DOI: 10.2478/v10163-012-0001-0

ORIGINAL ARTICLE

Detection of Large Deletions and Duplications in Moderate Risk Breast Cancer Susceptibility Genes in Breast Cancer Patients Negative for the *BRCA1* and *BRCA2* Mutations

Dagnija Kalniete*, **, Arvīds Irmejs*, **, Ilze Štrumfa*, **, Jekaterina Žestkova*, **, Karīna Aksenoka *, **, Jānis Gardovskis*, **, Edvīns Miklaševičs*, **
*Hereditary Cancer Institute, Riga Stradins University, Riga, Latvia
**Pauls Stradins Clinical University Hospital, Riga, Latvia

Summary
Introduction. Breast cancer is the most frequently diagnosed malignancy among woman in Latvia. Approximately 40% of all hereditary breast cancer cases can be explained due to the point mutations in the *BRCA1* and *BRCA2* genes. It is estimated that more than 10% of breast cancer predisposing mutations are large genomic rearrangements such as large deletions and duplications.
Aim of the study. The aim of the study was to determine a large deletions, or duplications in the moderate risk breast cancer susceptibility genes in breast cancer patients negative for the point mutations in the *BRCA1* and *BRCA2* genes.
Materials and methods. Study group consisted of 23 breast cancer patients negative for the 4153delA, 5382insC and 873delG, 886delTG mutations in the *BRCA1* and *BRCA2* genes, respectively. Multiplex ligation-dependent probe amplification was performed for the detection of large genomic deletions and duplications.
Results. Large genomic deletions were detected in the two cases. In one case was determined a large genomic deletion in the *CHEK2* gene spanning exons 9 and 10. In the second case exons 2 and 5 in the *CHEK2* gene and exons 29 and 46 in the *ATM* gene were deleted.
Conclusions. Large genomic deletions in the moderate risk breast cancer susceptibility genes can be found in Latvia. For the first time, a large genomic *CHEK2* gene deletion spanning exons 9 and 10 has been detected in Latvia.
Key Words: breast cancer, moderate risk breast cancer susceptibility genes, multiplex ligation-dependent probe amplification.

INTRODUCTION

Breast cancer is the most frequently diagnosed malignancy and leading cause of cancer death among women worldwide. It was estimated that approximately 1.38 million new cases were diagnosed and 558.400 females died from breast cancer in 2008 (Ahmed *et al.*, 2011). Breast cancer is the most common form of malignancy among female with approximately 1000 new cases diagnosed yearly in Latvia (Central Statistical Office of Latvia). The two major breast cancer susceptibility genes, *BRCA1* and *BRCA2,* are responsible for about 4% of all breast cancer cases in Latvia (Gardovskis *et al.*, 2009; Žestkova *et al.*, 2010) In the 3% of all breast cancer cases are found 4153delA, 5382insC mutations in the *BRCA1* gene and in the 1% of all breast cancer cases are found 886delTG and 873delG mutations in the *BRCA2* gene (Gardovskis *et al.*, 2009; Žestkova *et al.*, 2010). The *BRCA1* and *BRCA2* genes are responsible for about 30% of hereditary breast cancer cases worldwide and for about more than 40% in Latvia (Claus *et al.*, 1996, Gardovskis *et al.*, 2009). There is a strong association between *BRCA1* mutation status and morphology of the breast cancer. Approximately in the 80% of patients with the germ-line mutations in the *BRCA1* gene are found triple negative (TN) breast cancers (Spearman *et al.*, 2008). Triple negative breast cancers are attributed to the ER-negative, progesterone-receptor (PR)-negative and HER2-negative tumors (Perou *et al.*, 2000). Numerous studies have shown that large genomic rearrangements such as large genomic deletions or duplications are associated with the development of variety different hereditary conditions. For the most of the hereditary conditions partial gene deletions or duplications accounts for less than 10 % of all disease causing mutations and in some of the conditions it is 30% or more (Schouten *et al.*, 2002; Taylor *et al.*, 2003; Aretz *et al.*, 2007; Kanno *et al.*, 2007; Redeker *et al.*, 2008). Deletions or duplications of one or more exons of *BRCA1* and *BRCA2* genes predispose carriers to breast cancer. The prevalence of large genomic deletions and duplications in the *BRCA1* genes varies within different population but the overall rate is more than 10% and in the some of the populations even reaching almost 30% (Hartmann *et al.*, 2004; Pylkas *et al.*, 2008; Smith *et al.*, 2011; Hogervorst *et al.*, 2003). The large genomic deletions and duplications in the moderate risk breast cancer genes are studied less therefore in this study we focused our attention to moderate risk breast cancer susceptibility genes. *ATM* gene is a large gene containing 66 exons spanning approximately 150 kb of genomic DNA and over 400 mutations have been identified (Uziel *et al.*, 1996; Campbell et al., 2003). Due to its relatively

large size mutation detection is labor-intensive and times consuming thus a large proportion of mutations remains undetected. *CHEK2* gene spans approximately 50 kilobases of genomic DNA and consists of 14 exons (Bartek *et al*., 2001). 1100delC, I157T, IVS2+IG>A and 5.4 kb deletion of the *CHEK2* gene has been associated with an icreased risk of breast cancer (Wu *et al*., 2001; Meijer-Heijboer *et al*., 2002; Kilpivaara *et al*., 2004, Cybulski *et al*., 2006).

AIM OF THE STUDY

The aim of this study was to determine a large genomic deletions or duplications in the moderate risk breast cancer susceptibility genes (*CHEK2* and *ATM*) in breast cancer patients negative for the 4153delA, 5382insC and 873delG, 886delTG mutations in the *BRCA1* and *BRCA2* genes, respectively.

MATERIALS AND METHODS

Study group consisted of 23 breast cancer patients negative for the 4153delA, 5382insC and 873delG, 886delTG mutations in the *BRCA1* and *BRCA2* genes, respectively. All breast cancer patients were hospitalized from 2006 to 2008 at the Pauls Stradins Clinical University Hospital and Oncology Center of Latvia. The ages of patients at the diagnosis of the disease were from 41 to 71 years. Four healthy control donors were used as reference samples. All individuals signed informed consent forms for participation in the research. The study was approved by the Ethics Committee of the Riga Stradins University.

Genomic DNA was extracted from 2 ml of peripheral blood samples by FlexiGene DNA Kit (250) (Qiagen, Dusseldorf, Germany) according to the manufacturer's instructions.

Multiplex Ligation Probe Dependent Amplification (MLPA) was done by MLPA kit P190 (MRC-Holland, Amsterdam, Netherlands) according to the instructions of the manufacturer. MLPA was performed in the TProfessional thermal cycler (Biometra, Gottingen, Germany). Fragments were separated on Applied Biosystems 3130 capillary genetic analyzer and analyzed by GeneMapper Software v4.0 (Applied Biosystems, Foster City, CA).

MLPA data were normalized using block normalization. The relative probe signals were obtained by dividing the peak area of each amplification product by the total peak area of only the reference probes in the probemix. Probe ratios were calculated by dividing the relative probe signals in the cancer samples by the average of the relative probe signals in the reference (healthy controls) samples. Probe ratios between 0.7-1.3 were defined as normal.

Multiplex PCR was used to confirm a large genomic deletion in the *CHEK2* gene spanning exons 9 and 10. Multiplex PCR was performed in the TProfessional thermal cycler (Biometra, Gottingen, Germany) using specific primers (Cybulski *et al*., 2007). The cycling conditions were 3 minutes at 95°C, followed by 10 cycles of 30 seconds at 95°C, 30 seconds at 65°C and 30 seconds at 72°C and 40 cycles of 30 seconds at 95°C, 30 seconds at 55°C and 30 seconds at 72°C and fallowed by 10 minutes at 72°C.

Sequencing by Applied Biosystems 3130 capillary genetic analyzer was performed to detect the range of the deleted fragment in the *CHEK2* gene spanning exons 9 and 10.

Data were analyzed by Sequencing Analysis v5.3.1. and SeqScape v2.6.

RESULTS

The immunohistochemistry data of 23 breast cancer cases were analyzed. Thirteen breast cancers were diagnosed with triple negative (TN) cancer morphology and ten breast cancers were diagnosed with HER2 3+ cancer morphology. From 13 TN breast cancers cases a hereditary breast cancer (HBC) was diagnosed in 2 cases, hereditary breast ovarian cancer (HBOC) was diagnosed in 1 case and as being suspicious for hereditary breast cancer (HBC susp.) were diagnosed 3 patients. From 10 HER2 3+ with HBOC was diagnosed 1 patient.

In two cases large genomic deletions were detected. In one case was found a large genomic deletion in the *CHEK2* gene spanning exons 9 and 10. In the second case exons 2 and 5 of the *CHEK2* gene and exons 29 and 46 of the *ATM* gene were deleted.

Patient E111 showed reduced probe ratios of exons 9 and 10 of the *CHEK2* gene. The values of the probe ratios of exons nine and ten were 0.43 and 0.36, respectively (Figure 1A). Probe ratios between 0.7 and 1.3 were defined as normal and probe ratio under 0.7 were defined as deletion. The capillary electrophoresis peak profiles of exons 9 and 10 showed more than two times lower peak profiles in the cancer sample (Figure 1B) than adjacent peak profiles in the reference sample (Figure 1C) thus implying to the deletion of the two sequential exons in the *CHEK2* gene. E111 was diagnoses with a triple negative breast cancer at age 51. E111 aunt was diagnosed with breast cancer at age 60 and she died at the same age. Patient has five siblings with unknown age and health status (Figure 2). DNA samples for the rest of the siblings were not available. All siblings refused to participate in the study. A large genomic deletion of exons 9 and 10 of the *CHEK2* gene was confirmed by multiplex PCR. Amplification product gave an additional 450 base pair fragment thus implying to large genomic deletion as was previously described (Cybulski *et al*., 2006). Sequencing results revealed that deletion break-point sites are at 39867 bp and 45262 bp positions at the *CHEK2* gene (NT_011520) and our estimated length of the deleted fragment is 5395 base pairs.

Patient E224 showed reduced probe ratios of exons 2 and 5 of the *CHEK2* gene and reduced probe ratios of exons 29 and 46 of the *ATM* gene. The probe ratio values of exons 2 and 5 of the *CHEK2* gene were 0.64 and 0.63, respectively. The values of the probe ratios of exons 29 and 46 of the *ATM* gene were 0.62 and 0.59, respectively (Figure 4A). Peak profiles of exons 2 and 5 were reduced when were compared with adjacent peak

profiles of the reference sample. The same trend was seen in the case of the *ATM* gene (Figure 4B and 4C). E224 was diagnosed with the HER2 3+ breast cancer at age 69 and she has three siblings with unknown age and health status. The DNA samples from siblings were not available.

DISCUSSION

This is the first report of a large genomic deletion in the *CHEK2* gene spanning exons 9 and 10 in Latvia. *CHEK2* gene is a tumor suppressor gene which initiates DNA repair after double-strand breaks and has been found to be a breast cancer predisposing gene in several populations (Matsuoka *et al*., 1987; Chehab *et al*., 2000). A large genomic deletion in the *CHEK2* gene has been previously reported and has been associated with the elevated risk of breast cancer (Walsh *et al*., 2006; Cybulski *et al*., 2007; Bogdanova *et al*., 2007). A deleterious *CHEK2* gene mutation is prevalent in Central and Eastern European populations. Initially deletion was found in 8 breast cancer patients from the Czech Republic and Slovakia and was considered to be a founder mutation for these particular populations (Walsh *et al*., 2006). Later, a large genomic deletion was found in the 1% of unselected breast cancer cases in Poland. Mutation was present in the 0.4% of the population and was associated with approximately twice the risk of breast cancer (Cybulski *et al*., 2007). Deletion has been also associated with the prostate cancer and is considered to be one of the most widespread protein-truncating mutations in the *CHEK2* gene in Poland (Cybulski *et al*., 2006). By our estimations, the length of the deleted fragment spanning exons 9 and 10 is 5395 base pairs and it is the same length as was found in Poland (Cybulski *et al*., 2006). As with Polish we have common 4153delA, 5382insC founder mutations in the *BRCA1* gene it is very likely that 5395 base pair deletion in the *CHEK2* gene is a founder mutation in Latvia (Gorski *et al*., 2000). It would be very important to verify the prevalence of large deletion in Latvian population and to estimate relative risk for breast and prostate cancers patients carrying particular large genomic deletion.

In second case exons 2 and 5 in the *CHEK2* gene and exons 29 and 46 in the *ATM* gene were found to be deleted. A protein kinase encoded by *ATM* gene is involved in mitogenic signal transduction, meiotic recombination, detection of DNA damage, and cell cycle control (Savitsky *et al*., 1995). Ataxia telangiectasia is an inherited condition characterized by progressive cerebella ataxia, oculomotor apraxia, frequent infections, immunodeficiency, sensitivity to ionizing radiation, and increased risk of malignancy (Jasper *et al*., 1998; Gatti *et al*., 1991). A female relatives being heterozygous for an *ATM* gene mutations in the families of individuals with ataxia telangiectasia have an approximately two to five fold increase in risk of breast cancer (Swift *et al*., 1987; Thompson *et al*., 2005; Renwick *et al*., 2006) A truncating variant Glu1978X of the *ATM* gene has been associated with a large increase in breast cancer risk (Ahmed *et al*., 2006; Renwick *et al*., 2006). Deletions of exons 29 and 46 of the *ATM* gene and exons 2 and 5 of the *CHEK2* gene have not been previously reported and ask for further research.

CONCLUSIONS

1. A large genomic deletions in the moderate risk (*CHEK2* and *ATM*) breast cancer susceptibility genes can be found in Latvia.
2. This is the first report of a large genomic deletion in the *CHEK2* gene spanning exons 9 and 10 in Latvia.

ACKNOWLEDGEMENT

This study was supported by project of European Social Fond, agreement number 2009/0203/1DP/1.1.1.2.0/09/APIA/VIAA/070.

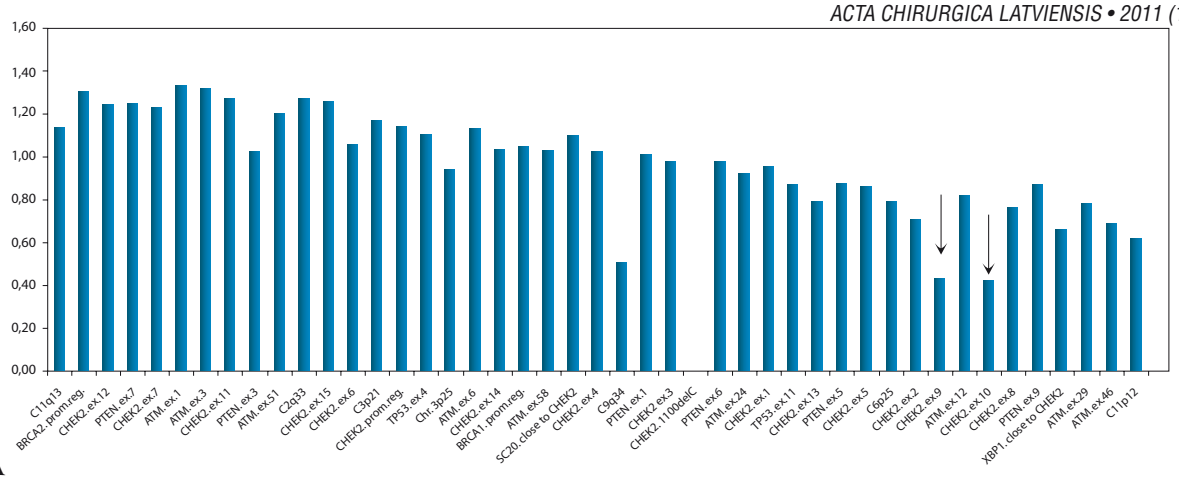
Conflict of interest: None

REFERENCES

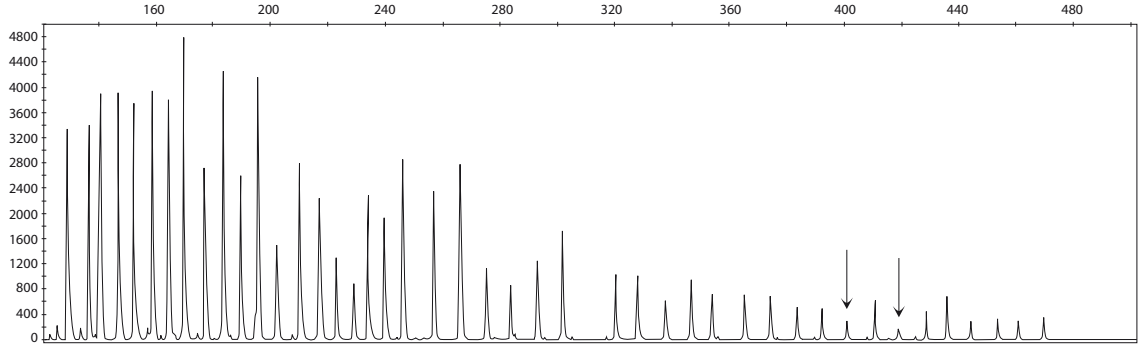
1. Ahmed J, Freddie B, Center MM, et al. Global cancer statistics // CA: Cancer J Clin, 2011; 61:69 – 90
2. Ahmed M, Rahman N. ATM and breast cancer susceptibility // Oncogene, 2006; 25: 5906 – 5911
3. Aretz S, Stienen D, Uhlhaas S, et al. High proportion of large genomic deletions and a genotype phenotype update in 80 unrelated families with juvenile polyposis syndrome // J Med Genet, 2007; 44:702 – 709
4. Bartek J, Falck J, Lukas J. chk2 kinase – a busy messenger // Nat Rev Mol Cell Biol, 2001; 2:877 – 886
5. Bogdanova N, Feshchenko S, Cybulski C, et al. CHEK2 mutation and Hereditary Breast Cancer // J Clin Oncol, 2007; 25:e26
6. Campbell C, Mitui M, Eng L, et al. ATM mutations on distinct SNP and STR haplotypes in ataxia-telangiectasia patients of differing ethnicities reveal ancestral founder effects // Hum Mutat, 2003; 21: 80 – 85
7. Chehab NH, Malikzay A, Appel M, Halazonetis TD. Chk2/hCds1 functions as a DNA damage checkpoint in G-1 by stabilizing p53 // Genes Dev, 2000; 14:278 – 288
8. Claus EB, Schildkraut JM, Thompson WD, Risch NJ. The genetic attributable risk of breast and ovaria cancer // Cancer, 1996; 77:2318 – 2324
9. Cybulski C, Wokolorczyk D, Huzarski T, et al. A deletion in CHEK2 of 5,395 bp predisposes to breast cancer in Poland // Breast Cancer Res Treat, 2007; 102: 119 – 122
10. Cybulski C., Wokolorczyk D. et al. A large germline deletion in the Chek2 kinase gene is associated with an increased risk of prostate cancer // J Med Genet, 2006; 43: 863 – 866
11. Gatti RA, Boder E, Vinters HV, et al. Ataxia-telangiectasia: an interdisciplinary approach to pathogenesis. Medicine, 1991; 70:99 – 117
12. Gardovskis A, Štrumfa I, Miklaševičs E, et al.

Epidemiological, clinical, molecular features and early detection strategy of most frequent hereditary cancers in Latvia // Proc Latvian Acad Sci, 2009; 63: 20 – 30
13. Gorski B, Byrski T, Huzarski T, et al. Founder mutations in the BRCA1 gene in Polish families with breast-ovarian cancer // Am J Hum Genet, 2000; 66:1963 – 1968
14. Hartmann C, John AL, Klaus R, et al. Large BRCA1 deletions are found in 3% of German high-risk breast cancer families // Hum Mutat, 2004; 6: 534
15. Hogevorst FB, NederloF PM, Gille JJ, et al. Large genomic deletions and duplications in the BRCA1 gene indentified by a novel quantitative method // Cancer Res, 2003; 63: 1449 – 1453
16. Jaspers NGJ, Gatti RA, Baan C, et al., Genetic complementation analysis of ataxia telangiectasia and Nijmegen breakage syndrome: a survey of 50 patients // Cytogenet. Cell Genet, 1988; 49:259 – 263
17. Kanno J, Hutchin T, Kamada F, et al. Genomic deletion within GLDC is a major cause of non-ketotic hyperglycinaemia // J Med Genet, 2007; 44:e69
18. Kilpivaara O, Vahteristo P, Falck J, et al. CHEK2 gene variant I157T may be associated with increased breast cancer risk // Int J Cancer, 2004; 111:534 – 547
19. Maserati E, Ottolini A, Veggiotti P, et al. Ataxia-without-telangiectasia in two sisters with rearrangements of chromosomes 7 and 14 // Clin Genet, 1988; 34:283 – 287
20. Matsuoka S, Huang M, Elledge SJ. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase // Science, 1998; 282:1893 – 1897
21. Meijers-Heijboer H, van den Ouweland A, Klijn J, et al. Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 and BRCA2 mutations // Nat Genet, 2002; 31:55 - 59
22. Pylkas K, Erkko H, Kikkilu J, et al. Analysis of large deletions in BRCA1, BRCA2 and PALB2 genes in Finish breast and ovarian cancer families // BMC Cancer, 2008; 8:146
23. Redeker EJ, de Visser ASH, Bergen AAB, Mannens MMAM. Multiplex ligation-dependent probe amplification (MLPA) enhances the molecular diagnosis of aniridia and related disorders // Mol Vis, 2008; 14: 836 – 840
24. Renwick A, Thompson D, Seal S, et al. ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles // Nat genet, 2006; 38:873 – 875
25. Savitsky K, Sfez S, Tagle DA, et al. The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species // Hum Molec Genet, 1995; 4:2025 – 2032
26. Schouten JP, McElgunn CJ, Waaijer R, et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification // Nucleic Acids Res, 2002; 30:e57
27. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumors // Nature, 2000; 406:747-752
28. Smith LD, Tesoriero AA, Wong EM, et al. Contribution of large genomic BRCA1 alterations to early-onset breast cancer selected for family history and tumor morphology: a report from The Breast Cancer Family Registry // Breast Canc Res, 2011; 13:R14
29. Spearman AD, Sweet K, Zhou XP, et al. Clinically applicable models to characterize BRCA1 and BRCA2 variants of uncertain significance // J Clin Oncol, 2008; 26:5393 – 5400
30. Swift M, Reitnauer PJ, Morrell D, Chase CL. Breast and other cancers in families with ataxia-telangiectasia // New Eng J Med, 1987; 316:1289 – 1294
31. Walsh T, Casadei S, Coats KH, et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer // JAMA, 2006; 295:1375 – 1388
32. Thompson D, Duedal S, Kirner J, et al. Cancer risks and mortality in heterozygous ATM mutation carriers // J Nat Cancer Inst, 2005; 97: 813 – 822
33. Taylor CF, Charlton RS, Burn J, et al. Genomic deletions in MSH2 or MLH1 are a frequent cause of hereditary non-polyposis colorectal cancer: identification of novel and recurrent deletions by MLPA // Hum Mutat, 2003; 6:428 – 433
34. Uziel T, Savitsky K, Platzer M, et al. Genomic organization of the ATM gene // Genomics, 1996; 33:317 – 320
35. Wu X, Webster SR, Chen J, et al. Characterization of tumor-associated Chk2 mutations // J Biol Chem, 2001; 276:2971 – 2974
36. Žestkova J, Štrumfa I, Bērziņa D, et al. Mutāciju noteikšana BRCA2 gēna 8. Ekzonā ar Reālā laika-PCR/HRM analīzi // RSU Zinātniskie raksti 2009, 2010; 71 – 74

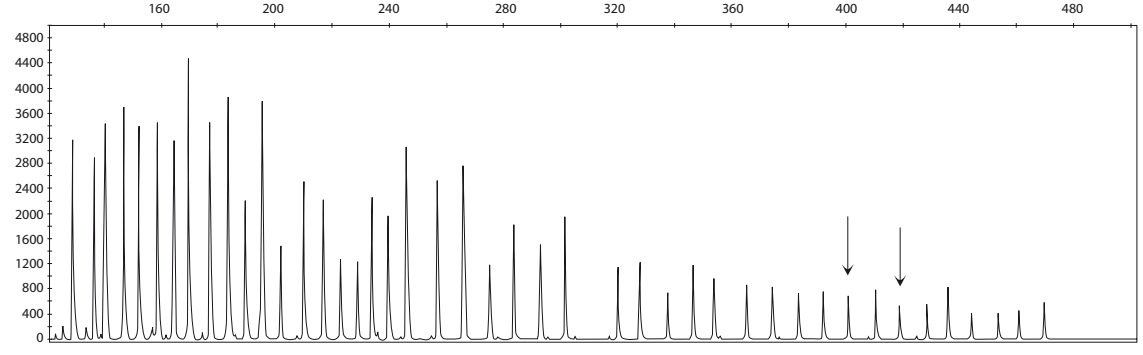
Address:
Dagnija Kalniete
Hereditary Cancer Institute,
Riga Stradins University,
Dzirciema Street 16, LV 1007, Riga, Latvia,
E-mail: dagnija.kalniete@rsu.lv



1A



1B



1C

Fig.1a, 1b, 1c. (1A) Probe ratios calculate for a breast cancer patient E111 with a large genomic deletion of the *CHEK2* gene spanning exons 9 and 10. (1B) MLPA electropherogram of a breast cancer patient E111 carrying a large genomic deletion in the *CHEK2* gene. MLPA electropherogram of a healthy control (1C). With arrows are indicated probes of exons 9 and 10 of the *CHEK2* gene.

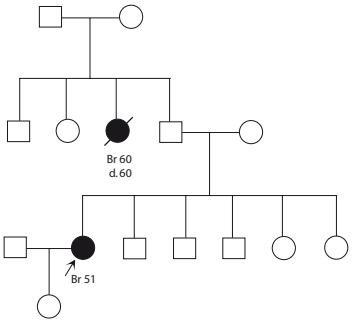


Fig. 2. Family pedigree of a breast cancer patient E111.

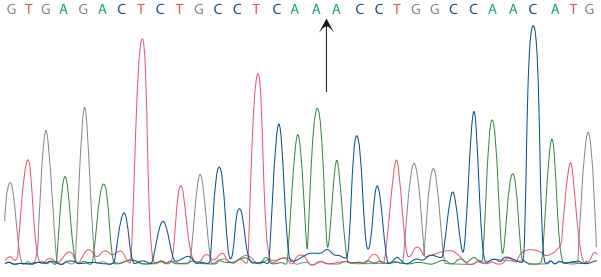
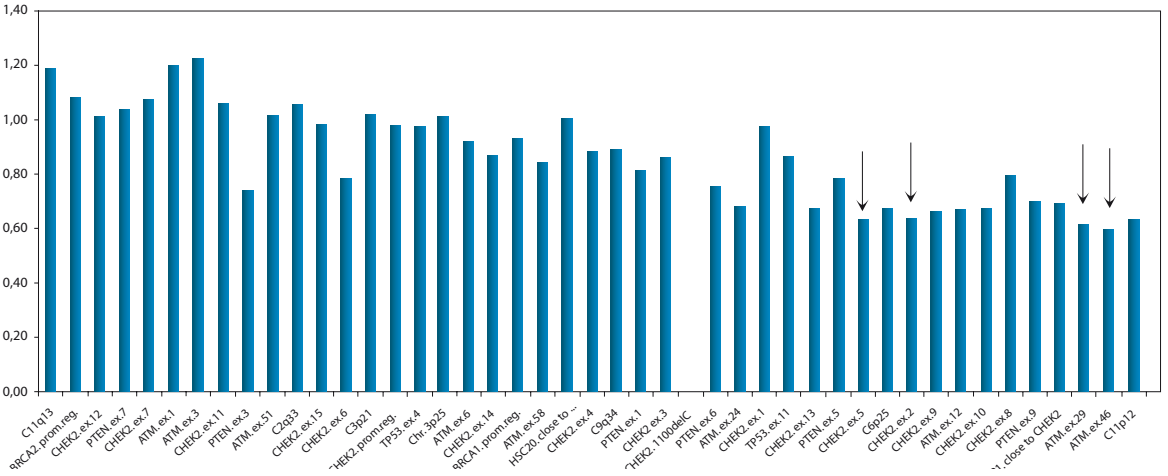
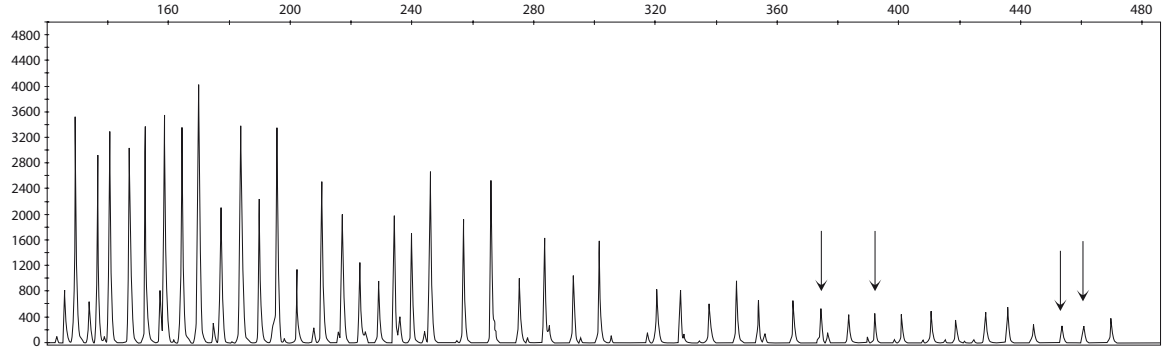


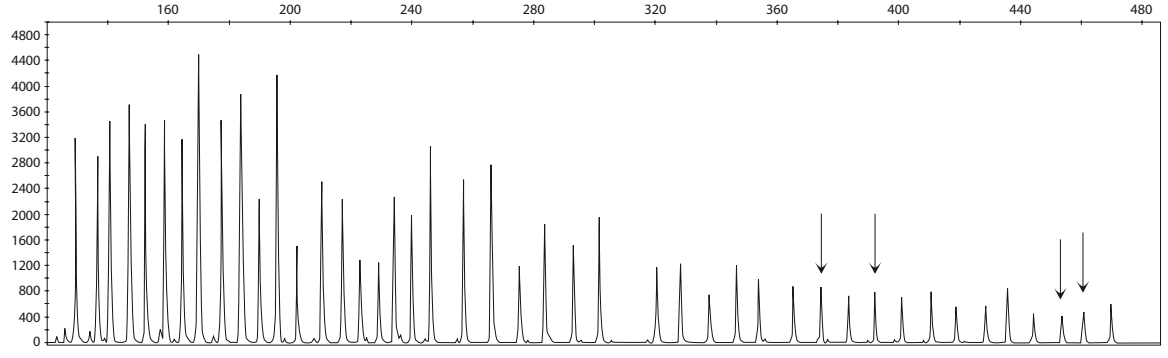
Fig. 3. Sequencing of a genomic breakpoint in a breast cancer patient E111 demonstrating a large deletion of exons 9-10 of the *CHEK2* gene.



4A



4B



4C

Fig. 4a, 4b, 4c. (4A) Probe ratios calculate for a breast cancer patient E224 with deleted exons 5 and 2 in the *CHEK2* gene and exons 29 and 46 in the *ATM* gene. (4B) MLPA electropherogram of a breast cancer patient harboring deleted exon 5 and exon 2 in the *CHEK2* gene and exons 29 and 46 in the *ATM* gene. MLPA electropherogram of a healthy control (4C). With arrows are indicated probes of exons 5 and 2 of the *CHEK2* gene and probes of exons 29 and 46 of the *ATM* gene.