	–
<i>BRCA2/2</i>	203	5'UTR	G to A	–	203G > A	P	P	10
<i>BRCA2/2</i>	282	18	C to T	Arg to Arg	R18R	P	P	–
<i>BRCA2/5</i>	IVS5		T > C		IVS5 + 26T > C	UV	UV	–
<i>BRCA2/8</i>	IVS8		C > T		IVS8 + 56C > T	UV	UV	3
<i>BRCA2/10</i>	1093	289	A to C	Asn to His	N289H	M	UV	7
<i>BRCA2/10</i>	1342	372	C to A	His to Asn	H372N	M	P	6
<i>BRCA2/11</i>	IVS10	–	delT		IVS10 + 12delT	P	P	2
<i>BRCA2/11</i>	3199	991	A to G	Asn to Asp	N991D	P	P	2
<i>BRCA2/11</i>	4035	1269	T to C	Val to Val	V1269V	P	P	3
<i>BRCA2/18</i>	IVS17	–	T to C		IVS17-12 T > C	P	P	1

^a Polymorphism within the beta-promoter sequence of *BRCA1* (accession number U37574).

^b Previously published mutations by us are also included in order to give a complete figure of the observed mutations in Greek population for future use as markers.

restricted to the Greek population. Mutation 2024del5 has been reported in Northern Europe [16]. There are three entries in BIC concerning patients from Sweden, Germany and Brazil. Mutation 6631del5 has been reported in Italy [9] but also in Germany (BIC) and Sweden [16] (five entries in BIC). Mutation 3034del4

is a recurrent mutation reported in very different ethnic groups including American, Spanish, Dutch Irish and other families (11 entries in BIC) [23,24].

It is surprising however that no deleterious mutation was identified in exon 11 of *BRCA1* although in our previous study there were four (out of 30) patients

carrying mutations in this exon. One possible explanation is that the three out of these four patients originate from northern Greece and were collected at AHEPA Hospital of Thessaloniki, while our current group of patients consists mainly from patients originating from Southern Greece. However, the relatively small number of samples may explain this inconsistency. Exons 2 and 5, where common mutations are described in other populations (185delAG and C61G), were sequenced directly in 150 patients but no sequence variation was observed, contrary to other highly variable exons as 11, 16, 20, etc.

Four out of five tumors carrying *BRCA1* deleterious mutations were ER negative contrary to the two *BRCA2* tumors that were ER positive. This observation is consistent with a previous study [25]. We were not able to do an extensive analysis of other prognostic factors mainly because of the lack of complete patient records.

Our results indicate that *BRCA1* and *BRCA2* are responsible for a part of hereditary site-specific breast cancer cases. It has to be noted that although the experimental approach used (combination of PTT and Sequencing) has a very high detection sensitivity, large genomic rearrangements are not detected, and some loss-of-function mutations located near the borders of PTT fragments may be missed; in addition, our screening strategy includes only exons 10 and 11 of *BRCA2* (50% of the coding region).

Although 5382insC is the most frequent deleterious mutation observed in the Greek population so far, mutation detection strategies should include complete analysis of *BRCA1* and *BRCA2* genes because many other unique or low frequency loss-of-function mutations exist in this population. Screening of a larger patient group remains also necessary.

Acknowledgements

We are indebted to the families for their collaboration. This work was supported by the Greek General Secretary for Research and Technology and the Greek Ministry of Health.

References

[1] Y. Miki, J. Swensen, D. Shattuck-Eidens, P.A. Futreal, K.

- Harshman, S. Tavtigian, Q. Liu, C. Cochran, L.M. Bennett, W. Ding, R. Bell, J. Rosenthal, C. Hussey, T. Tran, M. McClure, C. Frye, T. Hattier, R. Phelps, A. Haugen-Strano, H. Katcher, K. Yakumo, Z. Gholami, D. Shaffer, S. Stone, S. Bayer, C. Wray, R. Bogden, P. Dayananth, J. Ward, P. Tonin, S. Narod, P.K. Bristow, F.H. Norris, L. Helvering, P. Morrison, P. Rosteck, M. Lai, J.C. Barrett, C. Lewis, S. Neuhausen, L. Canon-Allbright, D. Goldgar, R. Wiseman, A. Kamb, M.H. Skolnick, A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*, *Science* 266 (1994) 66–71.
- [2] P.A. Futreal, Q. Liu, D. Shattuck-Eidens, C. Cochran, K. Harshman, S. Tavtigian, L.M. Bennett, A. Haugen-Strano, J. Swensen, Y. Miki, *BRCA1* mutations in primary breast and ovarian carcinomas, *Science* 266 (1994) 120–122.
- [3] R. Wooster, G. Bignell, J. Lancaster, S. Swift, S. Seal, J. Mangion, N. Collins, S. Gregory, C. Gumbs, G. Micklem, Identification of the breast cancer susceptibility gene *BRCA2*, *Nature* 378 (1995) 789–792.
- [4] C.I. Szabo, M.C. King, Population genetics of *BRCA1* and *BRCA2*, *Am. J. Hum. Genet.* 60 (1997) 1013–1020.
- [5] T. Kainu, S.H. Joo, R. Desper, A.A. Schaffer, E. Gillanders, E. Rozenblum, D. Freas-Lutz, D. Weaver, D. Stephan, J. Bailey-Wilson, O.P. Kallioniemi, M. Tirkkonen, K. Syrjakoski, T. Kuukasjarvi, P. Koivisto, R. Karhu, K. Holli, A. Arason, G. Johannesdottir, J.T. Berghthorsson, H. Johannsdottir, V. Egilsson, R.B. Barkardottir, O. Johannsson, K. Haraldsson, T. Sandberg, E. Holmberg, H. Gronberg, H. Olsson, A. Borg, P. Vehmanen, H. Eerola, P. Heikkila, S. Pyyhonen, H. Nevanlinna, Somatic deletions in hereditary breast cancers implicate 13q21 as a putative novel breast cancer susceptibility locus, *Proc. Natl. Acad. Sci. USA* 97 (2000) 9603–9608.
- [6] E.B. Claus, J.M. Schildkraut, W.D. Thompson, N.J. Risch, The genetic attributable risk of breast and ovarian cancer, *Cancer* 77 (1996) 2318–2324.
- [7] S.L. Neuhausen, Ethnic differences in cancer risk resulting from genetic variation, *Cancer* 86 (1999) 2575–2582.
- [8] S.V. Hodgson, N.E. Haines, M. Caligo, J. Chang-Claude, D. Eccles, G. Evans, P. Moller, P. Morrison, C.M. Steel, D. Stoppa-Lyonnet, H. Vasen, A survey of the current clinical facilities for the management of familial cancer in Europe. European Union BIOMED II Demonstration Project: Familial Breast Cancer: audit of a new development in medical practice in European centres, *J. Med. Genet.* 37 (2000) 605–607.
- [9] M. Santarosa, R. Dolcetti, M.D. Magri, D. Crivellari, M.G. Tibiletti, A. Gallo, S. Tumolo, L. Della Puppa, D. Furlan, M. Boiocchi, A. Viel, *BRCA1* and *BRCA2* genes: role in hereditary breast and ovarian cancer in Italy, *Int. J. Cancer* 83 (1999) 5–9.
- [10] J. Papp, L. Raicevic, J. Milasin, B. Dimitrijevic, S. Radulovic, E. Olah, Germline mutation analysis of *BRCA1* and *BRCA2* genes in Yugoslav breast/ovarian cancer families, *Oncol. Rep.* 6 (1999) 1435–1438.
- [11] H. Yazici, O. Bitisik, E. Akisik, N. Cabioglu, P. Saip, M. Muslu-manoglu, G. Glendon, E. Bengisu, S. Ozbilen, M. Dincer, S. Turkmen, I.L. Andrulis, N. Dalay, H. Ozcelik, *BRCA1* and *BRCA2* mutations in Turkish breast/ovarian families and young breast cancer patients, *Br. J. Cancer* 83 (2000) 737–742.

- [12] I. Konstantopoulou, C. Kroupis, A. Ladopoulou, A. Pantazidis, D. Boumba, E.S. Lianidou, M.B. Petersen, L. Florentin, E. Chiotellis, G. Nounesis, E. Efstathiou, D. Skarlos, C. Tsiou, G. Fountzilas, D. Yannoukakos, BRCA1 mutation analysis in breast/ovarian cancer families from Greece, *Hum. Mutat.* 6 (2000) 272–273.
- [13] C. Mettlin, Global breast cancer mortality statistics, *CA Cancer J. Clin.* 49 (1999) 138–144.
- [14] L.S. Friedman, E.A. Ostermeyer, C.I. Szabo, P. Dowd, E.D. Lynch, S.E. Rowell, M.C. King, Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families, *Nat. Genet.* 8 (1994) 399–404.
- [15] F.B. Hogervorst, R.S. Cornelis, M. Bout, M. van Vliet, J.C. Oosterwijk, R. Olmer, B. Bakker, J.G. Klijn, H.F. Vasen, H. Meijers-Heijboer, Rapid detection of BRCA1 mutations by the protein truncation test, *Nat. Genet.* 10 (1995) 208–212.
- [16] S. Hakansson, O. Johannsson, U. Johannsson, G. Sellberg, N. Loman, A.M. Gerdes, E. Holmberg, N. Dahl, N. Pandis, U. Kristoffersson, H. Olsson, A. Borg, Moderate frequency of BRCA1 and BRCA2 germ-line mutations in Scandinavian familial breast cancer, *Am. J. Hum. Genet.* 60 (1997) 1068–1078.
- [17] O.M. Serova, S. Mazoyer, N. Puget, V. Dubois, P. Tonin, Y.Y. Shugart, D. Goldgar, S.A. Narod, H.T. Lynch, G.M. Lenoir, Mutations in BRCA1 and BRCA2 in breast cancer families: are there more breast cancer-susceptibility genes? *Am. J. Hum. Genet.* 60 (1997) 486–495.
- [18] F. Hayes, C. Cayan, D. Barilla, A.N. Monteiro, Functional assay for BRCA1: mutagenesis of the COOH-terminal region reveals critical residues for transcription activation, *Cancer Res.* 60 (2000) 2411–2418.
- [19] J. Vallon-Christersson, C. Cayan, K. Haraldsson, N. Loman, J.T. Bergthorsson, K. Brondum-Nielsen, A.M. Gerdes, P. Moller, U. Kristoffersson, H. Olsson, A. Borg, A.N. Monteiro, Functional analysis of BRCA1 C-terminal missense mutations identified in breast and ovarian cancer families, *Hum. Mol. Genet.* 10 (2001) 353–360.
- [20] X. Zhang, S. Morera, P.A. Bates, P.C. Whitehead, A.I. Coffey, K. Hainbucher, R.A. Nash, M.J. Sternberg, T. Lindahl, P.S. Freemont, Structure of an XRCC1 BRCT domain: a new protein-protein interaction module, *EMBO J.* 17 (1998) 6404–6411.
- [21] H.A. Risch, J.R. McLaughlin, D.E. Cole, B. Rosen, L. Bradley, E. Kwan, E. Jack, D.J. Vesprini, G. Kuperstein, J.L. Abrahamson, I. Fan, B. Wong, S.A. Narod, Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer, *Am. J. Hum. Genet.* 68 (2001) 700–710.
- [22] T.M. Wagner, R.A. Moslinger, D. Muhr, G. Langbauer, K. Hirtenlehner, H. Concin, W. Doeller, A. Haid, A.H. Lang, P. Mayer, E. Ropp, E. Kubista, B. Amirimani, T. Helbich, A. Becherer, O. Scheiner, H. Breiteneder, A. Borg, P. Devilee, P. Oefner, C. Zielinski, BRCA1-related breast cancer in Austrian breast and ovarian cancer families: specific BRCA1 mutations and pathological characteristics, *Int. J. Cancer* 77 (1998) 354–360.
- [23] H. Takahashi, H.C. Chiu, C.A. Bandera, K. Behbakht, P.C. Liu, F.J. Couch, B.L. Weber, V.A. LiVolsi, M. Furusato, B.A. Rebane, A. Cardonick, I. Benjamin, M.A. Morgan, S.A. King, J.J. Mikuta, S.C. Rubin, J. Boyd, Mutations of the BRCA2 gene in ovarian carcinomas, *Cancer Res.* 56 (1996) 2738–2741.
- [24] E.L. Schubert, M.K. Lee, H.C. Mefford, R.H. Argonza, J.E. Morrow, J. Hull, J.L. Dann, M.C. King, BRCA2 in American families with four or more cases of breast or ovarian cancer: recurrent and novel mutations, variable expression, penetrance, and the possibility of families whose cancer is not attributable to BRCA1 or BRCA2, *Am. J. Hum. Genet.* 60 (1997) 1031–1040.
- [25] R. Lidereau, F. Eisinger, M.H. Champeme, C. Nogues, I. Bieche, D. Birnbaum, C. Pallud, J. Jacquemier, H. Sobol, Major improvement in the efficacy of BRCA1 mutation screening using morphoclinical features of breast cancer, *Cancer Res.* 60 (2000) 1206–1210.