Population-Based Estimate of the Contribution of TP53 Mutations to Subgroups of Early-Onset Breast Cancer: Australian Breast Cancer Family Study

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Abstract

Although germline TP53 mutations have been identified in women with breast cancer from families meeting Li-Fraumeni criteria, their contribution to breast cancer per se is not well known, but is thought to be minimal. We aimed to determine the prevalence of germline TP53 mutations in subgroups of early-onset breast cancer. Germline TP53 mutation status was assessed by DNA sequencing, screening for heterozygous single-nucleotide polymorphisms, and Multiplex Ligation-Dependent Probe Amplification analyses. From an Australian population-based series of invasive breast cancers, we studied (a) 52 women diagnosed before age 30 years unselected for family history [very early-onset (VEO)] and (b) 42 women diagnosed in their 30s with two or more first- or second-degree relatives with breast or ovarian cancer [early-onset family history (EO-FH)]. Of the VEO group, two (4%) had a mutation: G13203A (exon 6 missense) in a 24-year-old and a large 5,338-bp genomic deletion in a 26-year-old. Neither had a family cancer history that met Li-Fraumeni criteria. Of the EO-FH group, three (7%) had a mutation: T13240G (a known intron 5 splicing mutation) in a 36-year-old from a classic Li-Fraumeni family; G12299A (exon 4 missense) in a 33-year-old from a Li-Fraumeni-like family; and 14058delG (exon 7 frame-shift) in a 39-year-old with a family cancer history that did not meet Li-Fraumeni criteria. Germline TP53 mutations play a larger role in early-onset breast cancer than previously thought, and in this context, can be evident outside clinically defined Li-Fraumeni families. Cancer Res 70(12): 4795–800. ©2010 AACR.

Introduction

Based principally on studies of families with multiple cancers that accord with the Li-Fraumeni syndrome, a number of different cancers have been found to occur in people with a germline mutation in TP53, including breast cancer (1–3). The breast cancers associated with germline TP53 mutations in the context of Li-Fraumeni families seem to occur at a very early age for that disease, the majority between 15 and 44 years (1–5).

It is not controversial that women with early-onset breast cancer who have a family history consistent with Li-Fraumeni syndrome have a high probability of carrying a germline TP53 mutation. The contribution of TP53 germline mutations to breast cancer predisposition overall, however, has been thought to be minimal—much less than 1%. The reports on which this estimate was based were limited in technique or genomic areas tested (6–8). Follow-up of earlier reports suggests that germline TP53 mutations might be more frequent (9).

Almost all of the previous studies that have screened for germline TP53 mutations have used convenience samples of subjects who met predetermined clinical criteria and have not focused on early-onset breast cancer unselected for family cancer history or on population-based samples of early-onset disease in multiple-case breast cancer families that do not meet the Li-Fraumeni syndrome criteria. Whereas mutations in BRCA1 or BRCA2 are thought to be most probable in the multiple-case families presenting to familial breast cancer services, population-based studies are finding that mutations in these genes are not as common as previously thought, even for multiple-case breast cancer families with early-onset disease, and a substantial proportion of carriers do not have a family history (10). Other genetic alterations in CHEK2, ATM, BRIP1, and PALB2 might be responsible, but these mutations are rare in most populations and, on average, might not necessarily be associated with a high risk except perhaps in the context of a strong family history (11, 12). It is possible, therefore, that a clinically significant portion of early-onset breast cancer cases that...
are not caused by \textit{BRCA1}, \textit{BRCA2}, \textit{CHEK2}, \textit{ATM}, \textit{BRIP1}, or \textit{PALB2} mutations is attributable to \textit{TP53} mutations, particularly if newer sequencing techniques are used.

To better elucidate the contribution of \textit{TP53} mutations to hereditary breast cancer, we have estimated the prevalence of \textit{TP53} mutations for two subgroups of a population-based sample of breast cancers: (a) cases with very early onset (<30 years) and (b) cases with early age of onset (30–39 years) and a strong family history of breast cancer.

**Materials and Methods**

**Subjects**

The Australian Breast Cancer Family Study (ABCFS) is a population-based, case-control-family study of breast cancer with an emphasis on early-onset disease, carried out in Melbourne and Sydney, Australia (10, 13), and comprises the Australian component of the Breast Cancer Family Registry (14, 15). Approvals for this study were received from the Human Research Ethics Committees of The University of Melbourne, The Cancer Councils of Victoria and New South Wales, and the Institutional Review Boards of the University of Colorado and Kaiser Permanente Colorado.

Cases were adult women (18 years and older), living in the metropolitan areas of Melbourne and Sydney, who were diagnosed with a histologically confirmed first primary cancer of the breast between 1992 and 1999 and identified through the Victorian and New South Wales cancer registries, to which notification of cancer diagnoses is a legislative requirement. Enrollment of cases involved completing an epidemiology and family history questionnaire, request for a blood sample, and permission to obtain a tumor sample. Family members of cases were also invited to enroll into the ABCFS. Attempts were made to interview all living female adult first- and second-degree relatives of the cases.

Consequently, pedigree information was based not only on self-report from the case but also often from one or more relatives. For any family member, cancer history was rarely based only on a report from a second-degree relative and often came from multiple sources within the family. Collection of family history included ascertainment of both adult-onset and childhood-onset cancers. Verification of all cancers reported in relatives was sought through cancer registries and death certificates.

For this study, we defined two subgroups of early-onset invasive breast cancer cases: (a) women diagnosed before the age of 30 years irrespective of family history [very early-onset (VEO)] and (b) women diagnosed between the ages of 30 and 39 years with two or more first- or second-degree relatives with breast or ovarian cancer [early onset-family history (EO-FH)]. We approached 1,208 cases diagnosed before age 40 years and 856 (71%) were interviewed. Of these, 82 women met the VEO criteria and 61 women met the EO-FH criteria, of whom 52 and 42, respectively, had sufficient DNA available for \textit{TP53} mutation testing. For each case found to have a germline mutation in \textit{TP53}, all of the relatives enrolled in the ABCFS from whom a blood sample was obtained were tested for the family-specific mutation.

Table 1 shows that there was sufficient DNA for testing 52 of the VEO cases for \textit{TP53} mutations. Of these, we have already identified that 11 (21%) carried a breast cancer predisposing gene mutation: 6 in \textit{BRCA1} (16, 17), 3 in \textit{BRCA2} (11, 16), and 2 in \textit{CHEK2} (17). Table 1 also shows that there was sufficient DNA for testing 42 of the EO-FH cases, and that 11 (26%) carried a breast cancer predisposing gene mutation: 7 in \textit{BRCA1} (10, 15–17), 3 in \textit{BRCA2} (11, 16), and 1 in \textit{ATM} (18). Therefore, of the 94 cases tested here for \textit{TP53} mutations, 22 (23%) were known to carry a breast cancer predisposing mutation in another gene (including 13 in \textit{BRCA1} and 6 in \textit{BRCA2}).

**Definitions of Li-Fraumeni criteria**

There are a number of definitions of Li-Fraumeni syndrome. A family was considered to meet the classic Li-Fraumeni (C-LF) criteria if it contained the following:

(a) an index person with a sarcoma diagnosed before the age of 45 years,

(b) at least one first-degree relative of the index person with a cancer diagnosed before the age of 45 years, and

(c) a third family member who is a first- or second-degree relative of the index person and who has been diagnosed with cancer before the age of 45 years or has a sarcoma diagnosed at any age (2).

A family was considered to meet the Li-Fraumeni–like (LF-L) criteria if it contained the following:

(a) an index person with any childhood cancer or sarcoma, brain tumor, or adrenal cortical tumor diagnosed before the age of 45 years,

(b) a first- or second-degree relative with a typical Li-Fraumeni syndrome cancer (sarcoma, breast cancer, brain tumor, adrenal cortical tumor, or acute leukemia) diagnosed at any age, and

(c) an additional first- or second-degree relative with any cancer diagnosed before the age of 60 years (3).

Families were considered to meet the Li-Fraumeni criteria defined by Chompret and colleagues (LF-C; ref. 19) if any of the following were present:

(a) a proband affected by sarcoma, brain tumor, breast cancer, or adrenocortical carcinoma occurring before 36 years if they also have one or more first- or second-degree relatives with one of these cancers (but must be other than breast cancer if the proband has breast cancer) before 46 years of age, or if they also have a relative with multiple primary tumors at any age;

(b) a proband with multiple primary tumors, two of which are sarcoma, brain tumor, breast cancer, and/or adrenocortical cancer, with the initial cancer occurring before 36 years of age; or

(c) a proband with adrenocortical cancer.

**DNA isolation, DNA sequencing, copy number determination, and identification of sequence variations**

DNA was isolated from peripheral blood samples using a Qiagen spin column protocol as described by the
manufacturer. Approximately 50 ng of genomic DNA (estimated by agarose gel electrophoresis with ethidium bromide staining) were used in each long-range PCR reaction spanning four overlapping regions of the TP53 gene (reference sequence that is used by the IARC TP53 Mutation Database: HSP53G, i.e., X54156): the variable number tandem repeat (VNTR) region of intron 1 [nucleotide positions 8,658–9,076; exons 2 through exon 9 (nucleotide positions 11,633–14,847)], VNTR–exon 9 (8,658–14,847), and exons 10–11 (17,492–18,785; refs. 20, 21).

The mutational status of TP53 was examined by purifying and bidirectional DNA sequencing of the overlapping long-range PCR products in the following regions of the reference sequence: 8,658–9,076, 11,633–14,847, and 17,492–18,785. The sequence of the PCR and sequencing primers and the exact PCR conditions are available on request. Sequence electropherograms were quality inspected before alignment to the TP53 reference sequence with either the Sequencher v. 4.2 (Gene Codes) program or the program SeqScape v. 2.5 (Applied Biosystems, Inc.). The consequences of any sequence differences in the DNA samples were characterized by comparing deduced effects on coding and splicing with those documented effects in the IARC database (20, 21).

For 38 of the 93 DNA samples screened, the DNA sequence seemed to be homozygous in the regions of exons and introns 2–9 and 10–11 (i.e., no mixed bases were found anywhere in the amplicons in these regions). This could be due to either the two copies of the TP53 gene being identical or only one copy of the gene being amplified and sequenced.

To determine the cause of this apparent homozygosity, two tests were performed. First, the VNTR region (22, 23), which is frequently variable for the number of repeats (we found between 6 and 10 copies of the repeated sequence AAAAT) and for two single-nucleotide polymorphisms (T8759C and 8913–8914insAAG), was amplified and sequenced using primers we had designed. Those DNA samples that were heterozygous for one or more of these sequence variations were used to PCR amplify the VNTR–exon 9 region. Three regions were subsequently sequenced (the VNTR region and across the exon 2 forward and the exon 9 reverse primer sequences) to try to verify that the longer products were also heterozygous in the VNTR region. If these longer products were also heterozygous in the VNTR region and the sequences of the exon 2 and exon 9 primer regions were normal, this would show that both copies of the TP53 gene had been initially amplified and sequenced and that the case was homozygous in these regions of TP53. Of the 38 apparently homo-/hemizygous samples, 19 were verified to be homozygous.

The remaining 19 were tested for the presence of multi-exonic deletions or amplifications of TP53 by determining the copy number of different regions of the gene using the Multiplex Ligation-Dependent Probe Amplification (MLPA) assay of Schouten and colleagues (23) with their P056 kit (MRC-Holland). Individual peak areas were normalized by

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**Table 1. Outcome of TP53 and previous mutation screening**

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>TP53 mutation</th>
<th>Other mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nucleotide</td>
<td>Exon</td>
</tr>
<tr>
<td>VEO group *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>G13203A</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>7568-12905del</td>
<td>2–4</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
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<tr>
<td>39</td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EO-FH group †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>G12299A</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>14058delG</td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>T13240G</td>
<td>Intron 5</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
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</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
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</tbody>
</table>

*Breast cancer diagnosed before age 30 y (irrespective of family history).
†Breast cancer diagnosed at age 30 to 39 y, with two or more first- or second-degree relatives affected with breast and/or ovarian cancer (a strong family history).
dividing their area by the sum of the peak areas, and these fractions were compared with the corresponding normalized peak areas for the control human DNA sample. This control DNA (Roche) had been purified from pooled blood samples obtained from 80 to 100 individuals of both genders.

Definition of a mutation
A mutation was defined as a nucleotide change predicted to produce a nonfunctional protein, including one that affects mRNA splicing.

Statistical analysis
Prevalences and their 95% confidence intervals (95% CI) were estimated under binomial sampling theory using the “cii” command of STATA, release 8.2, StataCorp. 2003.

Results
Table 1 shows that, of the 52 tested cases in the VEO group, 2 (4%; 95% CI, 0.5–13%) had a TP53 mutation. Of the 42 tested cases in the EO-FH group, 3 (7%; 95% CI, 1.5–20%) had a TP53 mutation.

VEO group
(a) The case carrier had the missense mutation G13203A (exon 6, codon R175H), which changes the arginine codon to that for histidine.

She had been diagnosed with breast cancer at the age of 24 years. Pathology information was not available and no blood sample was available from family members. The only reported history was a cancer registry–verified throat cancer in a second-degree relative, diagnosed in their late 50s.

(b) The case carrier was identified by MLPA screening the samples potentially hemizygous for the TP53 region and shown to have lost one copy of exons 2, 3, and 4. Long-range PCR analysis verified that there was a large deletion in this region. DNA sequencing established that 5,383 bp (spanning base pairs 7,568–12,905 from intron 1 to intron 4, deleting exons 2–4) had been lost from one copy of TP53. This deletion, which does not seem to have been previously described, might have arisen by recombination between two copies of an 8-bp repeat in the two introns. One of the repeats lies within the Alu Sx element at 7,508–7,826 bp and the other repeat is not in an Alu element as judged by analysis with the program Repeat Masker (http://www.RepeatMasker.org).

She had been diagnosed at age 26 years. The family history included one lineage of a reported leukemia diagnosed in a first-degree relative in their late 30s and a probable stomach cancer in a third-degree relative. The other lineage included an intestinal cancer in a third-degree relative. There were a number of unaffected relatives, and no blood samples were available from family members.

EO-FH group
(a) The case carrier had a silent codon change of T125T in the last codon of exon 4 (G12299A) that has been shown to block the splicing excision of intron 4 from the TP53 transcript and thereby blocking TP53 synthesis by introduction of a stop codon (24).

She had been diagnosed with breast cancer at the age of 33 years. Her family cancer history was consistent with the LF-L and LF-C criteria. In the same lineage, two first-degree relatives had primary diagnoses of pediatric-onset brain cancer (both gliomas), one second-degree relative had adrenal and breast cancers, both diagnosed in their 30s, another had breast cancer diagnosed in her late 30s, another had breast cancer diagnosed in her late 60s, and a third-degree relative had pediatric-onset adrenal cancer. An obligate carrier in this lineage died at a young age, cancer-free. The other lineage included one first-degree relative with both leukemia and intestinal cancers in their 30s. All family cancers were cancer registry verified. No blood sample was available from family members.

(b) The case carrier had a deletion of a G from one of three G’s in exon 7 mutating codon 244. This mutation (14058delG) lies in the region encoding the DNA binding region of TP53 and gives rise to a reading frameshift that changes codon 246 to a stop.

She had been diagnosed with breast cancer at the age of 39 years with a reported family cancer history that included two other cases of breast cancer diagnosed in second-degree relatives before the age of 40 years in the same lineage. The obligate carrier had cancer registry–verified Hodgkin lymphoma diagnosed in their 30s. Also reported was a second-degree relative with throat cancer, a second-degree relative with pediatric-onset leukemia, and a third-degree relative with adult-onset nasal cavity cancer. None of these reports were verified. Blood samples were available from three relatives, none of whom were from the affected lineage, and none were found to carry the TP53 mutation.

(c) The case carrier had a heterozygous mutation T13240G of the invariant second base of intron 5 within the consensus splice donor site at the exon 5/intron 5 junction. This woman, her family, and this mutation have previously been reported (25). The authors have confirmed with us that there was a typographical error in their Fig. 1b, which stated that the variant was identified in exon 5/intron 5, but illustrated exon 6/intron 6 junction in the figure.6

She had been diagnosed with breast cancer at the age of 36 years. Her family history was consistent with the CL-F criteria. She had two first-degree relatives diagnosed with breast cancer in their 30s (one was cancer registry verified), two other first-degree relatives with verified pediatric osteosarcoma, and another first-degree relative with a verified pediatric sarcoma. There were two second-degree relatives reported to be diagnosed with breast cancer in their late 20s, two third-degree relatives with breast cancer diagnosed in their late 20s, and one third-degree relative with an intestinal cancer diagnosed before the age of 50 years. Blood samples were available for two unaffected first-degree relatives and both were found not to carry the mutation. A potential obligate carrier died cancer-free in their mid-30s.

8 Personal communication from G. Lindeman to M.C. Southey, April 11, 2008.
Discussion

In this study, we used a population-based series of cases and applied extensive direct sequencing to estimate the contribution of germline TP53 mutations to two subgroups of early-onset breast cancer likely enriched for genetic causes, and found that 5 of 94 tested cases were carriers (5%; 95% CI, 2–12%). That is, germline mutations in TP53 might play a larger role in breast cancer than previously thought. Our study also showed that, in the context of early-onset breast cancer, TP53 mutation carriers can be evident outside the traditional clinically defined Li-Fraumeni family syndrome.

Few studies have tested for germline TP53 mutations in women with breast cancer outside the context of pedigrees meeting Li-Fraumeni criteria (6, 7, 26), and only one using population-based sampling (27). For example, although Walsh and colleagues (28) selected a clinic-based series of 300 women with breast cancer and four or more relatives with breast or ovarian cancer, they tested for germline mutations in TP53 only for 31 families, with 10 meeting C-LF or LF-L criteria and 21 with at least two cases of breast cancer diagnosed before the age of 35 years. They found three carrier families, two with CL-F syndrome and one with LF-L syndrome. Similarly, Gonzalez and colleagues (29) tested for TP53 germline mutations in 525 women submitted for diagnostic testing without specifying the ascertainment criteria. They found that 95% of mutation carriers who had family history recorded met either the CL-F syndrome or the LF-C criteria. Due to their study design, however, neither of these studies can address the prevalence of mutations in women from families not meeting clinical CL-F, LF-L, or LF-C criteria.

On the other hand, and similar to our study, Lalloo and colleagues (27) tested 100 women from the United Kingdom with very early onset breast cancer diagnosed at age 30 years or younger, unselected for family history, and found a TP53 mutation prevalence of 4% (95% CI, 1.1–10%). Two case carriers met the study’s definition for “familial breast cancer” and both fulfilled the CL-F or LF-L criteria. The other two case carriers had no family history of breast or ovarian cancer and did not meet either the CL-F or the LF-L criteria.

The finding of TP53 mutation carriers outside of the definition of CL-F, LF-L, and LF-C syndrome might in part be due to more extensive TP53 mutation screening. Lalloo and colleagues (27) performed direct sequencing of 10 coding exons, splice site junctions, and the promoter region of TP53 (27). We conducted extensive sequencing of the TP53 region (=4,500 bp) with determination of whether the PCR products represented both copies of the gene.

Our findings are reliable because subjects were drawn from a population-based source with a thorough method for collecting family history. The ABCFS collection of family cancer history included asking relatives about their personal and family history. This enabled us to categorize pedigrees according to the C-LF, LF-L, and LF-C criteria using any affected individual as the “index” case, thereby increasing the likelihood of identifying pedigrees meeting the criteria. For each of the cases in our study with a germline TP53 mutation, four generations of pedigree information were available for review. Thus, we are confident that we did not under-categorize pedigrees into meeting Li-Fraumeni criteria.

Whereas our previous work had identified that 20% of these cases carried a disease-predisposing mutation in a known breast cancer susceptibility gene, we have now found that an additional 5% carried a pathogenic mutation in TP53. Therefore, almost as many cases in these targeted subgroups are caused by TP53 mutations as they are by mutations in BRCA2. Yet, even within the EO-FH group, less than 30% could be attributed to known mutations in BRCA1, BRCA2, CHEK2, ATM, or TP53. Thus, whereas TP53 might contribute to early-onset breast cancer more so than previously thought, a large proportion of early-onset breast cancer—even within multiple-case families—is due to genetic mutations in yet to be identified genes or mutations in known genes that current methods do not detect.

Our work and that of others suggest that TP53 mutation testing for subgroups of families with early-onset breast cancer might be warranted (27, 29). Future work could refine the use of clinical TP53 germline testing for pedigrees not fitting the current Li-Fraumeni criteria and incorporate factors such as age of onset of breast cancer and tumor histology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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