

# Investigation of single-strand conformational polymorphism of the TP53 gene in women with a family history of breast cancer

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## Abstract

Breast cancer in families with germ line mutations in the TP53 gene has been described in the medical literature. Mutation screening for susceptibility genes should allow effective prophylactic and preventive measures. Using single-strand conformational polymorphism, we screened for mutations in exons 5, 6, 7 and 8 of gene TP53 in the peripheral blood of 8 young non-affected members (17 to 36 years old) of families with a history of breast cancer. Studies of this type on young patients (mean age, 25 years) are very rare in the literature. The identification of these mutations would contribute to genetic counseling of members of families with predisposition to breast cancer. The results obtained did not show any polymorphism indicating mutation. In our sample, the familial tumorigenesis is probably related to other gene etiologies.

## Key words

- TP53
- Family history of breast cancer
- Mutation screening
- Early breast cancer

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Research supported by UFPA,  
HEMOPa, UFPb, USP, FAPESP,  
CAPES and CNPq.

Received January 19, 2000  
Accepted August 7, 2000

Numerous risk factors for the development of breast cancer have been identified. Family history, suggesting an inherited component in the development of some breast cancers, is one of the strongest known risk factors. It is estimated that 15 to 20% of women with breast cancer have a family history of the disease (1). Alterations of tumor suppressor gene TP53 (encoding p53) are found in half of all tumors (2). The strongest link between TP53 mutations and breast cancer is based on the increased incidence of early-onset breast cancer seen in Li-Fraumeni syndrome, the family cancer syn-

drome caused by germ line alterations in the TP53 gene (3).

In sporadic breast tumors as well as in the germ line of family members with a history of breast cancer, most of the mutations in the TP53 gene are found in exons 5 to 8 (4-6). By using single-strand conformational polymorphism (SSCP), we performed a mutation screening in exons 5, 6, 7 and 8 of the tumor suppressor gene TP53 in the peripheral blood of 8 young women with a family history of breast cancer. Previous identification of the carriers of mutations in the TP53 gene would contribute to genetic counseling and treat-

ment of young patients with cancer.

In the present study, 8 blood samples from young women belonging to families from Pará, Brazil, with a history of breast cancer were analyzed. The family history and the detailed assessment of the age-specific risk of breast cancer in the women of our sample with relatives affected with the disease were based on data from a population-based, case control cancer and steroid hormone study, which can be used for individual risk assessment (Table 1) (7). A blood sample from young women with no family

history of breast cancer was used as the control.

DNA was extracted from 10 ml of whole blood as described by Sambrook et al. (8).

Primers (9) were used to amplify exons 5, 6, 7 and 8 of the TP53 gene. Approximately 200 ng of genomic DNA was mixed with 50 pmol of each primer and *Taq* polymerase buffer (Cenbiot, Porto Alegre, RS, Brazil), at a final concentration of 10 mM Tris-HCl, pH 8.4, 50 mM KCl and 1.5 mM MgCl<sub>2</sub>, 50 mM of each triphosphated deoxynucleotide, and 1.25 units of *Taq* polymerase. Amplifications were carried out using a Perkin Elmer/Cetus (Norwalk, CT, USA) thermal cycler under the following conditions: 1 cycle at 95°C for 5 min, 35 cycles at 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, and 1 cycle at 72°C for 7 min.

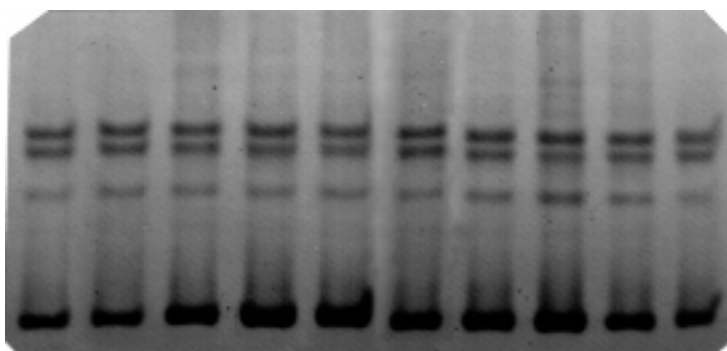
For SSCP analysis, the DNA was amplified by PCR using the specific p53 gene primers described above. After the reaction, the products were diluted in 100 µl of a solution of 0.1% SDS and 10 mM EDTA. Three microliters of the diluted sample was mixed with 4 µl of formamide stain, denatured for 10 min at 95°C and kept refrigerated prior to application to the gel. Electrophoresis was carried out on 40 cm of 5-10% polyacrylamide gel containing 10% glycerol for 16-18 h at 6 W and room temperature (10). The single-strand DNA was stained with silver nitrate.

The deactivation of tumor suppressor genes is a common genetic mechanism in breast cancer, and is of considerable significance for the pathogenesis of human cancer (11). Thus, kindreds with germ line mutations of the TP53 gene can now be screened closely from an early age for the development of tumors, as their risk is extremely high (12). We checked whether the conformational alterations in TP53 exons 5, 6, 7 and 8 detected in the PCR-SSCP test were initial events in the genesis of familial breast cancer. No alterations were found in our sample (Figure 1).

Table 1 - Family history of breast cancer for the women of our sample and assessment of the age-specific risk of breast cancer in women whose relatives developed breast cancer (from Ref. 7).

<sup>a</sup>Population risk for breast cancer at age 40-50 is 1 in 100 (1%). <sup>b</sup>Population lifetime risk (age 20-80 years) is 1 in 12. 'Relatives' include first-degree relatives (mother, father, brother, sister, child). A relative with bilateral breast cancer counts as two. In our sample, all relatives are dead.

Case (initials)	Age (years)	Family history of breast cancer	Expected risk of breast cancer between 40 and 50 years <sup>a</sup>	Lifetime risk <sup>b</sup>
1 (MSS)	17	2 relatives >60 years	<1 in 45	<1 in 7.5
2 (RSP)	36	2 relatives >60 years	<1 in 45	<1 in 7.5
3 (EQL)	18	2 relatives >60 years	<1 in 45	<1 in 7.5
4 (YPS)	35	2 relatives >60 years	<1 in 45	<1 in 7.5
5 (MEL)	22	2 relatives >60 years	<1 in 45	<1 in 7.5
6 (ECC)	22	2 relatives 50-60 years	>1 in 25	1 in 4
7 (ECF)	17	2 relatives 50-60 years	>1 in 25	1 in 4
8 (SRA)	35	2 relatives 50-60 years	>1 in 25	1 in 4



Control Case 1 Case 2 Case 3 Case 4 Control Case 5 Case 6 Case 7 Case 8

Figure 1 - Single strand of exon 8 of gene TP53. Polyacrylamide gel with 10% glycerol. The gel was stained with silver nitrate. Single-strand conformational polymorphism did not show any polymorphism indicating mutation.

The family history can be an indicator of a hereditary disorder. Family characteristics indicating hereditary cancer are a) two or more first-degree relatives with the same malignancy and/or b) two or more first-degree relatives with rare tumors (13). The women in our sample present these characteristics (Table 1).

Literature studies also did not detect constitutional mutations of gene TP53 in members of families with a history of breast cancer or in patients with multiple primary tumors (4,14,15). Germ line mutations in TP53 have been identified as rare causes of hereditary breast cancer (16), although TP53 mutations may be a more important cause of cases of very early-onset breast cancer (12). Mutations in this gene seem to be exclusive characteristics of patients with Li-Fraumeni syndrome, the rare syndrome of family cancer characterized by a high incidence of breast carcinoma and other neoplasias (6).

Therefore, germ line alterations in gene TP53 probably are not a significant cause of familial breast cancer (17,18).

In our sample, familial tumorigenesis may have been related to other genic etiologies or to other segments of the TP53 gene not analyzed in this study. Two other major breast cancer susceptibility genes (BRCA1 and BRCA2) have been identified (19,20) and others are being actively sought. It is very likely that other, lower penetrance genes are responsible for inherited susceptibility to breast cancer in families in which the incidence of breast cancer is higher than that in the general population but the inheritance pattern does not fit the classic model of Mendelian inheritance (1).

The study of breast cancer susceptibility genes has greatly expanded our knowledge of inherited breast cancer, and of development markers for genetic counseling of women with a family history of breast cancer.

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