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BRCA1/2 mutation screening and LOH analysis of lung adenocarcinoma tissue in a multiple-cancer patient with a strong family history of breast cancer

Melanie Barbara Boettger[1], Consolato Sergi[2] and Peter Meyer*[1,3]

Address: [1]Institute of Human Genetics, University of Heidelberg, 69120 Heidelberg, Germany, [2]Institute of Pathology, University of Heidelberg, 69120 Heidelberg, Germany and [3]Genefinder Technologies Ltd., Sperberstr. 2, D-81827 Munich, Germany

Email: Melanie Barbara Boettger - melanie.boettger@onkogenetik.de; Consolato Sergi - C.Sergi@bristol.ac.uk; Peter Meyer* - Peter.Meyer@onkogenetik.de

* Corresponding author

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Abstract

Background: Germline mutations in *BRCA1/2* greatly elevate risks of breast and ovarian cancers, but the role of these genes in tumourigenesis of other cancer types is still being investigated.

Objective: We report on an investigation of *BRCA1/2* mutations and their loss of heterozygosity (LOH) in a patient with a strong family history of breast cancer who was diagnosed with consecutive primary cervical, ovarian and lung carcinomas.

Methods and results: *BRCA1/2* mutation screening of the proband revealed a common familial breast- and ovarian cancer-associated germline *BRCA2* mutation (3034del4bp). We then performed LOH analysis for *BRCA2* in lung adenocarcinoma tissue of the patient. Using the laser-capture microdissection (LCM) technique, we obtained pure populations of neoplastic cells from which DNA could be extracted. Mutation analysis by denaturing high-performance liquid chromatography (DHPLC) and direct sequencing revealed loss of the mutant allele in the adenocarcinoma tumour tissue.

Conclusion: To our knowledge, this is the first report of investigation for LOH for *BRCA2* in primary lung adenocarcinoma tissue of a patient with multiple primary tumours related to a familial germline *BRCA2* mutation. Interestingly, it was the mutant, not the wild-type, allele which was lost in the lung adenocarcinoma tissue.

Introduction

Germline mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* are strongly associated with an elevated risk of breast and ovarian cancers [1]. Mutation carriers also have a statistically increased risk for several other cancers; i.e. pancreatic cancer and carcinoma of the uterine body and cervix for *BRCA1* [2], and prostate, pancreatic, biliary, and gastric cancers, and malignant melanoma for *BRCA2* [3]. LOH at the *BRCA2* locus has been observed in several tumours other than breast and ovary, i.e. tumours of the prostate, cervix, colon and ureter in patients with germline mutations in that gene [4]. LOH

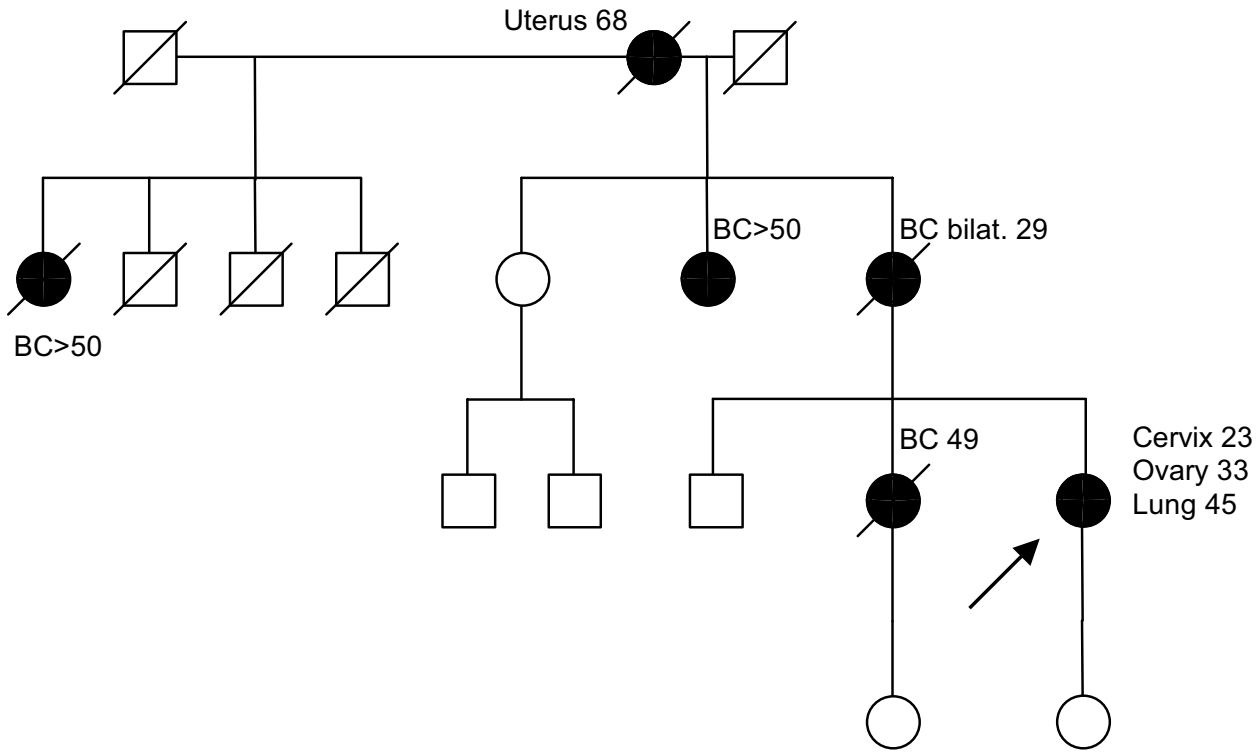

Figure 1
Pedigree of the patient's family showing strong family history of breast cancer (BC). Filled symbols (black) represent cancer cases. Ages of disease onset are shown in years. The index patient is indicated with an arrow.

analysis of tumour tissues in multiple-cancer patients who harbour mutations in cancer-related genes is one means for investigating the contribution of these genes to the oncogenesis of these cancers. Using this strategy, we performed *BRCA1/2* mutational analysis of germline cells and LOH analysis of lung adenocarcinoma tumour cells to investigate the role of *BRCA1/2* tumourigenesis in lung adenocarcinoma in this patient with primary cervical, ovarian and lung cancers and a strong family history of breast cancer.

Case Report

A 46-year-old woman presented to the Department of Clinical Genetics, University of Heidelberg with multiple primary carcinomas and a family history of breast cancer. Her mother was diagnosed with bilateral breast cancer at the age of 29 years. The patient's sister was found to have breast cancer at age 49 years. Additionally, two maternal aunts had postmenopausal breast cancer (Fig. 1). Our patient had three primary tumours: cervical carcinoma at age 23, right ovarian carcinoma at age 33 and primary lung carcinoma at age 45 years. None of the tumours were treated with chemotherapy. The patient smoked one pack of cigarettes per day for ten years prior to her lung cancer diagnosis.

Methods and Results

Screening for BRCA1 and BRCA2 germline mutations

We obtained prior written informed consent from our patient using protocols approved by the Ethics Committee of the Medical Faculty at the University of Heidelberg, Germany. Genomic DNA was isolated from blood using standard procedures. To amplify exon and exon-intron boundaries, we used primer pairs and polymerase chain reaction (PCR) protocols as previously published [5]. Mutational screening for *BRCA1* and *BRCA2* mutations was carried out by prescreening with denaturing high-performance liquid chromatography (DHPLC) analysis and direct sequencing as described elsewhere [5]. DHPLC analysis of amplified genomic DNA of our patient showed a distinctive elution profile in exon 11 of *BRCA2* (Fig. 2A,2B,2C), and dye terminator sequencing identified the exact sequence alteration. We detected the common breast cancer-associated frameshift mutation 3034del4bp (Fig.

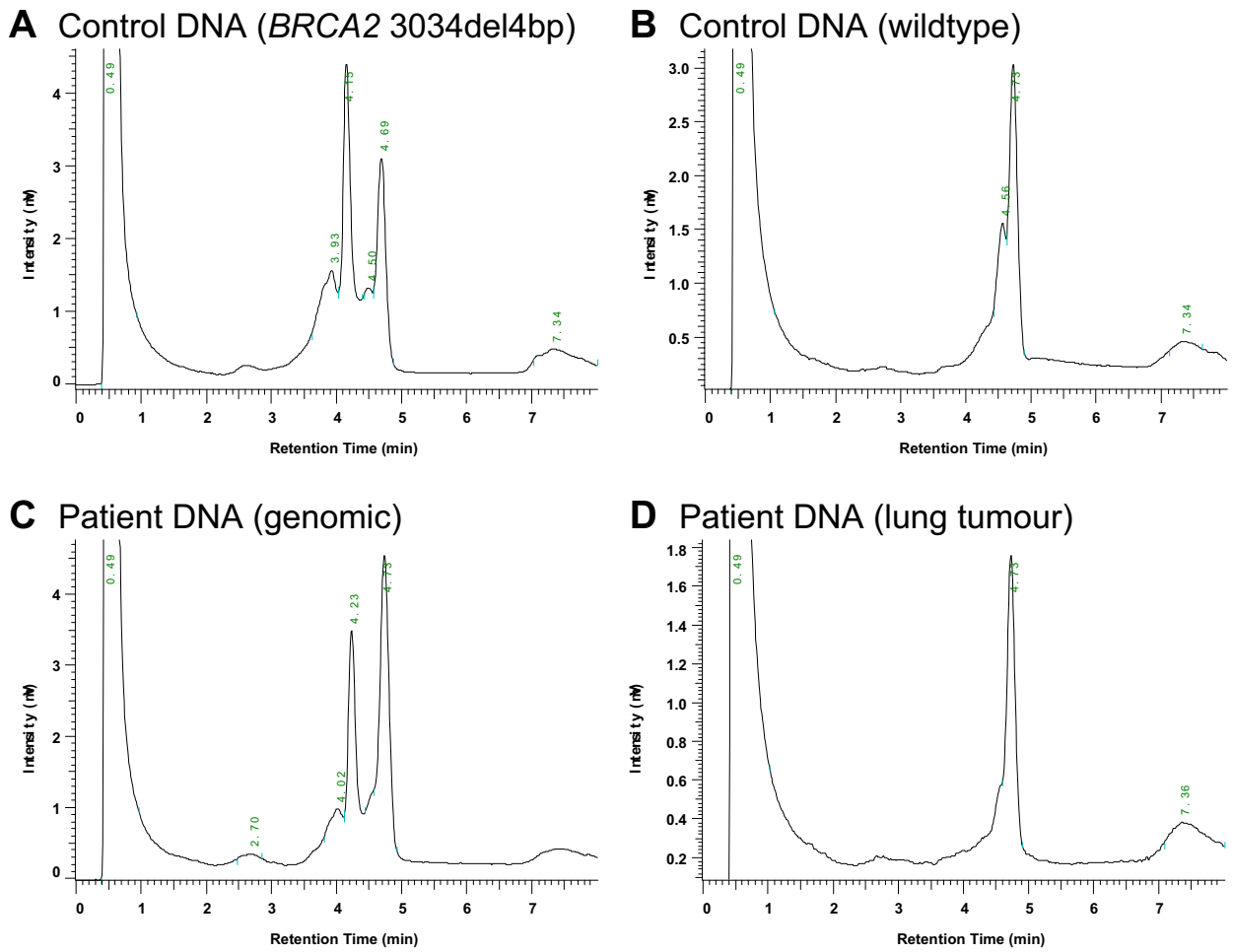

Figure 2
DHPLC elution profiles of 463 base pair PCR fragments of *BRCA2* exon 11. **A:** Heteroduplex profile with two peaks from control DNA that was found to be heterozygous for the common exon 11 *BRCA2* mutation 3034del4bp. **B:** Homoduplex profile (DHPLC) in amplified wild-type DNA. **C:** DHPLC analysis of amplified genomic DNA of our patient showing a heteroduplex profile similar to the profile of the mutant control DNA sample. **D:** DHPLC elution profile of amplified lung tumour DNA showing only one peak indicating LOH, either of the wild-type or the mutant allele.

3A), which causes premature protein termination at codon 958.

Loss of heterozygosity (LOH) analysis of tumour tissue

LOH analysis was performed in the bronchial adenocarcinoma tissue only, as tissue samples of the other primary tumours were not available. Sections (5 μm) of the paraffin-embedded lung carcinoma sample were stained by hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) using standard protocols. Distinct neoplastic cells (approx. 500) were collected by laser-mediated microdissection and subsequent laser pressure catapulting method as previously described [6]. In brief, three 5 μm thin sections including tumourous and nontumourous lung tissue were placed in xylene and deparaffinated. The slide was placed on the stage of a laser-guided zoom stereo microscope and the tumour tissue was excised by laser microdissection. A plane of cleavage could easily be developed between cells for analysis and those to be left behind. As the periphery of the target area became elevated, the laser tip could segment the contralateral piece, until the whole area was undermined and detached. Single fragments were aspirated into a micropipette tip and transferred to a microcentrifuge tube. The remaining tissue was stained with H&E to test the appropriateness of the microdissection. We used a commercially-available

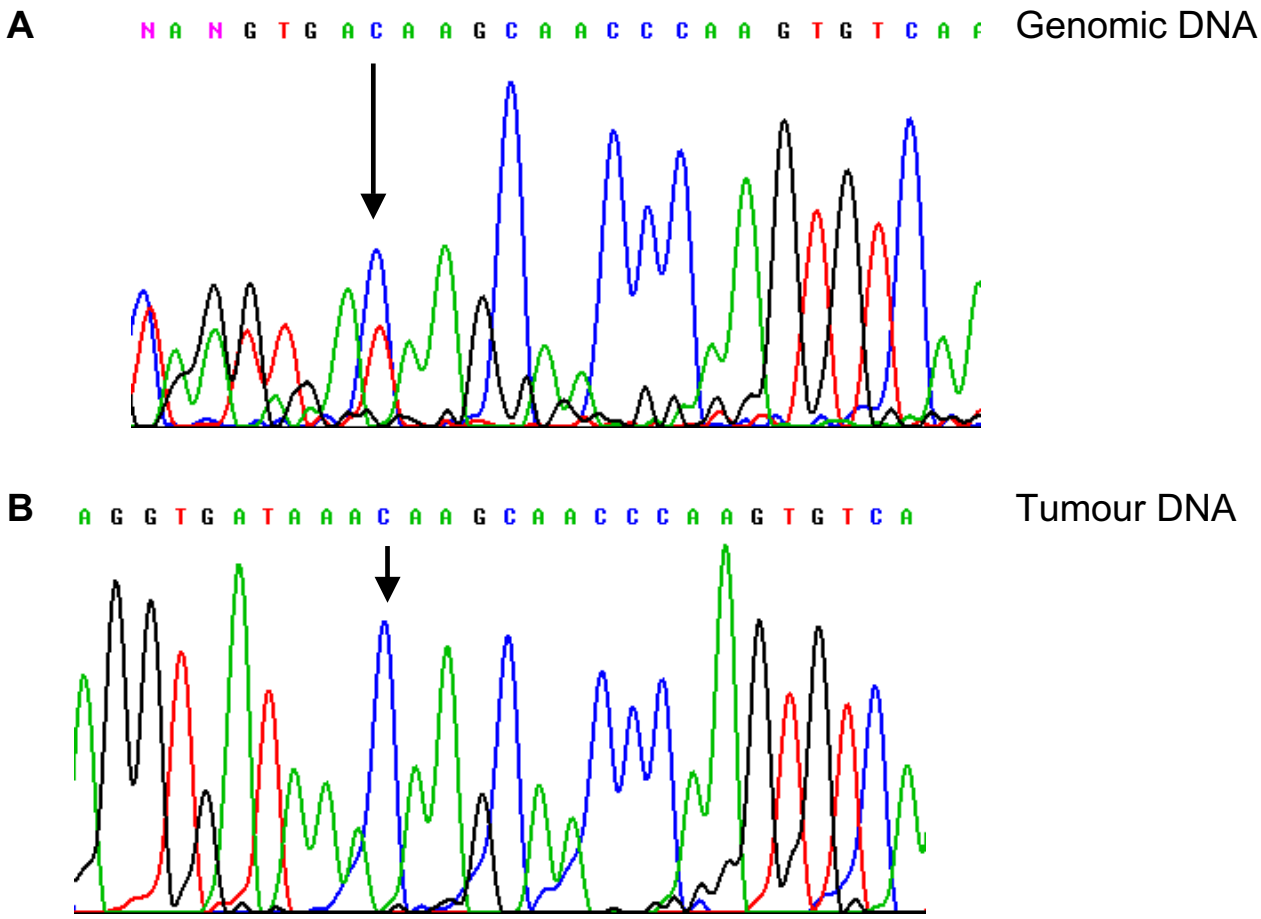

Figure 3
Results of direct sequencing-amplified genomic (A) and lung tumour DNA (B) of our patient. **A:** Direct sequencing reveals heterozygosity beginning from position 3034 of the *BRCA2* gene (C/T, **arrow**), that was shown to be a deletion of 4 base pairs (AAAC) leading to frame shift and premature termination of translation in position 958 of BRCA2 protein. **B:** "Homozygosity" for the wild-type allele around position 3034 of *BRCA2*, most likely as a result of chromosomal deletion of the mutant allele, was found by sequencing analysis (**arrow**).

UV-Laser Microbeam System (Robot-MicroBeam, P.A.L.M. GmbH, Bernried, Germany). The dissected cells were digested in PCR buffer with proteinase K (2 µl of 20 mg/ml stock solution). Samples were incubated with shaking at 56°C for 12 hr followed by inactivation of proteinase K at 96°C for 10 min. Two µl of this solution containing tumour DNA was amplified using PCR and analysed by DHPLC as described above. Elution profiles of wild-type control DNA and tumour tissue DNA showed similar single peaks (Fig. 2B,2D), indicating homoduplices. Sequence analysis of the same fragment revealed loss of the mutant allele but not of the wild-type allele in the tumour cells (Fig. 3).

Discussion

Knudson's two-hit hypothesis for the genesis of hereditary cancer predicts that cancer may develop when the wild-type allele of a tumour suppressor gene is lost in an individual with a germline mutation in that gene [7]. Our female patient had multiple tumours and strong family history of breast cancer. Sequence analysis of germline cells revealed a frameshift mutation in exon 11 of *BRCA2*. This mutation, 3034del4bp, is listed in the Breast Cancer Information Core (BIC) 18 times and is known to be associated with familial breast and ovarian cancer [8]. BRCA genes conform to the classic paradigm for tumour suppressor genes in that loss of the wild-type allele can be found in tumour cells isolated from predisposed individ-

uals [9]. Therefore, we performed LOH analysis on our patient's lung adenocarcinoma tissue to investigate the involvement of *BRCA2* in the genesis of this tumour. Unfortunately, neither cervical nor ovarian cancer tumour tissues were available for analysis. Subsequent DNA analysis by DHPLC and direct sequencing in the lung adenocarcinoma revealed loss of the mutant, but not of the wild-type allele. The loss of an abnormal cancer-associated germline *BRCA2* mutation rather than a normal protective allele would intuitively seem to argue against a causative role of the germline *BRCA2* mutation in the lung adenocarcinoma in this patient. *BRCA2* is comprised of 27 exons encoding 3,418 amino acids. Our patient carries a germline mutation in exon 11 of the *BRCA2* gene leading to a termination of the protein sequence after 958 aminoacids. We assume that the protein encoded by the mutant allele is strongly reduced in its function, and loss of an essentially nonfunctional protein product, leaving only the normal tumour suppressor gene product should not lead to tumour formation. It remains the possibility that the wild-type allele of *BRCA2* in the tumour tissue was knocked out by a deleterious point mutation that could not be identified with the here described methods. To test this more unlikely hypothesis, an extensive mutation screen of the entire coding region of *BRCA2* in the lung tumour tissue would have been necessary. Alternatively, deletions of other genes located near the *BRCA2* locus of the deleted mutant allele might also participate in lung adenocarcinoma oncogenesis. Investigation of the extensiveness of the chromosomal deletion in the neoplastic cells might be helpful in suggesting mechanisms of disease in this case. Certainly, other mechanisms of tumourigenesis for the lung adenocarcinoma in this patient unrelated to *BRCA2* mutation must be considered, especially in light of a history of cigarette smoking.

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