Detection of the most frequent mutations in *BRCA1* gene on polyacrylamide gels containing Spreadex Polymer NAB*

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Heterozygous carriers of germ-line mutations in the *BRCA1* gene are at high risk for the development of breast and ovarian cancer. Inactivating mutations have been identified in the whole coding region of the gene, however, repeatedly occuring mutations can explain a large proportion of gene alterations detected in certain ethnic groups. In Czech patients, the 5382insC and 185delAG mutations may account for approximately 50% of all *BRCA1* abnormalities (unpublished data). In the present study, a rapid and simple method to identify these short insertions and deletions that alter the size of the polymerase chain reaction (PCR) product is described. The analysis involves the separation of fragments amplified with primers that flank altered sites in the *BRCA1* gene on non-denaturing polyacrylamide gels containing Spreadex Polymer NAB. The increased resolving power of Spreadex gels enables full separation of two DNA fragments that differ by 1-bp on gels that are 5 cm long. The method gave interpretable results with the genetic material obtained from all tested mutation carriers and control persons. Defective alleles were also detected in DNA samples from carriers of the 1135delA mutation in *BRCA1* and the 4206ins4 mutation in *BRCA2.* These results suggest that electrophoresis on Spreadex gels can be used universally for detection of the most frequent frameshift mutations in *BRCA* genes. The method is suitable even for rapid screening of frequent germ-line mutations in *BRCA* genes in breast and ovarian cancer patients not selected for family history of cancer or age at diagnosis.

Key words: Hereditary breast and ovarian cancer, BRCA1, founder mutations, Spreadex Polymer NAB.

Breast cancer is the most frequently diagnosed malignancy affecting women in Europe. A positive family history appears to be a major risk factor for the development of this disease. Hereditary breast cancer is characterized by an early age of onset and by its association with ovarian cancer incidence. Mutations in two highly penetrant susceptibility genes *BRCA1* and *BRCA2* (breast cancer gene 1 and 2) are responsible for the majority of hereditary breast and ovarian cancers. Approximately 45% of families with breast cancer only and 80%–90% of families with multiple cases of breast and ovarian cancer have linkage to the *BRCA1* gene [4]. The gene was mapped to chromosome band 17q21 in 1990 [7] and cloned in 1994 [13]. *BRCA1* contains 24 exons distributed over more than 80 kb of genomic DNA [17] and encodes a protein of 1863 amino acids.

Several hundred distinct mutations spread over the whole coding region of the *BRCA1* gene have been identified. However, population-specific, founder mutations are known in particular ethnic groups. In Ashkenazi Jews, two ancestral mutations, the 185delAG and 5382insC, occur at population frequencies of ~1% and ~0.11%, respectively [18]. These most common abnormalities of *BRCA1* also appear repeatedly in non-Jewish population. The 5382insC mutation occurs most frequently in central and Eastern Europe [5, 6, 19, 20]. This defect accounts for approximately one-half of the identified mutations in Poland [6] and may also be the predominant mutation of *BRCA1* gene occurring in the Czech Republic [15].

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Knowledge of the nature of population specific mutations enables to develop simple diagnostic tests for their detection. Mutation specific tests can be useful for rapid screening of frequent germ-line mutations in patients from breast and/or ovarian cancer families and for predictive testing of family members.

Elchrom Scientific (Switzerland) has recently developed a variety of precast gels, which are used for applications that require highest resolution in a narrow size range of DNA fragments. Non-denaturing submerged electrophoresis on Spreadex EL300 gels was used to detect loss of heterozygosity (LOH) of tetranucleotide and dinucleotide microsatellite markers [8, 10, 12]. Germ-line mutations in the APC gene that result in deletions of 4 to 5 bp [9] were also detected on these gels. Spreadex Polymer NAB is a product that is added to standard non-denaturing polyacrylamide gels to increase the resolving power of electrophoretic separation. The purpose of our study was to evaluate the usefulness of these modified polyacrylamide gels in the rapid screening of the two most frequent *BRCA1* gene alterations, 2-bp deletion in exon 2 (185delAG) and 1-bp insertion in exon 20 (5382insC).

Material and methods

Genetic material. Genetic material was obtained from 13 symptomatic or presymptomatic mutation carriers detected in risk families by the direct sequencing of appropriate PCR products [15] and from the same number of control persons. All participants in the study have given their informed consent for analysis. The investigation has been approved by the Ethical Committee at the Charles University. Wizard genomic DNA purification kit (Promega) was used for isolation of DNA from EDTA-treated peripheral blood.

PCR conditions. Sequences of primers that flank altered sites in *BRCA1* gene and predicted sizes of PCR fragments are listed in Table 1. The PCR reactions of 12.5 μl were performed in a reaction buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$), containing 0.4 μM of each primer, 200 μM of each dNTP, 0.4 unit of Taq DNA polymerase (Finnzymes) and 100 ng of genomic DNA. Following initial denaturation at 94 °C for 2 min, 32 cycles (at 94 °C and 58 °C for 30 s and at 72 °C for 1min) and final extension at 72 °C for 5min were performed in a thermal cycler (MJ-Research).

Analysis of PCR products. The 0.5–1.0 μl portions of PCR samples were mixed with 2 μl of loading buffer containing 50% glycerol, 0.05% bromphenol blue and 0.05% xylene cyanol in standard TAE buffer [16] and resolved on vertical 5 cm long 11% non-denaturing acrylamide:bisacrylamide minigels (29:1) containing 10% of Spreadex Polymer NAB. The upper 1 cm long low- percentage (4%) polyacrylamide gel without Spreadex polymer was used to avoid irregularities of DNA bands caused by uneven polymerization at the interface with the comb. Electrophoresis was performed in standard TAE buffer and gels were run for 3:30 h at a constant voltage of 200 V at room temperature in a MiniProtean apparatus (Bio-Rad Laboratories). After electrophoresis, gels were stained in a solution of SYBR Gold (Molecular Probes Inc.) in TAE buffer for 20 min and photographed by a Polaroid camera.

The aliquots of PCR samples were further electrophorezed on 11% polyacrylamide minigels without Spreadex Polymer NAB. The gels were run for 60 min at room temperature. Other conditions of analysis were as described above.

Table 1. Primers used for PCR

Primer	Gene	Exon	Mutation[a]	Sequence 5'-3'	Position in gene[b]	Size of PCR product [bp]
P1	BRCA1	2	185delAG	AATGCTATGCAGAAAATCTTAG	4684–4705	133 bp
P2				CATGTCTTTTCTTCCCTAGTA	4796–4816[c]	
P3	BRCA1	20	5382insC	GGTTGTGTTTGGTTTCTTTCAG	71576–71597	131 bp
P4				GTCAACTTGAGGGAGGGAG	71688–71706[c]	
P5	BRCA1	11	1135delA	GCGGACTCCCAGGACAGAAAAAA	34194–34216	132 bp
P6				AGTGTTATCCAAGGAACATCTTC	34303–34325[c]	
P7	BRCA2	11	4206ins4	ATTGAAATGACTACTGGCAC	77572–77591	131 bp
P8				GAATCACTGCCATCAAATTC	77683–77702[c]	

[a]Nomenclature is according to BEAUDET and TSUI [3]. [b]Position according to sequences *BRCA1* and *BRCA2* by Genbank, accession numbers L78833 and Z74739. [c]The reverse complement of the given *BRCA1/2* sequence.

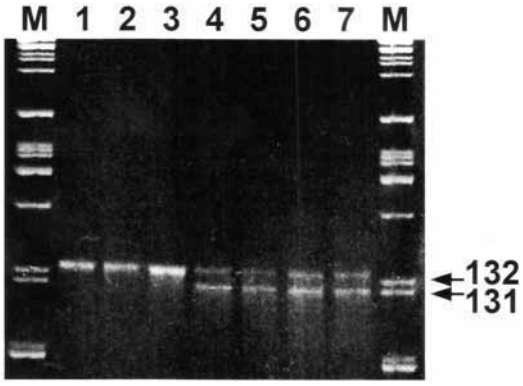

Figure 1. Detection of the *BRCA1* 185delAG mutation. PCR fragments containing exon 2 of *BRCA1* gene were analyzed on 11% polyacrylamide gels containing Spreadex Polymer NAB. Lanes M, M3 markers with separated fragments of 123, 124, 131, 132, 141, 147, 151, 152, 153, 160, 174, 180 and 184 bp; lanes 1–3, control DNA samples; lanes 4–7, DNA samples from mutation carriers.

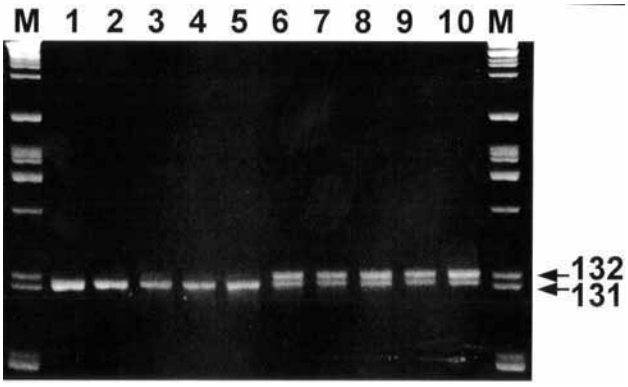

Figure 2. Detection of the *BRCA1* 5382insC mutation. PCR fragments containing exon 20 of *BRCA1* gene were analyzed on 11% polyacrylamide gels containing Spreadex Polymer NAB. Lanes M, M3 markers; lanes 1–5, control DNA samples; lanes 6–10, DNA samples from mutation carriers.

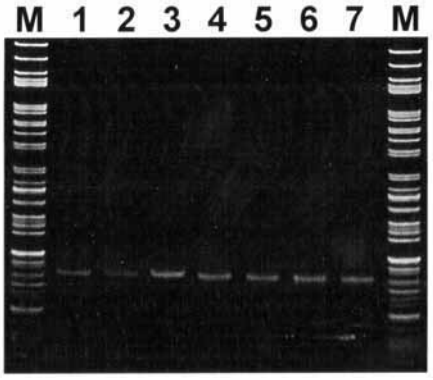

Figure 3. Analysis of PCR fragments with 185delAG mutation in polyacrylamide gels. PCR products containing exon 2 of *BRCA1* gene were analyzed on 11% polyacrylamide gels. Lanes M, M3 markers; lanes 1–3, control DNA samples; lanes 4–7, DNA samples from mutation carriers.

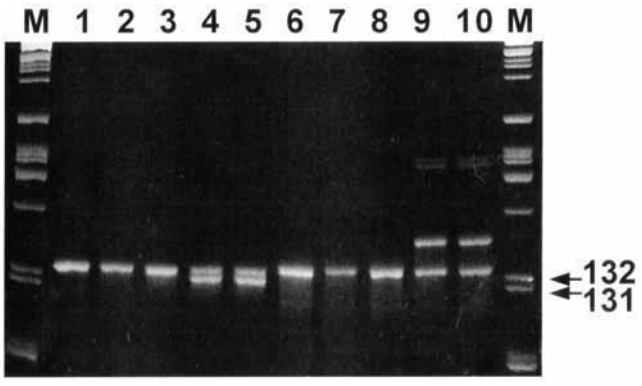

Figure 4. Detection of the *BRCA1* 1135delA and the *BRCA2* 4206ins4 mutations. PCR products were resolved on 11% polyacrylamide gels containing Spreadex Polymer NAB. Lanes M, M3 markers; lanes 1–3 and 6–8, control DNA samples; lanes 4 and 5, DNA samples from the *BRCA1* 1135delA mutation carriers.

Results

To determine the applicability of Spreadex gels in screening mutations in *BRCA1*, a set of DNA samples isolated from blood of mutation carriers and control individuals was analyzed. To resolve a deletion of 2-bp (185delAG) and an insertion of 1-bp (5382insC) in the gene, we have employed non-denaturing TAE polyacrylamide minigels containing Spreadex Polymer NAB that enable accurate sizing of PCR products. After 3.5 h of electrophoresis on 11% gels, the M3 molecular weight markers (Elchrom Scientific) gave optimal separation of fragments within the size range of 123-184 bp (Fig. 1, lanes M). Under these conditions, we tested the resolution of additional bands in PCR fragments carrying mutations.

Analysis of PCR fragments containing exon 2 from 185delAG mutation carriers showed a mutation in one of the *BRCA1* alleles as, in addition to the normal PCR product of 133 bp, a deleted product of 131 bp was detected (Fig. 1, lanes 4–7). Only normal, wild type products of 133 bp were observed in control DNA samples (Fig. 1, lanes 1–3). Figure 2 illustrates results obtained with carriers of 5382insC mutation. The PCR products from these samples including exon 20 contained longer fragments of 132 bp, besides the normal 131 bp products (Fig. 2, lanes 6–10). PCR fragments of 131 bp and 132 bp were completely resolved and matched the bands from the M3 marker. In control samples, only wild type products of 131 bp were detected (Fig. 2, lanes 1–5).

Figure 3 shows the analysis of PCR fragments with 185delAG mutation on 11% non-denaturing polyacrylamide gels, as a comparison. The resolution of 133 bp and 131 bp fragments in samples containing germ-line mutations was poor on these gels (Fig. 3, lanes 4–7). The M3 marker also did not show good separation in this gel (Fig 3, lanes M)

and the sizes of bands from PCR fragments did not match the bands from the marker.

Discussion

The analysis of population specific frequently occurring mutations in *BRCA1* gene gives an opportunity for simple and rapid detection of symptomatic and presymptomatic mutation carriers. To identify the most common short deletions or insertions in *BRCA1* gene, a variety of rapid tests can be used. The mutations 5382insC and 185delAG were detected by allele-specific PCR or by PCR assays using primers containing an introduced mismatch (mismatch PCR assay) [1, 2, 11, 14]. In mismatch PCR assays, the fragments amplified on the normal or mutated allele acquired restriction site that the second allele did not have. Consequently, restriction analysis was required for mutation detection. Both mutations were also identified by resolving of the multiplex PCR products on long denaturing polyacrylamide gels [1].

In this study, the mutations frequently occurring in the Czech Republic that alter the size of PCR fragments were detected on polyacrylamide minigels containing Spreadex Polymer NAB. Our experiments demonstrated that the addition of Spreadex polymer increased the resolving power of polyacrylamide gels and a resolution to 1-bp was achieved on 5 cm long minigels. Analysis performed in a standard electrophoretic equipment gave interpretable results in all tested samples.

To confirm the general applicability of Spreadex gels for the detection of mutation alleles that have either a short deletion or insertion, we analyzed other alterations in *BRCA1* and *BRCA2* genes found in our population. Besides normal 132 bp products, smaller fragments of 131 bp were found in carriers of the 1135delA mutation in *BRCA1* (Fig. 4, lanes 4 and 5) and longer fragments of 136 bp were detected in DNA samples containing the 4206ins4 mutation in *BRCA2* (Fig. 4, lanes 9 and 10). A heteroduplex bands above the 147 bp fragment of the M3 marker was observed in samples containing this mutation.

Our experiments show that electrophoresis on Spreadex gels can be used as an alternative method for the detection of common frameshift mutations in *BRCA* genes. Low costs of testing make possible to analyze patients who were not selected for age at diagnosis or family history of cancer and to investigate the impact of the most common founder mutations on the development of breast and ovarian cancer in the general population.

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