[CANCER RESEARCH 52, 3234–3236, June 1, 1992]

Advances in Brief

Screening for Germ Line *TP53* Mutations in Breast Cancer Patients[1]

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Abstract

The constant denaturant gel electrophoresis technique was used to screen for *TP53* germ line mutations in 237 women with breast carcinoma (167 unselected patients, 30 patients with at least one first-degree relative with breast cancer, and 40 women diagnosed with breast cancer before age 35). A germ line mutation at codon 181 was noted in one of the unselected patients and a codon 245 mutation in one of the early-onset patients. Both had a family history of breast cancer and other malignancies suggestive of Li-Fraumeni syndrome. The codon 245 mutation was also present in this patient's affected mother.

Introduction

Breast carcinomas account for approximately 20% of cancer-related deaths in women. In Norway, Iceland, and the United States, the incidence of breast cancer ranges from 90 to 110 cases/100,000 women/year, and deaths from breast cancer in these three populations approximate 45,000 annually (1–3). Although most cases are sporadic, a frequent familial aggregation of breast cancer indicates that it will be of critical importance to understand the genetic elements of susceptibility. Between 5 and 10% of all cases are estimated to be hereditary, with an autosomal dominant mode of inheritance (4). Approximately 20% of women with breast cancer are estimated to have one or more first-degree relatives with the disease (5). Both epidemiological studies and linkage analysis suggest heterogeneity with respect to the genetic basis of breast cancer (6). In some families, breast cancer is associated with the occurrence of ovarian, uterine, or colorectal cancer (7). Survivors of childhood soft tissue sarcoma and their relatives have also been observed to be at increased risk for developing breast and adrenocortical carcinomas, sarcomas, leukemias, and brain tumors (Li-Fraumeni syndrome) (8).

Recently, two specific genetic factors thought to be important for breast cancer susceptibility have been identified. A region on the long arm of chromosome 17 has been shown to be closely linked to breast cancer susceptibility in many families with early onset site specific breast cancer, and in families with breast and ovarian cancer (6). The gene defect in breast cancer patients who have the Li-Fraumeni syndrome often appears to be germ line mutations in the tumor suppressor gene *TP53* on chromosome arm 17p (9, 10). The frequencies of germ line *TP53* mutations among patients with various cancers whose family histories are not consistent with Li-Fraumeni syndrome have not yet been fully defined. We have recently developed a rapid screening technique for detection of *TP53* mutations (11), using CDGE (12),[3] which is a modification of the denaturing gradient gel electrophoresis system (13). In this technique altered DNA fragments migrate with a constantly different rate through the gel, allowing separation of several centimeters between mutants and wild-type DNA. In the present study, the CDGE technique has been used to explore the occurrence of germ line *TP53* mutations in exons 5 through 8, in which the conserved regions are located, and where more than 95% of the mutations in sporadic tumors have been located (14). Specifically, we have studied 167 unselected breast cancer patients, 30 breast cancer patients with positive family history, and 40 patients who developed breast cancer before age 35.

Materials and Methods

Patients. The unselected breast cancer patients consisted of a consecutive series of 65 breast cancer patients with mean age 56.8 years (ranging from 31 to 80) admitted to The Norwegian Radium Hospital, and a consecutive series of 102 Icelandic patients with mean age 58.6 (ranging from 33 to 94) admitted to the three main Reykjavik hospitals. All cases were checked for family history of breast cancer in collaboration with the Norwegian and Icelandic Cancer Registries. Fourteen of the Norwegian and 12 of the Icelandic patients had at least one first-degree relative with the disease. The selected series of 30 patients with familial aggregation of breast cancer (at least one first-degree relative with breast cancer) consisted of patients admitted to the Norwegian Radium Hospital and patients from the Oslo area asking for genetic counseling because of a familial aggregation of breast cancer. The patients in this series were interviewed with respect to family history by standardized questionnaires, and all cancer diagnoses reported were confirmed by the Norwegian Cancer Registry.

The series of 40 breast cancer patients diagnosed before the age of 35 consisted of 38 patients identified through the Breast Evaluation Center at the Dana Farber Cancer Institute, the Joint Center for Radiation Therapy at the Harvard Medical School, the Toronto-Bayview Regional Cancer Center in Toronto, and from calls from interested patients and physicians regarding genetic testing. The other two patients under the age of 35 were identified at the Georgetown Medical School. Breast cancer diagnoses were confirmed by using medical records, and family history information was recorded but not routinely confirmed. Ten to 20 ml of blood were drawn from each patient into EDTA-containing test tubes and stored at −40°C until DNA analysis.

***TP53* Mutation Analysis with the Use of CDGE.** DNA was extracted from whole blood, using standard procedures (phenol/chloroform and ethanol precipitation). The PCR was performed by using four sets of primers, covering 80% of the conserved region of the *TP53* gene (codons 128-153 and 155-185 of exon 5, codons 237-253 of exon 7, and codons 265-301 of exon 8) as previously described, using 100–300 ng of template DNA (11). CDGE and perpendicular denaturing gradient gels were performed by using 12.5% acrylamide in 0.04 M Tris-acetate, 0.001 M EDTA (pH 8.0), and varying denaturant concentrations consisting of urea and formamide (100% denaturant corresponds to 7

Received 2/19/92; accepted 4/15/92.

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[1] This work was supported by grants from the Norwegian Cancer Society, the Norwegian Council for Science and Humanities, The Nordic Fund for Technology and Industrial Development, Thorsteds Legacy, Grethe Harbitz Legacy for Cancer Research, The Icelandic Cancer Society Science Fund, The Research Fund of the University of Iceland, and The Medical Research Council of Canada.

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[3] The abbreviations used are: CDGE, constant denaturant gel electrophoresis; PCR, polymerase chain reaction.

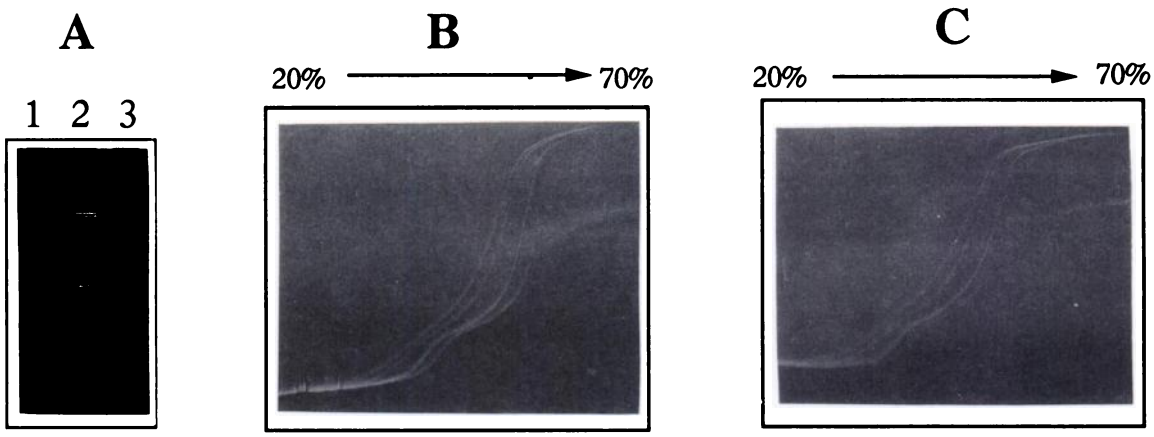
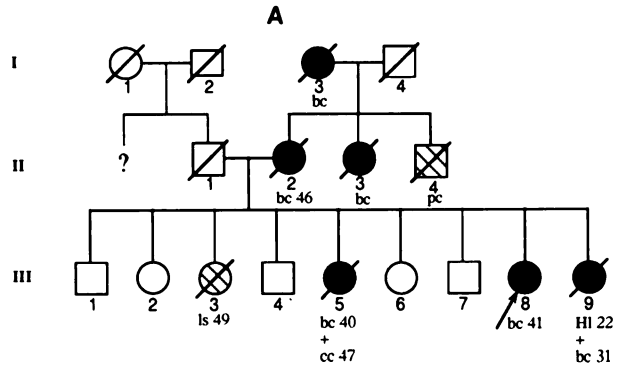


Fig. 1. Denaturing gels of PCR amplified B fragments. *A*, CDGE run at 52% denaturant. *Lane 1*, constitutional DNA from Patient 101; *Lane 2*, tumor DNA from Patient 101; *Lane 3*, normal DNA. *B*, perpendicular gel of constitutional DNA from Patient 101. *C*, perpendicular gel of tumor DNA from Patient 101. The perpendicular gels contained 20–70% denaturant.



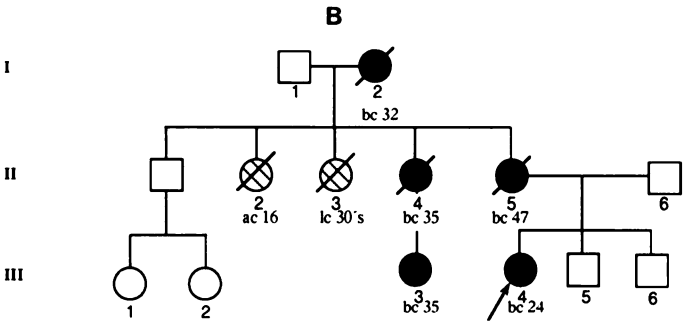


Fig. 2. Pedigrees of the patients with germ line *TP53* mutations. *A*, Patient 101 with a mutation at codon 181. *B*, Patient 144 with a mutation at codon 245. *bc*, breast cancer; *pc*, prostate cancer; *ls*, leiomyosarcoma; *cc*, colorectal carcinoma; *Hl*, Hodgkin's lymphoma; *ac*, abdominal cancer; *lc*, lung cancer. *Numbers* indicate age at diagnosis. *Arrows*, probands.

M urea and 40% formamide) as previously described (11, 12). In perpendicular gels a denaturant gradient was used perpendicular to the electrophoretic direction. In CDGE a uniform denaturing concentration through the gel was used. After electrophoresis, the gels were stained for 2 min in ethidium bromide, 2 mg ethidium bromide/liter and 0.04 M tris-acetate, 0.001 M EDTA (pH 8.0), and photographed by using a UV transilluminator.

Sequencing. Samples that had aberrantly migrating bands in the CDGE were all submitted to PCR for direct sequencing. PCR was performed with one biotinylated primer. The biotinylated PCR products were sequenced directly with standard dideoxy sequencing reactions, using Dynabeads M280-Streptavidin (Dynal AS, Norway) as solid support, or using a sequencing kit (Stratagene).

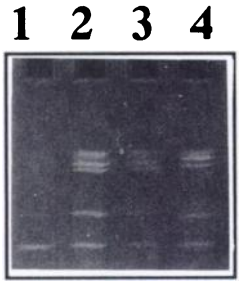


Fig. 3. CDGE of PCR amplified C fragments run at 48% denaturant. *Lane 1*, normal DNA; *Lane 2*, constitutional DNA from Patient 143 (mother of Patient 144); *Lane 3*, constitutional DNA from Patient 144; *Lane 4*, mutant control (constitutional DNA from a previously identified patient, wt/248).

Results

Analysis of the constitutional DNAs from the 167 unselected patients yielded an aberrantly migrating band in fragment B (codon 155-185) in one sample (Patient 101). This indicated the presence of a germ line mutation in this region of the gene of this patient. A tumor sample from the patient was analyzed by using both CDGE and perpendicular gels (Fig. 1). In the tumor, the wild-type fragment that accompanied the mutated fragment was present in reduced concentration, indicating loss of the normal allele. Sequencing was performed to determine the exact nature of the mutation. A mutation at codon 181 (CGC → CAC / Arg → His) was detected. This confirmed the CDGE results. Review of this patient's family history revealed six cases of breast cancer, one leiomyosarcoma at age 49, and other neoplasms (Fig. 2*A*). The presence of sarcoma and breast cancer in this kindred is suggestive of Li-Fraumeni syndrome, with the exception of the late onset of the sarcoma.

An aberrantly migrating band in fragment C was found in 1 of the 40 patients with early onset of disease (Patient 144; Fig. 3, *Lane 3*). The mutation was confirmed in a perpendicular gel and by sequencing, and was located at codon 245 (GGC → AGC / Gly → Ser). The patient had a positive maternal family history of four premenopausal breast cancers, abdominal cancer at age 16, and early-onset lung cancer (Fig. 2*B*). The identical germ line mutation at codon 245 was identified in the mother, who was diagnosed with breast cancer at age 47 (Fig. 3, *Lane 2*). No germ line carriers were found in the selected group of 30 Norwegian patients with a positive family history.

In order to verify that the CDGE technique was able to detect mutations in exons 5 through 8, we sequenced all of the 40

samples from the early-onset patients. No additional mutants were found.

Discussion

TP53 gene mutations currently represent the most common genetic alteration detected in human malignancies (14). *TP53* appears to be a tumor suppressor gene, encoding a nuclear phosphoprotein that normally negatively regulates cell growth and differentiation (15).

Recently, it has been shown that germ line *TP53* mutations are present in affected members of some Li-Fraumeni syndrome families. In the first five families investigated, the affected family members all carried an altered *TP53* gene (9). The finding of germ line *TP53* mutations in Li-Fraumeni syndrome families has been confirmed by several groups (10, 16, 17). Since then, we have found some Li-Fraumeni families who do not have *TP53* mutations in exons 5-8.[4]

Little is known about the frequency of *TP53* germ line mutations in breast cancer patients outside families with the classical Li-Fraumeni syndrome. Prosser *et al.* (18) screened five families with early onset breast cancer for constitutional mutations in all 11 exons of the *TP53* gene, using the hydroxylamine mismatch base pair technique, without finding mutations. We have detected two *TP53* germ line carriers, 1 among 167 unselected breast cancer patients, and 1 among 40 early-onset breast cancer patients (approximately 1%). This frequency is lower than the 4 carriers detected among 196 sarcoma patients (2%) (19) and the 4 detected among 59 patients with second malignancies (7%) (20). These studies analyzed exons 5 through 8 of the *TP53* gene. We restricted our study to the same parts of the gene. Germ line *TP53* mutations may, however occur outside this region, and CDGE is well suited for eventually extending these studies.

The first study describing germ line mutations in Li-Fraumeni syndrome reported mutations in exon 7 only, clustering from codon 245 to 258 (9). Recently, a mutation in exon 5 (at codon 133) have been identified as well (16). In another recent report, where exon 7 was investigated, 2 of 8 Li-Fraumeni syndrome families showed germ line mutations (17). The mutations found in patients with sarcomas and patients with second malignancies were found in several exons (19, 20).

The two germ line mutations detected in the present material were both missense mutations. They were found in exons 5 and 7, respectively. Since the codon 181 (Arg → His) mutation has not been noted previously in families with Li-Fraumeni syndrome or any sporadic tumors, we have tested whether this mutation inactivates *TP53* function and whether it is associated with cancer risk. Preliminary studies suggest that this codon 181 mutation may be functionally silent and may not impart any increased cancer risk (21). In contrast, mutation at codon 245 is found in a highly conserved domain and has been noted in families with Li-Fraumeni syndrome, and in sporadic tumors (9, 14). The codon 245 mutation, like the classic Li-Fraumeni syndrome *TP53* mutations, appears to be associated with cancer risk.

Surveying large populations to determine the frequency of germ line *TP53* mutations is prohibitively expensive when the frequency is less than 1%. CDGE, or similar further improvements in rapid screening techniques, offers an alternative method for surveying such populations.

Our findings indicate that germ line *TP53* mutations occur in extremely few breast cancer patients who do not have family histories suggestive of Li-Fraumeni syndrome.

[4] S. H. Friend, personal communication.

Acknowledgments

We wish to thank Sigrid Lystad for excellent technical assistance, and Dr. Marc Lippman, Dr. Jay R. Harris, Dr. Catherine Pritchard, and Dr. Johannes Bjørnsson for contributing samples.

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