Elevated Frequency and Functional Activity of a Specific Germ-Line p53 Intron Mutation in Familial Breast Cancer 1

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ABSTRACT

Previous studies have determined that the frequency of germ-line p53 mutations in familial breast cancer patients is 1% or less, but these reports have not investigated the importance of polymorphic intron base changes in the p53 gene. Therefore, we investigated the frequency of both exon and intron germ-line p53 base changes in 42 breast cancer patients with a strong family history of breast cancer. The mean age of presentation of these patients was 44.0 years (range, 29–69), and 12 of 42 (29%) were of known Ashkenazi ancestry. Purified DNA obtained from the 42 index cases was screened for germ-line p53 mutations in exons 2–11 and surrounding introns using a combination of intron based primers for PCR-single strand conformation polymorphism analysis, direct sequencing, and microarray sequencing using the Affymetrix p53 gene chip methodology. Morphological analysis of apoptosis and cell survival determination were performed on EBV-immortalized lymphoblastoid cell lines from two patients with the p53 intron 6 mutation. A germ-line mutation in the p53 gene at nucleotide 13964 with a G to C base change (13964GC) was identified in 3 of 42 (7.1%) hereditary breast cancer patients. Two patients were heterozygous for this mutation, and one patient had a homozygous mutation. In comparison, 0 of 171 (0%) of sporadic breast cancer patients had the p53 13964GC mutation (P = 0.0003). We found that 0 of 42 (0%) of these hereditary breast cancer patients had other germ-line p53 mutations in exons 2–11. However, pedigree analysis demonstrated that all three patients had strong family histories of multiple types of cancers consistent with Li-Fraumeni syndrome but with late age of onset. Comprehensive BRCA1 and BRCA2 nucleotide analysis from patients with the p53 13964GC mutation revealed no concomitant deletorious BRCA1 or BRCA2 mutations, although they were found in the other hereditary breast cancer patients. Functional analysis of two immortalized lymphoblastoid cell lines derived from patients with the p53 13964GC mutation demonstrated prolonged in vitro survival in response to cisplatinum treatment and showed decreased chemotherapy-induced apoptosis. Immunohistochemical analysis of breast tumors from these patients revealed high levels of mutant p53 protein, suggesting a functional mutation in the p53 gene. In summary, we have identified a single p53 intron mutation in familial breast cancer patients that is present at elevated frequency and has functional activity.

INTRODUCTION

The p53 gene is a tumor suppressor gene localized to chromosome 17 that has been demonstrated to have many important biological functions, including regulation of cellular transformation, cell cycle checkpoint following DNA damage, and DNA repair, and is an important mediator of apoptosis following DNA damage in certain cell lineages (1–7). LFS 3 is characterized by germ-line p53 mutations and a clinical phenotype that includes premenopausal breast cancers, childhood sarcomas, brain tumors, leukemias/lymphomas, and adrenocortical carcinomas (8, 9). Approximately 70–80% of LFS patients have germ-line missense mutations in the p53 gene that are generally localized to the conserved exons (9–11). Interestingly, 20–30% of affected LFS families do not have detectable exon mutations, but many studies have not fully examined intron and promoter sites, which may be important regulators of gene expression (10).

p53 intronic base substitutions have been noted in patients including a p53 G to C transversion at base 13964 (p53 13964GC) in women diagnosed with both breast and ovarian cancer, but this base change has previously been thought to represent a polymorphism of unclear significance (12). This is an important because these base changes may results in functional distinction and women with LFS have an 80% lifetime risk of developing breast cancer (10). The clinical significance of germ-line p53 gene mutations in familial breast cancer has not been considered important because less than 1% of all breast cancer patients have germ-line p53 mutations; this percentage is small compared to those with BRCA1 and BRCA2 mutations (13–17).

There is accumulating evidence that novel mechanisms of gene regulation, including mutations in splice donor and acceptor sites, enhancer, intron, and promoter elements, may be important in regulating gene expression, including p53 (18, 19). The identification of these unique mutations that result in aberrant gene expression may provide for the detection of patients at risk for the development of breast cancer. The identification of these mutations may someday allow physicians to optimize treatment because these genetic changes may alter specific biochemical pathways. We now provide evidence that the p53 13964 base is mutated in patients who have familial breast carcinoma but not sporadic breast cancer. Furthermore, this mutation inhibits apoptosis and prolongs cell survival following DNA damage and thus may indeed affect breast cancer risk. The identification of women at risk for the development of breast cancer will have important implications for the prevention of cancers, treatment strategies, and improved cure rates of these patients.

PATIENTS AND METHODS

Patient Population. We identified 42 breast cancer patients with an autosomal dominant pattern of inheritance of breast cancer from the breast cancer database at Yale University School of Medicine. A strong family history of breast cancer was determined by having at least three first- or second-generation family members with a pathological diagnosis of breast cancer or 1 or more family members with premenopausal breast cancer. From this population, we also identified 171 breast cancer patients with no identifiable family history of breast and ovarian cancer, who are thus considered sporadic (Table 1). These patients were all treated with radiation therapy and closely followed in the clinic at Yale University School of Medicine. A protocol for the study was approved by the Human Investigations Committee at the Yale University
were performed as described previously with the addition of 5 μCi of \([\text{\[^{32}\text{P}\]dCTP}}\) per 15-
ml of blood was collected for genomic DNA isolation from lymphocytes.

**Germ-line \(p53\) Gene PCR-SSCP Analysis.** The peripheral blood mono-
uclear cells were isolated, and genomic DNA was extracted with phenol and chloroform and resuspended in sterile TE. Genomic DNA (100 ng) was used in PCR-SSCP screening assay with intron primers that flank exons 5–9 (BioServe Biotechnologies, Ltd., Laurel, MD; Ref. 20).

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**Stage I/II**

<table>
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<th>55</th>
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<td>43/171 (25%)</td>
<td>NS</td>
</tr>
<tr>
<td>Infiltrating ductal carcinoma</td>
<td>42 (100%)</td>
<td>171 (100%)</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of breast/ovarian cancer</td>
<td>42 (100%)</td>
<td>0 (0%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
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School of Medicine, and all patients signed a written informed consent form. Samples were coded and entered into a double-blinded database. The enrolled familial breast cancer patients underwent an extensive interview for complete family history with a three-generation pedigree profile. After the interview, all enrolled patients underwent standard sterile phlebotomy, from which 10–15 ml of blood was collected for genomic DNA isolation from lymphocytes.

**Germ-line \(p53\) Gene PCR-SSCP Analysis.** The peripheral blood mono-
uclear cells were isolated, and genomic DNA was extracted with phenol and chloroform and resuspended in sterile TE. Genomic DNA (100 ng) was used in PCR-SSCP screening assay with intron primers that flank exons 5–9 (BioServe Biotechnologies, Ltd., Laurel, MD; Ref. 20).

**Statistical Analysis.** The frequency of base changes in the \(p53\) allele between the patients with familial and sporadic breast cancer was compared using the Fischer’s exact test. Differences in categorical variables between the patient groups has been compared using the Spearman \(\chi^2\) test. A \(P\) of less than or equal to 0.05 (two-tailed) was considered to indicate statistical significance.

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**RESULTS**

**Clinical Characteristics of Familial Breast Cancer Patients.** Forty-two breast cancer patients with a strong family history of breast cancer (index cases) consistent with an autosomal dominant pattern of inheritance were included for germ-line \(p53\) analysis. The mean age of presentation of this cohort of patients was 44.0 years (range, 29–69), and 12 of 42 (29%) were of Ashkenazi ancestry. Comprehensive \(BRCA1/BRCA2\) analysis was performed on these patients, and 7 of 42 (17%) were found to have a deleterious \(BRCA1\) or \(BRCA2\) mutation. This finding is consistent with recent reports that suggest that 10–40% of women with strong family histories of breast/ovarian cancer unselected for age have deleterious \(BRCA1\) or \(BRCA2\) mutations (27–29).

**Germ-line \(p53\) Mutation Analysis.** Previous studies have determined that the frequency of germ-line \(p53\) mutations in familial breast cancer patients is 1% or less, but many of these reports have not investigated the importance of intron base changes in the \(p53\) gene (13–17). Therefore, we investigated the frequency of both exon and intron germ-line \(p53\) base changes in 42 breast cancer patients with a strong family history of breast cancer. Purified DNA obtained from the 42 index cases was screened for germ-line \(p53\) mutations in exons 2–11 and surrounding introns using a combination of intron based primers for PCR-SSCP analysis, direct sequencing, and microarray sequencing using the Affymetrix \(p53\) gene chip methodology. We identified two samples (BT-16 and BT-31) that had intron 6/exon 7/intron 7 gel shift alterations (Fig. 1A, lanes 2 and 10) and a single sample (BT-102) that had a homozygous gel shift alteration by SSCP (Fig. 1A, lane 13). Because examination of exon 7 and the adjoining splice sites revealed no mutation by direct sequencing, we concentrated on sequencing all of the intron 6 and intron 7 regions that were present in the PCR-SSCP fragment. We identified a \(p53\) 13964GC intron 6 mutation by direct sequencing which was verified by cloning the PCR products (TA cloning system) and sequencing. We determined that BT-16 and BT-31 were heterozygous (3 of 6 and 4 of 10 clones were mutants), and BT-102 was homozygous for the \(p53\) 13964GC base change by automated sequencing (9 of 9 clones were mutants; Fig. 1B). Comprehensive scanning of \(p53\) exons 2–11 was performed by gene array analysis (Affymetrix) and revealed no missense mutations in exons 2–11 of the \(p53\) gene in any of the 42 familial breast cancer samples.

**Genotyping Assay for Familial Breast Cancer Patients.** Examination of the intron 6 \(p53\) 13964GC base change revealed that the mutation destroys a \(HhaI\) cleavage site (recognition sequence GC/GC), and its presence could thus be detected by restriction enzymatic

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**Table 1 Characteristics of familial and sporadic breast cancer cases**

<table>
<thead>
<tr>
<th>Data</th>
<th>Familial</th>
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<td>Stage I/II</td>
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<td>171 (100%)</td>
<td>NS</td>
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<td>1/42 (29%)</td>
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<td>Infiltrating ductal carcinoma</td>
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<tr>
<td>Family history of breast/ovarian cancer</td>
<td>42 (100%)</td>
<td>0 (0%)</td>
<td>&lt;0.001</td>
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</table>

\(\ast\) The population base for this study was composed of 1450 patients with stage I or II breast cancer treated with lumpectomy and radiation therapy to the intact breast at Yale-New Haven Hospital. N/A, not applicable; NS, not significant.
digestion of PCR amplified products analyzed on 4% NuSieve/agarose gels (Fig. 2). Using the genotyping assay, we screened the 42 familial breast cancer patients and identified only those patients previously found on SSCP and sequencing to have the heterozygous (BT-16 and BT-31) and homozygous (BT-102) mutations at base 13964 of the \textit{p53} gene (Fig. 2). Two samples (BT-36 and BT-52) that contained \textit{p53} wild-type sequences were used as controls and produced the expected 172- and 24-bp fragments on \textit{HhaI} digestion (Fig. 2).

**Genotyping Assay for Sporadic Breast Cancer Patients.** The \textit{HhaI} genotyping assay was used to screen 171 sporadic breast cancer patients, 43 of whom (25\%) were of Ashkenazi ancestry but none of whom had a family history of breast/ovarian cancer (Table 1). The sporadic and familial breast cancer cohorts were found to be matched for histopathology (adenocarcinoma), stage (I/II), and Ashkenazi ancestry as determined by \textit{\chi}^2 analysis (Table 1). We found the age of presentation of familial breast cancer patients to be 44 years compared to 55 years for sporadic breast cancer patients (\textit{P}, 0.001; Table 1).

We identified 0 of 171 sporadic breast cancer patients to have the \textit{p53} 13964 GC base change compared to 3 of 42 (7\%) index cases (Fisher’s exact test, \textit{P}, 0.0003). Thus, there was a highly statistically significant association between the intron 6 \textit{p53} 13964 GC mutation and familial breast cancer.

**Clinical and Molecular Characteristics of Patients with the \textit{p53} 13964\textsuperscript{GC} Base Change.** The ages of presentation of the three patients were 30, 42, and 59 years; all three patients presented with stage I or II invasive ductal carcinoma, and none developed metastatic disease (Table 2). We found that both patients with the heterozygous \textit{p53} 13964\textsuperscript{GC} base change had a family history of pre- and post-menopausal breast cancers and that both had bilateral breast cancer.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{SSCP analysis and sequencing of germ-line \textit{p53} from familial breast cancer patients. A, SSCP analysis of the \textit{p53} exon 7 region that includes flanking introns. Lanes 1–13, familial breast cancer samples; Lanes 14 and 16, homo- and heterozygous mutant controls, respectively; Lane 15, wild-type control; Lane 17, negative control. Samples with altered mobility are present in Lanes 2 (BT-16), 10 (BT-31), and 13 (BT-102). B, automated sequencing analysis of the \textit{p53} intron 6 nucleotide 13964 region. PCR products were sequenced from patient BT-16 (top) and a sporadic breast cancer patient (bottom).}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{PCR-RFLP genotyping assay for \textit{p53} intron 6 13964\textsuperscript{GC} mutation. PCR-amplified fragments of 196 bp were subjected to digestion with \textit{HhaI} enzyme and analyzed on 4% NuSieve/agarose gels. Samples with wild-type sequence contain a \textit{HhaI} site and are digested to 172 and 24 bp (the smaller fragment is not visible on gel; Lanes 2, 4, 5, and 7). Heterozygous samples display both the 196-bp and the 172-bp fragments (Lanes 1 and 6). A homozygous \textit{p53} intron 6 mutation at nucleotide 13964 destroys the \textit{HhaI} site, so an undigested fragment of 196 bp is visible (Lane 3).}
\end{figure}
consistent with the inheritance of a dominant gene (30). Interestingly, both of these patients were of Ashkenazi ancestry, although no previously defined relationship between p53 gene mutations and Ashkenazi ancestry has been described. We were able to obtain the paraffin-embedded breast tumor blocks from a proband’s (BT-16) mother and maternal aunt, who had a history of breast cancer diagnosed at ages 42 and 55, respectively. Genotyping revealed the p53 13964GC base change in both affected relatives, demonstrating that the genetic change appears to track with the cancer-prone family members. Tissue from other family members to evaluate for the p53 13964GC mutation was not available. The single patient with the homozygous p53 13964GC mutation has a strong family cancer history, including postmenopausal breast cancers, early and late onset leukemias, brain tumors, and lung cancers from both maternal and paternal lineages with no evidence of consanguinity within this family (Fig. 3). We performed complete sequencing of all coding exons and intron/exon junctions of BRCA1/BRCA2 genes in these patients and found all patients with the p53 13964GC mutation to have wild-type BRCA1/BRCA2 genes.

Functional Importance of the p53 Intron 6 13964GC Base Change. We immortalized lymphoblastoid cells from patients BT-16 and BT-102 and created cell lines containing the heterozygous and homozygous p53 13964GC base change, respectively. The cell lines did not contain any exons 2–11 mutations, as determined by PCR-SSCP and p53 gene chip analysis. Functional p53 studies were carried out to determine the importance of the p53 intron base change at nucleotide 13964. We examined whether cells with the heterozygous or homozygous p53 intron 13964GC base change would be resistant to chemotherapy-induced apoptosis, as demonstrated previously for p53 missense mutations (5, 31, 32). BT-16 and BT-102 lymphoblastoid cells containing the p53 13964GC base change and two p53 wild-type lymphoblastoid cell lines were treated with varying doses of cisplatinum, and cell survival was determined at 48 h (Fig. 4A). The survival of BT-16 and BT-102 cells at 48 h following treatment with 5, 10, and 20 μg/ml cisplatinum was 79 ± 2, 75 ± 2, and 62 ± 1% for BT-16 and 89 ± 2, 82 ± 2, and 76 ± 2% for BT-102, respectively, compared to 42 ± 3, 31 ± 3, and 18 ± 2% for the p53 wild-type lymphoblastoid cell line VDS (Fig. 4A). The survival of p53 wild-type 012 cells demonstrated similar survival characteristics to VDS cells and generally had poor survival following chemotherapy treatment compared to BT-16 and BT-102 cells (Fig. 4A). Chemotherapy-induced apoptosis was determined in two p53 wild-type lymphoblastoid cell lines and was found to be maximal at 48 h following drug treatment (data not shown). Treatment of BT-16 and BT-102 lymphoblastoid cells with 10 μg/ml cisplatinum for 48 h resulted in apoptosis in 15 ± 3 and 10 ± 3%, respectively. In comparison, p53 wild-type cells VDS and 012 (data not shown) demonstrated 47 ± 5 and 38 ± 5%, respectively (Fig. 4B). Transfection assays with the pC53-SN3 plasmid containing wild-type p53 into VDS, BT-16, and BT-102 cells demonstrated partial restoration of normal chemotherapy-induced apoptosis in the BT-102 cells containing the homozygous p53 13964GC base change but not in BT-16 cells containing the heterozygous base alteration. Following treatment with cisplatinum, apoptosis was found in 30 ± 4% of BT-102/p53T cells compared with only 10 ± 3% of untransfected BT-102 cells (Fig. 4B). The morphological changes of nuclear blebbing and membrane fragmentation characteristic of apoptosis are shown by staining of cells with bis-benzimide Hoechst stain are prominent in p53 wild-type cell lines VDS and 012 (Fig. 5, C and D) but poorly demonstrated in the BT-16 and BT-102 lymphoblastoid cell lines (Fig. 5, A and B).

Somatic p53 Mutations. Because previous investigators have identified increased mutant p53 protein in tumor specimens from familial breast cancer patients without germ-line p53 missense mutations, we evaluated for mutant p53 protein expression in tumor specimens from patients with the p53 13964GC mutation (33). We did not use an antigen retrieval technique because this has been associated with increased background signal, making the interpretation of the staining pattern difficult. Mutations in the p53 gene have previously been shown to prolong protein stability, thus allowing the detection of mutant p53 protein by using an antibody specific to this protein.

We obtained the paraffin-embedded breast tumor blocks from patients BT-16, BT-31, and BT-102, which were found to have the

<table>
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<th>Identification no.</th>
<th>Age at diagnosis (yr)</th>
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<td>59</td>
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Table 2 Clinical, pathological, and molecular characteristics of breast cancer patients with germ-line p53 13964GC mutation

Fig. 3. Pedigree of patient with the homozygous p53 13964GC germ-line mutation (BT-102). Br, breast; Leuk, leukemia, BT, brain tumor.
germ-line $p53^{13964\text{GC}}$ mutation. Immunostaining of breast tumor specimens from BT-102 (Fig. 6A), BT-31 (Fig. 6B), and BT-16 (Fig. 6C) revealed strong nuclear staining within the invasive ductal component of all three patients. Previously, LFS patients with $p53$ mutations have been shown to have high levels of mutant $p53$ protein in benign tissue containing only the germ-line heterozygote $p53$ mutation, but there was little benign tissue on these sections, and expression of mutant $p53$ in benign tissue could not be evaluated. Staining of sections with a secondary antibody alone revealed no nuclear reactivity, demonstrating the lack of nonspecific cross-reactive proteins (Fig. 6D).

**DISCUSSION**

We now present evidence that the germ-line $p53^{13964\text{GC}}$ base substitution is identified in familial breast cancer patients but not in sporadic breast cancer patients and that the base change alters normal gene function. The functional studies support our theory that the $p53^{13964\text{GC}}$ base change functions as a dominant mutation similar to the more common missense, nonsense, and splice-site mutations described previously for LFS. $BRCA1$ and $BRCA2$ mutations have been thought to account for 10–40% of hereditary breast cancers, but there are approximately 200–300 different mutations with different phenotypes, and detection of these mutations is often difficult and expensive. The $p53^{13964\text{GC}}$ mutation represents a single noncoding base change that is easily detected and may be responsible for a significant number of hereditary breast cancers; this mutation is the first demonstration of the importance of intronic sequences in regulating $p53$ function, as suggested by other studies (18).

In a cohort of breast cancer patients with a strong family history of breast cancer, 3 of 42 (7%) have the specific $p53^{13964\text{GC}}$ mutation compared with 0 of 171 sporadic breast cancer patients ($P = 0.003$). A detailed analysis of the pedigrees of these patients reveals a pattern of late-onset cancers that appear to be inherited in an autosomal dominant pattern. Late-onset Li-Fraumeni cancers have been described previously in families with exon 4 mutations and indicate that some germ-line $p53$ mutations may allow for partial function of the protein, which results in the late-onset phenotype of tumors (19, 34). Genetic analysis of one of the proband’s mother and maternal aunt, both of whom developed breast cancer, demonstrated that they carried the $p53^{13964\text{GC}}$ mutation. Examination of the pedigree from the patient with the homozygous $p53^{13964\text{GC}}$ substitution is striking, revealing many early and late-onset LFS types of cancers including breast...
It was important to establish whether this intron base change represented a functional mutation. Therefore, we established an EBV immortalized lymphoblastoid cell line from two patients with the p53 13964GC base change. The p53 protein is a transcription factor which is a downstream regulator of many signal transduction pathways, cancers. It is noteworthy that there are both maternal and paternal relatives with late onset breast cancers and other Li-Fraumeni-like cancers. Two of the breast cancer patients with the p53 13964GC mutation were of Ashkenazi ancestry, suggesting that this may represent a founder mutation.
especially those that mediate an apoptotic response to chemotherapy and ionizing radiation. By using p53 knockout and transgenic mice that overexpress dominant negative p53, several independent groups have shown that p53 is essential for apoptosis following treatment with ionizing radiation, chemotherapy, or serum withdrawal (4, 7). A report from another group describes lymphoblastoid cell lines from LFS patients with heterozygous p53 germ-line mutations at codons 282 and 286 to be associated with resistance to radiation-induced apoptosis (35). Our in vitro experiments demonstrating that the p53 13964 GC mutation results in prolonged cell survival and inhibits chemotherapy-induced apoptosis is consistent with these findings.

The p53 13964 GC base substitution was first reported by Buller et al. (12), who found the p53 13964 GC base change in 3 of 73 (4%) patients with both breast and ovarian cancer. The authors concluded that the p53 13964 GC base change represents a nonfunctional polymorphism because there was no direct functional activity to implicate it in malignant transformation. Furthermore, a recent study examining children from Belarus with possible radiation-induced thyroid tumors demonstrated that 5 of 70 (7.1%) had the p53 13964 GC base change, whereas 1 healthy child was also found to carry this base change (36). The family history of these patients were not available, so no firm conclusions were made from this study with regard to associations with inherited cancers, although many childhood malignancies are often due to inherited genetic mutations. Other p53 intron 6 base substitutions have also been identified that may be associated with tumor development. A substitution at nucleotide 13961TGCA was identified in 6 children with the Li-Fraumeni spectrum of tumors, whereas only 1 of 184 healthy controls was found to have this base change (P = 0.036; Ref. 37). Tissue specimens from these patients were found to contain mutant p53 protein immunoreactivity in both tumor and normal tissue. Recently, investigators have identified another intron 6 germ-line base substitution 13492–13499TGCS that was present in several members of a three generation Li-Fraumeni family characterized by breast cancers, medulloblastoma, and rhabdomyosarcoma but not identified in 85 normal control samples (38). Both benign and malignant tissue from these family members demonstrated strong p53 protein immunoreactivity, but mRNA analysis did not demonstrate aberrant transcript production. Other intron 6 base substitutions have been reported that include changes at the regions of 13487–13494 (39, 40). It appears that intron 6 of the p53 gene is potentially a hot spot for mutation with novel mechanisms of gene regulation that appear to be important for tumor formation. The presence of a CpG-rich Alu repetitive element adjacent to the p53 13964 base may explain the frequency of mutation at this site (41). It has been reported previously that breast tumors from patients with familial breast cancer have elevated levels of mutant p53 protein without evidence of exon mutations, suggesting that mutations may occur at sites other than in exons, further supporting our findings (33).

We found that breast cancer specimens from patients with the p53 13964 GC mutation demonstrated strong nuclear p53 immunoreactivity, suggesting that the germ-line mutation causes overexpression of mutant p53 protein, similar to what occurs with missense mutations. Elevated levels of p53 protein in paraffin-embedded tumor specimens from a single Li-Fraumeni family with an intron 6 mutation was reported (38). Identification of p53 mutations may be important for treatment-related decisions because previous studies demonstrated that breast cancer patients with p53 mutations have poor survival rates (31, 32). The outcome of patients with LFS including the novel p53 13964 GC mutations we now describe treated with standard modern therapies, including surgery, radiation, and chemotherapy, has not yet been defined.

We now propose that the noncoding, p53 13964 base is mutated in familial breast cancer. Furthermore, intron 6 appears to be a critical location for mutation in patients with familial tumors of the Li-Fraumeni spectrum that do not have detectable germ-line p53 coding or splice site mutations. This group of Li-Fraumeni patients has late age of onset, which suggests that the function of p53 in these patients may only be partially impaired (19, 34). The mechanism by which the p53 13964 GC mutation results in altered genetic signals remains to be determined. This site is located adjacent to an intron 6 CpG-rich Alu-repetitive element, which may explain the high rate of mutations at this specific region (41). The detection of patients at risk for malignancies is critical in identifying candidates for screening and prevention programs and someday may allow optimal treatment. Genetic testing to identify these subgroups of patients at risk for familial breast cancer and to determine their respective outcome to modern treatments has only just begun. Our finding that BRCA1 and BRCA2 wild-type familial breast cancer patients have the germ-line p53 13964 GC mutation is one step toward identifying genetic mutations that predispose women to breast cancer. Larger epidemiological studies are now warranted to further define the frequency of this mutation and perhaps detect other important intronic mutations.

ACKNOWLEDGMENTS

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35. Hillenbrandt, S., Stellner, C., Demidchik, E. P., Biko, J., and Reiners, C. Polymor-
36. Hillebrandt, S., Streffer, C., Demidchik, E. P., Biko, J., and Reiners, C. Poly-
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