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Mutation analysis of *BRCA1* and *BRCA2* genes in Iranian high risk breast cancer families

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Abstract Purpose: Germline mutations in either *BRCA1* or *BRCA2* genes are responsible for the majority of hereditary breast and ovarian cancers. At present, over thousand distinct *BRCA1* and *BRCA2* mutations have been identified. Specific mutations are found to be common within particular populations, resulting from genetic founder effects. To investigate the contribution of germline mutations in these two genes to inherited breast cancer in Iran, we performed *BRCA1/BRCA2* mutation analyses in ten Iranian high risk breast cancer families. This is the first study analysing the complete coding sequences of both genes that concerns the Iranian population. **Methods:** *BRCA1/BRCA2* mutation detection included sequencing of the coding and the 3' and 5' untranslated regions. To detect large genomic rearrangements in the *BRCA1* gene semi-quantitative multiplex PCR was performed. **Results:** Two pathogenic mutations in the *BRCA2* gene were detected: a novel deletion c.4415_4418delAGAA and a previously described insertion c.6033_6034insGT. In addition, one intronic variation g.5075–53C>T and a deletion/insertion

g.*381_389del9ins29 in the 3' untranslated region of *BRCA1* were found in two of the investigated families. Both sequence alterations were absent in an age matched Iranian control group. The *BRCA2* homozygous variation p.N372H, previously associated with an increased risk for developing breast cancer, was not identified in this study. We did not detect large genomic rearrangements in *BRCA1* in patients tested negatively for disease causing mutations in both genes by standard sequencing. **Conclusions:** At present, the *BRCA2* mutations c.4415_4418delAGAA and c.6033_6034insGT have not been identified in any investigated population except the Iranian. Whether both mutations are specific for the Iranian population or a special subgroup remains to be investigated in larger studies. The absence of *BRCA1* mutations in the analysed families may suggest that penetrance or prevalence of *BRCA1* mutations may be lower in Iran.

Keywords Breast cancer · Ovarian cancer · *BRCA2* · *BRCA1* · Mutation · Unclassified variants · Iran

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Introduction

Worldwide, breast cancer is one of the most frequent malignancies in females. Within the Iranian population, neoplasms of the oesophagus and cancers of the breast and cervix are leading malignancies among women (Mosavi-Jarrahi et al. 2001). The majority of breast cancer cases appear sporadically. About 5–10% of breast cancer affected women have a familiar background for the disease. To date, family history remains one of the strongest predictors of breast cancer risk (Mehdipour et al. 2003). Therefore, attention has been focused on the role of the breast cancer susceptibility genes *BRCA1* (MIM# 113705) and *BRCA2* (MIM# 600185). Germline mutations in either of these genes are responsible for a lifetime risk of breast cancer (82%) and ovarian cancers (54% for *BRCA1* and 23% for *BRCA2*

mutation carriers; King et al. 2003). At present, over thousand distinct *BRCA1* and *BRCA2* mutations, polymorphisms and unclassified sequence variants have been identified [The Breast Cancer Information Core (BIC) database, January 2005]. In addition, recent studies have revealed large rearrangements within *BRCA1* resulting from homologous recombination events (Gad et al. 2002; The *BRCA1* Exon 13 Duplication Screening Group 2000; Unger et al. 2000). Specific *BRCA1/2* mutations are found to be common within particular populations, resulting from genetic founder effects (Neuhausen 2000). To date, only a small number of germline mutations in *BRCA1* and *BRCA2* have been identified in women with early-onset breast cancer with or without a family history of the disease within the Iranian population (Ghaderi et al. 2001; Yassaee et al. 2002). To investigate the contribution of germline mutations of these genes to inherited breast cancer in Iran, we have focused our interest on ten Iranian high risk breast cancer families with three or more individuals affected with the disease. The *BRCA1/2* mutation screening was performed by direct sequencing. Furthermore, large rearrangements within *BRCA1* were searched for using semi-quantitative multiplex PCR as described previously (Hofmann et al. 2002).

Materials and methods

Study subjects

Based on the following criteria, ten unrelated Iranian high risk breast or breast-ovarian cancer families were included in this study: at least three breast and/or ovarian cancer cases as first-degree relatives, multiple cases as first, second and distant relatives in the same lineage, bilateral breast cancer, occurrence of breast and/or ovarian cancer with onset under the age of 50 years (Table 1). Blood samples from these families were collected in the Department of Surgery, Day hospital and Cancer Institute, Tehran University of Medical Sciences, in Tehran. Information concerning the pedigrees were gathered on the basis of multiple interviews and DNA samples were extracted from peripheral blood lymphocytes using the standard phenol-chloroform procedure in the Department of Medical Genetics, Tehran University of Medical Sciences. DNA samples of 20 unrelated healthy female Iranian individuals served as an age matched control group and were a gift from the Iranian Human Mutation Gene Bank (Najmabadi et al. 2003).

Table 1 Characterization of the Iranian breast cancer families

Family	Number of breast cancer cases (age at diagnosis or <i>age at death</i>)	Number of bilateral breast cancer cases	Additional cancer cases among the families (age at diagnosis or <i>age at death</i>)	<i>BRCA1/2</i> sequence change ^a
I	8 (? , 30, 44, 48, 48, 48, 65, 70)	–	Liver (51), colon (40), prostate (57), leukaemia (60, 16)	–
II	8 (25, 30, 35, 36, 50, 53, 56, 60)	–	Gastric (27)	–
III	5 (? , 40, 40, 43, 60)	2	–	<i>BRCA1</i> : g.*381_389del9 ins29
IV	3 (30, 40, 45)	–	–	–
V	5 (37, 38, 42, 42, 44)	–	Leukaemia (3), ovary (70), brain (38), uterus (65), lung (80), prostate (55), brain (40), colon (?)	–
VI	8 Maternal (? , 31, 35, 50, 56, 63, 64, 70) and 3 paternal (26, 45, 60)	–	Paternal: leukaemia (80, 40), uterus (62, 39), larynx (60), brain (39), lung (57) maternal: ovary (28)	–
VII	12 Paternal (? , 25, 25, 27, 30, 37, 50, 50, 58, 58, 62, 65, 67) and 1 maternal (60)	1	Paternal: uterus (76, 54, 56, 45), stomach (52), leukaemia (58, ?, ?) maternal: bladder (60), lung (60), uterus (70)	<i>BRCA1</i> : g.5075–53C > T
VIII	4 Maternal (42, 58, 62, 64) and 2 paternal (50, 62)	–	Maternal: pancreas (54), thyroid gland (56), uterus (80)	–
IX	5(? , 27, 32, 33, 60)	–	Liver (62)	<i>BRCA2</i> : c.6033_6034 insGT
X	3 (37, 39, 50)	–	Unknown (79, ?)	<i>BRCA2</i> : c.4415_4418del AGAA

^aName of sequence alteration according to the nomenclature recommendations by den Dunnen and Antonarakis (<http://www.dmd.nl/mutnomen.html> 2004; den Dunnen and Antonarakis 2001)

Table 2 Sequence variations in *BRCA1* and *BRCA2* among the Iranian breast cancer families

<i>BRCA1/2</i> alteration ^a	Exon (intron)	Effect on aminoacid	Mutation type	Allele frequency (in investigated Iranian families)
BRCA1:				
g.-1075C > G	(1a)	–	P ^{1,3}	7/20 (35%)
g.-235A > G	(1b)	–	P ¹	6/20 (30%)
g.-134T > C	(1b)	–	P ¹	6/20 (30%)
g.442–34C > T	(7)	–	P ²	4/20 (20%)
g.548–58delT	(8)	–	P ¹	6/20 (30%)
c.2077G > A	11	p.Asp693Asn	P	1/20 (5%)
c.2082C > T	11	p.Ser694Ser	P ¹	6/20 (30%)
c.2311T > C	11	p.Leu771Leu	P ¹	6/20 (30%)
c.2612C > T	11	p.Pro871Leu	P ^{1, 3}	7/20 (35%)
c.3113A > G	11	p.Glu1038Gly	P ¹	6/20 (30%)
c.3119G > A	11	p.Ser1040Asn	P ²	2/20 (10%)
c.3548A > G	11	p.Lys1183Arg	P ¹	6/20 (30%)
c.4308T > C	13	p.Ser1436Ser	P ¹	6/20 (30%)
c.4837A > G	16	p.Ser1613Gly	P ¹	6/20 (30%)
g.4987–68A > G	(16)	–	P ¹	6/20 (30%)
g.4987–92A > G	(16)	–	P ¹	6/20 (30%)
g.5075–53C > T	(17)	–	UV	1/20 (5%)
g.5152 + 66G > A	(18)	–	P ¹	6/20 (30%)
g.*381_389del9ins29	24	–	UV	1/20 (5%)
g.*421G > T	24	–	P ¹	6/20 (30%)
g.*1286C > T	24	–	P ¹	6/20 (30%)
BRCA2:				
g.-1235G > A	-	–	P	1/20 (5%)
g.-26G > A	2	–	P	4/20 (20%)
g.681 + 56C > T	(8)	–	P	2/20 (10%)
c.865A > C	10	p.Asn289His	P	3/20 (15%)
c.1114A > C	10	p.Asn372His	P	3/20 (15%)
c.1365A > G	10	p.Ser455Ser	P	2/20 (10%)
c.2229T > C	11	p.His743His	P	2/20 (10%)
c.2971A > G	11	p.Asn991Asp	P	2/20 (10%)
c.3396A > G	11	p.Lys1132Lys	P	5/20 (25%)
c.3516G > A	11	p.Ser1172Ser	P	1/20 (5%)
c.3807T > C	11	p.Val1269Val	P	8/20 (40%)
c.4415_4418delAGAA	11	p.Lys1472fsX5	FS	1/20 (5%)
c.5529A > C	11	p.Ala1843Ala	P	1/20 (5%)
c.6033_6034insGT	11	p.Ser2012fsX28	FS	1/20 (5%)
c.7242A > G	14	p.Ser2414Ser	P	4/20 (20%)
g.7435 + 53C > T	(14)	–	P	2/20 (10%)
g.7806–14T > C	(16)	–	P	8/20 (40%)
g.8755–66T > C	(21)	–	P	8/20 (40%)

UV unclassified variant,
FS Frame-shift

^aName of sequence alteration according to the nomenclature recommendations by den Dunnen and Antonarakis (<http://www.dmd.nl/mutnomen.html> 2004; den Dunnen and Antonarakis 2001)

¹Correlating *BRCA1* polymorphisms (P) defining the most frequent haplotype after the wild type (wt)
^{2, 3}Additional more rare *BRCA1* haplotypes

Direct sequencing

PCR products of all exons and the flanking intronic regions and the 5' and 3' untranslated regions (UTR) of *BRCA1* and the 5' UTR of *BRCA2* were analysed using the Applied Biosystem *BigDye* Terminator v.3.0 Ready Reaction Cycle Seqkit and the Applied Biosystem ABI 377 sequencer in the Department of Tumor Genetics, Max Delbrück Center, Berlin, according to the manufacturer's instructions. The sequences of the primers used may be obtained from the corresponding author upon request.

Semi-quantitative fluorescent multiplex PCR

To detect large rearrangements, the protein-coding exons of the *BRCA1* gene and the 5' UTR containing a promoter fragment of *BRCA1* were amplified by semi-

quantitative PCR from genomic DNA of the probands, as described by Hofmann et al. (2003).

Results

Frameshift mutations were found in the *BRCA2* gene of two Iranian breast cancer families leading to a premature stop codon (Tables 1, 2). An insertion of GT at nucleotide position 6034 in exon 11 (*BRCA2* c.6033_6034insGT, corresponding to the traditional nomenclature 6262insGT) results in a translation stop at codon 2040 in family IX (Fig. 1).

A deletion of AGAA at nucleotide-position 4415 in exon 11 (*BRCA2* c.4415_4418delAGAA, corresponding to the traditional nomenclature 4643delAGAA) leads to a premature stop codon at position 1477 in family X (Fig. 2).

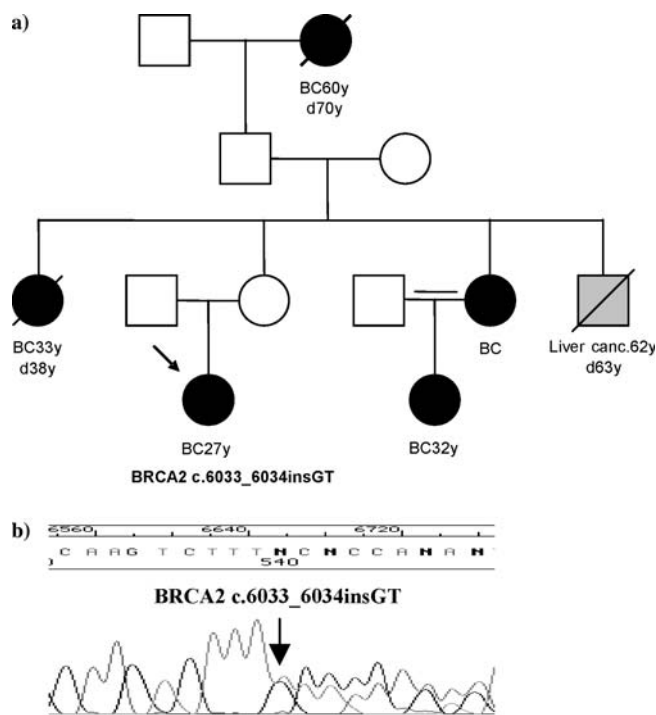


Fig. 1 a Pedigree of family IX. b Electropherogram of the mutation *BRCA2* c.6033_6034insGT

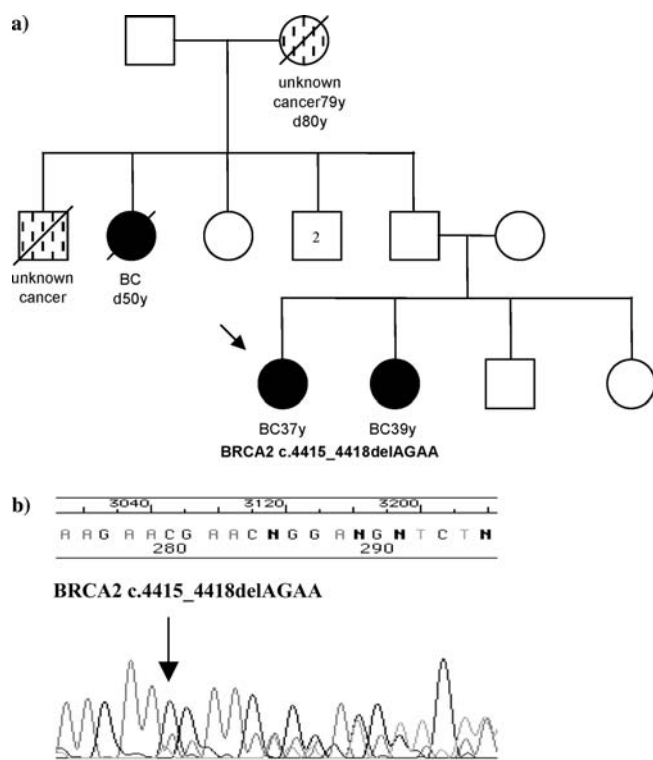


Fig. 2 a Pedigree of family X. b Electropherogram of the detected mutation *BRCA2* c.4415_4418delAGAA

Sequence analysis of *BRCA1* showed a nucleotide change in intron 17 (g.5075–53C>T, corresponding to the traditional nomenclature IVS17–53C>T) in family

VII (Fig. 3). Segregation analysis could not be performed because DNA of affected and unaffected relatives was not available. However, g.5075–53C>T did not appear in the analysed alleles of the 20 healthy female control individuals from the Iranian population. Furthermore, the alteration g.*381_389del9ins29 in the 3'UTR of *BRCA1* was identified in family III (Fig. 4). This sequence change did not show complete segregation with the disease in this family. This alteration was absent in the healthy mother, as well as in two breast cancer affected sisters of the proband. Another healthy sister was also a carrier of this variation.

In this study, many alterations, previously described as polymorphisms (BIC 2005), have been identified in *BRCA1* and *BRCA2* (Table 2). In addition, certain polymorphisms in *BRCA1* were found to correlate with each other (Table 2). No large rearrangements in *BRCA1* were detected using semi-quantitative fluorescent multiplex PCR.

Discussion

Since the identification of the breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2*, the Caucasian populations in North America, Europe, Israel and Australia have been investigated for germline mutations in both genes using different PCR-based mutation detection methods. Few studies concerning *BRCA1* and *BRCA2* alterations in the Iranian population have been published: In a study performed by Yassaee and colleagues, 83 early-onset breast cancer patients from Tehran were investigated (Yassaee et al. 2002). Based on this study, the prevalence of *BRCA1/2* mutations among early-onset breast cancer patients (<45 years) with or without a family history for the disease is thought to be approximately 6%. Ghaderi and colleagues performed *BRCA1* mutation screening in a study comprising 80 patients with breast cancer with a median age of 42 years at onset of the disease from Shiraz, Iran (Ghaderi et al. 2001). Only two of the patients had a family history of breast and/or ovarian cancer. Analysis of exons 2, 20 and 11 of *BRCA1* and exons 10 and 11 of *BRCA2* has been restricted to a single Iranian family with hereditary breast and ovarian cancer syndrome (Moslehi et al. 2003). In this family a novel nonsense mutation in *BRCA1* was identified (G2031T, using the traditional nomenclature).

In our study, both *BRCA1* and *BRCA2* genes were screened in ten high risk breast cancer families of non-jewish origin. Two of the investigated families were found to inherit disease-causing frameshift mutations (Table 1). Both mutations, c.4415_4418delAGAA and c.6033_6034insGT, were located in exon 11 of *BRCA2*. Alterations in this region confer a higher risk for developing ovarian cancer (OCCR) (Thompson et al. 2001). In contrast, the pedigrees of the families involved did not show cases of ovarian cancer. In a

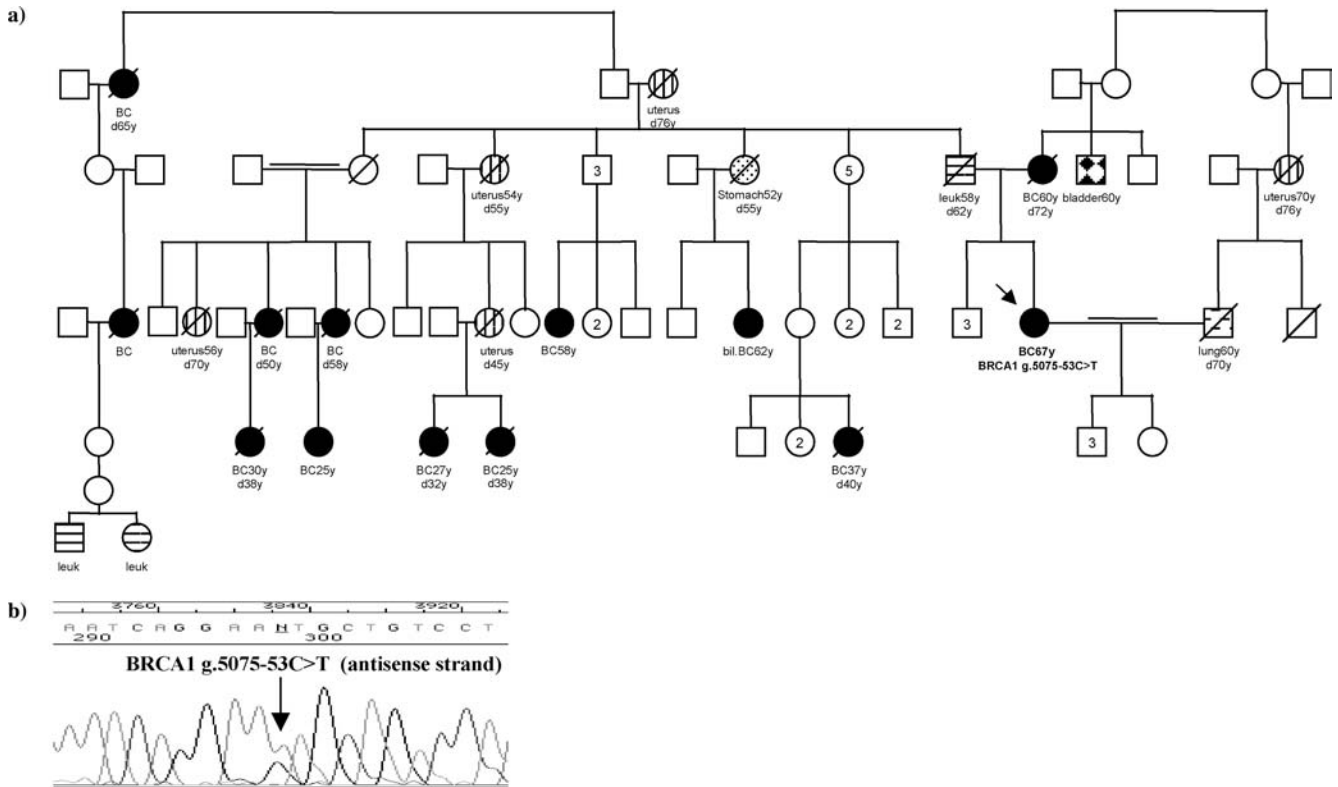


Fig. 3 a Pedigree of family VII. **b** Electropherogram of the *BRCA1* variation g.5075-53C>T

study conducted on Spanish breast/ovarian cancer patients, no association with mutations situated in the ovarian cancer cluster region (OCCR) of *BRCA2*, but rather an association between ovarian cancer and mutations localized in the 5' end of *BRCA1* was found (Diez et al. 2003). Whether additional environmental factors or other genetic modifiers may influence the development of certain phenotypes remains unclear.

To our knowledge, the *BRCA2* mutation c.4415_4418delAGAA is novel (BIC 2005, January). *BRCA2* c.6033_6034insGT has been described previously in a single Iranian woman with early-onset breast cancer (Yassaee et al. 2002). It remains to be investigated whether these sequence changes represent isolated cases or founder mutations in the Iranian population.

The clinical consequences of the intronic *BRCA1* sequence variation g.5075-53C>T and the 3' UTR alteration g.*381_389del9ins29 are unclear. The *BRCA1* variation g.5075-53C>T was found neither in the control subjects nor in the remaining investigated families. Concerning the alteration g.*381_389del9ins29 in the 3' UTR of *BRCA1*, we conclude that this variation was not causal for breast cancer occurrence in family III, because of missing segregation with the disease. Investigation of the control group did not show this variant.

In this study, many polymorphisms spreading over the whole gene regions of *BRCA1* and *BRCA2* have been found. In *BRCA1* there is a correlation

between several of the polymorphisms. The strong correlation could allow certain haplotypes to be defined and this finding can help in understanding the evolutionary development of variants (Shattuck-Eidens et al. 1997; Liu and Barker 1999). Whether certain haplotypes are associated with an increased breast cancer risk remains unclear. To date, some studies have found an increased risk of developing breast cancer in individuals who show homozygous genotypes for special variants, for instance the *BRCA2* p.N372HH genotype (Healey et al. 2000; Goode et al. 2002). Three of our investigated patients show a heterozygous genotype (p.N372NH).

Using the sequencing method, we did not find any frameshift and nonsense mutations leading to a premature translation stop in *BRCA1*. In addition, sequencing methods are not suitable for detecting large genomic rearrangements (Unger et al. 2000). Therefore, the semi-quantitative multiplex PCR method to screen these patients for large deletions or duplications within the *BRCA1* gene was used in this study. None of the investigated patients showed a *BRCA1* rearrangement.

In summary, two pathogenic mutations in the *BRCA2* gene have been detected in this study: c.6033_6034insGT previously described in a single Iranian woman affected with breast cancer and a novel c.4415_4418delAGAA mutation. Whether these alterations are specific for the Iranian population or for special subgroups remains to be investigated in larger studies. Interestingly, we did not detect a disease causing mutation in *BRCA1*. Penetrance or prevalence of *BRCA1* mutations may be lower in Iran (Liede and Narod 2002). Whether the remaining eight

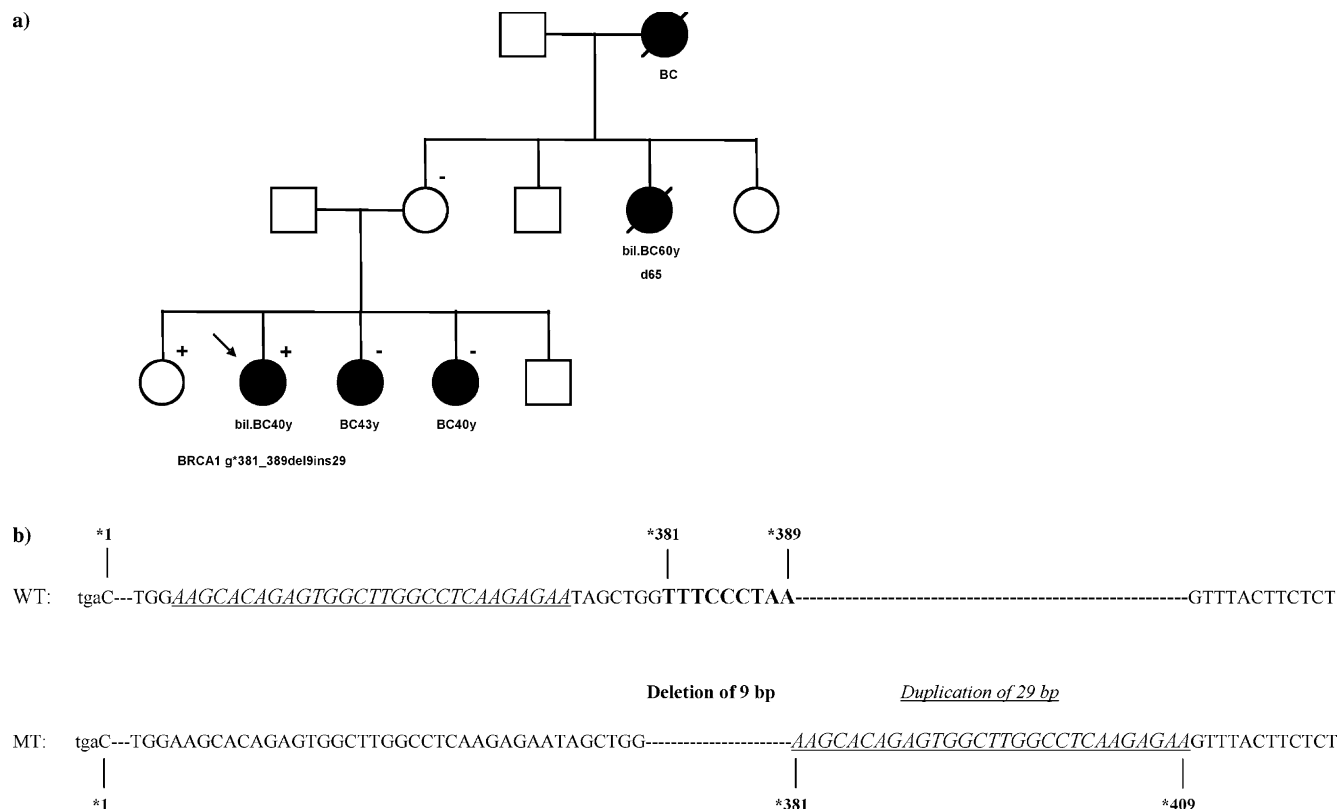


Fig. 4 a Pedigree of family III and segregation analysis (+ variation found, -variation not found). **b** Schematic description of *BRCA1* g.*381_389del9ins29

investigated high risk breast cancer families carry disease causing mutations in unknown breast cancer susceptibility genes or in rare modifier genes is still unclear. Investigation of the influence of additional environmental factors in connection with genetic variants such as polymorphisms or special haplotypes are therefore necessary.

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