Identification of a Novel TP53 Cancer Susceptibility Mutation Through Whole-Genome Sequencing of a Patient With Therapy-Related AML

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Context The identification of patients with inherited cancer susceptibility syndromes facilitates early diagnosis, prevention, and treatment. However, in many cases of suspected cancer susceptibility, the family history is unclear and genetic testing of common cancer susceptibility genes is unrevealing.

Objective To apply whole-genome sequencing to a patient without any significant family history of cancer but with suspected increased cancer susceptibility because of multiple primary tumors to identify rare or novel germline variants in cancer susceptibility genes.

Design, Setting, and Participant Skin (normal) and bone marrow (leukemia) DNA were obtained from a patient with early-onset breast and ovarian cancer (negative for BRCA1 and BRCA2 mutations) and therapy-related acute myeloid leukemia (t-AML) and analyzed with the following: whole-genome sequencing using paired-end reads, single-nucleotide polymorphism (SNP) genotyping, RNA expression profiling, and spectral karyotyping.

Main Outcome Measures Structural variants, copy number alterations, single-nucleotide variants, and small insertions and deletions (indels) were detected and validated using the described platforms.

Results Whole-genome sequencing revealed a novel, heterozygous 3-kilobase deletion removing exons 7-9 of TP53 in the patient’s normal skin DNA, which was homozygous in the leukemia DNA as a result of uniparental disomy. In addition, a total of 28 validated somatic single-nucleotide variations or indels in coding genes, 8 somatic structural variants, and 12 somatic copy number alterations were detected in the patient’s leukemia genome.

Conclusion Whole-genome sequencing can identify novel, cryptic variants in cancer susceptibility genes in addition to providing unbiased information on the spectrum of mutations in a cancer genome.

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Cancer susceptibility is often suspected in individuals presenting with cancers at an early age, with multiple primary cancers, or with a suggestive family history. The identification of the genetic basis for cancer susceptibility has important clinical implications for the prevention and early detection of associated neoplasms. However, genetic testing is expensive, and in many cases, mutations in cancer susceptibility genes...
are not identified. This stems from both technical limitations of commercial assays and limited knowledge regarding the genes that contribute to cancer susceptibility. Thus, a comprehensive, unbiased approach to identify mutations in genes contributing to cancer susceptibility is needed. We describe the use of whole-genome sequencing to identify a novel deletion in \textit{TP53} (NCBI Entrez Gene 7157) in the normal (skin) genome of an individual with early-onset breast and ovarian cancer who subsequently developed therapy-related acute myeloid leukemia (t-AML).

**CASE HISTORY**

A 37-year-old woman presented with stage II estrogen receptor–positive, progesterone receptor–positive, Her2-positive breast cancer and was treated with surgery, local radiotherapy, and chemotherapy (cyclophosphamide, etoposide, and doxorubicin). At age 39 years, she was diagnosed with stage IIIC ovarian serous cystadenocarcinoma (FIGO grade III), and she was treated with surgery and chemotherapy (carboplatin and paclitaxel). Her ovarian cancer recurred at age 42 years, and she received additional chemotherapy. Six months later, she presented with t-AML. Analysis of her bone marrow revealed 76% blast cells with monocytic features and a complex karyotype involving monosomy 7, del(5q), and 2 marker chromosomes that could not be resolved by standard cytogenetic analysis (eFigure 1, available at http://www.jama.com). She developed respiratory failure and died 8 days after presentation. Of note, her family history did not suggest an inherited cancer susceptibility syndrome, as no first-degree relatives, including her parents, brother, and 3 children, had cancer. Nonetheless, the early onset of both breast and ovarian cancer prompted commercial testing for \textit{BRCA1} and \textit{BRCA2} mutations, the results of which were negative.

To identify genetic variants contributing to cancer susceptibility and leukemic transformation in this indi-

![Figure 1. TP53 Germline Deletion in Patient With t-AML](image-url)
We analyzed the leukemia (bone marrow) and normal (skin) genome in this patient. Specifically, bone marrow and skin biopsies were obtained after informed consent and analyzed in the following ways: whole-genome sequencing of leukemia and skin DNA, single-nucleotide polymorphism (SNP) genotyping to detect copy number alterations (deletions and amplifications) and uniparental disomy, RNA expression profiling on leukemia RNA to assess gene expression, and spectral karyotyping to assess chromosomal alterations.

**METHODS**

**Patient Samples**

All AML samples were obtained from a study at Washington University to identify genetic factors contributing to AML initiation and progression. The Washington University institutional review board approved these studies. After we obtained written informed consent from the patient, we collected a bone marrow sample. In addition, a 6-mm punch biopsy of the skin was obtained for analysis of the “normal” genome for the patient. In addition, a blood sample was obtained from the mother of the patient with t-AML. Samples were collected between October 2008 and July 2010.

**Library Generation, Sequence Production, and Data Analysis for Whole-Genome Sequencing**

Whole-genome sequencing of leukemia and skin DNA was performed on the Illumina platform (Illumina, San Diego, California) using paired-end reads with an average read length of 75 base pairs (bp). Library generation, sequence production, and data analysis for whole-genome sequencing were performed as previously described. All genomic coordinates are based on the NCBI36/hg18 assembly. Somatic mutations were identified using our in-house program glfSomatic (Washington University) and a modified version of the SAMtools indel caller (http://samtools

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**Figure 2. TP53 Messenger RNA Expression in Patient With t-AML and Controls**

**Figure 3. Immunostaining for TP53 of Bone Marrow Cells**

Bone marrow was obtained from (A) our patient with therapy-related acute myeloid leukemia (t-AML) and from (B) a healthy donor. Immunohistochemistry was performed using a monoclonal anti-p53 DO-7 antibody and a 3,3’ diaminobenzidine detection test. TP53 is indicated by a dark brown stain; counterstain, hematoxylin and a bluing reagent. Original magnification, ×40.
Putative mutations in gene coding regions and splice sites were manually reviewed and validated by 454 sequencing. The mutational spectrum analysis included all “uber” high-confidence single-nucleotide variant (SNV) calls in our patient’s t-AML leukemia genome, including some that were not validated by orthogonal sequencing methods. To minimize false positives, we increased the stringency of our somatic SNV calling algorithm. Specifically, we set the minimum mapping quality score (confidence in the mapping position) at 48 and the minimum somatic score (confidence that a SNV is a somatic mutation) at 55. These uber high-confidence SNV calls have a false-positive rate of less than 10%. All genetic variants identified in this study will be contributed to the database of Genotypes and Phenotypes (dbGaP accession number phs000159.v3.p2; http://www.ncbi.nlm.nih.gov/gap).

**Confirmation of TP53 Germline Deletion**

DNA was isolated from the patient’s skin and leukemic bone marrow and the peripheral blood of the patient’s mother, and polymerase chain reaction (PCR) was performed using Platinum Taq and the PCRx enhancer system (Invitrogen, Carlsbad, California). Refer to the eMethods for primer sequences and PCR conditions.

**RNA Expression Profiling**

RNA (approximately 1 µg) isolated from the bone marrow of our t-AML patient or each of 6 patients with de novo AML (all >70% blast cells) was analyzed using the Affymetrix Exon 1.0 array (Affymetrix, Santa Clara, California) according to the manufacturer’s instructions. These 6 control samples were previously subjected to whole-genome sequencing and lack any mutation in TP53.

**Confirmation of Mutant TP53, DGKG-BST1, and BST1-DGKG**

Normal and t-AML patient bone marrow complementary DNA (cDNA) was generated using the SuperScript III First-Strand Synthesis System for reverse-transcription (RT) PCR (Invitrogen), following the manufacturer’s instructions and using oligo (dT)20 primers. cDNA was then amplified using Titanium Taq (Clontech, Mountain View, California), visualized on a 1.5% agarose gel and purified using the Wizard SV gel and PCR cleanup system (Promega, Madison, Wisconsin). Products were cloned into the pCR 2.1-TOPO vector using the TOPO TA cloning kit (Invitrogen) and sequenced using the ABI Big Dye sequencing system (Applied Biosystems, Foster City, California) using the M13 forward and reverse primers. Refer to the eMethods for primer sequences.

**TP53 Immunohistochemistry**

TP53 immunohistochemistry was performed on the t-AML patient’s leukemic bone marrow cells and normal control bone marrow cells as previously described, using the monoclonal anti-p53 DO-7 antibody.

![Figure 4. Transcriptional Activity of Mutant TP53](image-url)
recognizing an epitope from amino acids 1 to 45 (Dako, Carpenteria, California). Briefly, cells were fixed in formalin, washed and resuspended in Histogel (Thermo Scientific, Waltham, Massachusetts), embedded in paraffin, cut to 4-µM sections, deparaffinized, and treated with heat-induced epitope retrieval with CC1 buffer (Ventana, Tucson, Arizona). Sections were then incubated with the DO-7 antibody at a 1:50 dilution for 32 minutes at 37°C, followed by detection using the iVIEW DAB detection kit (Ventana), producing a dark brown precipitate. Slides were counterstained with hematoxylin and bluing reagent.

**TP53 Reporter Studies**

The human osteosarcoma cell line SaOS2 was transfected in duplicate with 250-ng wild-type TP53-responsive p50-2Luc promoter-reporter4 and 100 ng or 1 µg of an expression vector with the cytomegalovirus-promoter driving expression of neomycin alone, wild-type TP53, t-AML mutant TP53, or hotspot DNA binding mutant p53-R175H as previously described.3 After 48 hours, the cells were collected and lysed in passive lysis buffer (Promega). Protein levels were quantified and normalized using the Bradford protein assay, and relative light units were determined by the Dual Luciferase Assay kit (Promega) according to the manufacturer's protocol.

To determine the expression levels of exogenous wild-type and mutant TP53, total cellular proteins (12 mg/sample) from the luciferase assay lysates were separated on 4% to 12% precast Nupage gels (Invitrogen), transferred to nitrocellulose filters, and probed with sheep polyclonal anti-p53 AB7 antibody (Calbiochem, San Diego, California) and mouse monoclonal /H9252-actin antibody (Sigma-Aldrich, St Louis, Missouri) as previously described.4 Secondary antibodies included sheep antimouse IgG horseradish peroxidase (GE Healthcare, Little Chalfont, United Kingdom) and rabbit antisheep IgG horseradish peroxidase. Proteins were detected using SuperSignal West Dura Extended substrate (Pierce, Rockford, Illinois).

Refer to the eMethods for reagents and methods for spectral karyotyping and fluorescence in situ hybridization analyses and for NCBI (National Center for Biotechnology Information) Entrez Gene identification numbers of all genes described in the article.

**RESULTS**

For the leukemia DNA, a total of 115 billion bases of sequence were obtained (28.7X haploid coverage), and for the skin DNA, a total of 114.6 billion bases of sequence were obtained (29.9X haploid coverage). To assess se-
sequence coverage, we determined whether SNPs identified in the genome of this patient using SNP arrays were detected by sequencing. In the leukemia and skin genomes, 96.2% and 97.8% of heterozygous SNPs were detected by sequencing, respectively, demonstrating adequate sequence coverage of these genomes.

Analysis of the sequence of the skin genome of this individual identified no SNVs or small insertion/deletions (indels) in common cancer susceptibility genes, including BRCA1, BRCA2, TP53, PTEN, CHEK2, CDH1, BRIPI (FANCJ), PALB2 (FANCN), STK11, and ATM. However, a 3-kilobase heterozygous deletion of TP53, encompassing exons 7-9, was detected in the skin genome (Figure 1A). Sequence analysis of leukemia DNA revealed a 17.6-megabase region of uniparental disomy on chromosome 17 (eFigure 2) that resulted in homozygous deletion of exons 7-9 of TP53 in the leukemia genome (Figure 1A). This particular mutation has not been previously reported in the Database of Genomic Variants (http://projects.tcag.ca/variation) or the International Agency for Research on Cancer TP53 database. To determine if this was an inherited or de novo mutation, we obtained blood from the patient’s mother to look for the mutation, we obtained blood from the patient’s mother to look for the

46,XX,der(3)ins(3;4)(q26.2; q13.3;q31.1)ins(3;3)(q26.2; q27q12), der(3)ins(3;3)(q26.2; q27q12), der(4)ins(3;4)(q26.2;q13.3;31.1)ins(3;4)(q26.2;p12), der(5)del(5)(p13.3q34);del(12)(q34;p12.3).idem,−(r7)p11.2q11.29)

Boxes indicate chromosomal rearrangements.

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(purine→pyrimidine change) was similar in the t-AML patient sample and the 2 AML samples (FIGURE 5A). A total of 26 validated somatic SNVs and 2 indels were detected in coding genes (Figure 5B and eTable 1). None of these genes (other than NUP98, which is frequently involved in translocations) has previously been reported to be mutated in either de novo AML or t-AML. Moreover, none of these mutations were detected by sequencing an additional 93 bone marrow samples from patients with t-AML. We identified 12 copy number alterations by sequencing that included, in addition to monosomy 7 and del(5q), small deletions and an amplification not detected by standard karyotyping (eTable 2).

Previous studies reported the association of germline TP53 mutations with complex chromosomal alterations in t-AML, most notably abnormalities of 5q. Consistent with these observations, spectral karyotyping revealed several complex chromosomal rearrangements in the leukemia genome (FIGURE 6). Through whole-genome sequencing, we further characterized these rearrangements and defined the break points. In total, we identified 8 novel chromosomal translocations (Figure 6 and eTable 3). Among them is a reciprocal translocation between chromosomes 3 and 4, resulting in the fusion of the genes diacylglycerol kinase gamma (DGKG) and bone marrow stromal antigen 1 (BST1). Transcripts for both the DGKG-BST1 and BST1-DGKG fusions were identified in the patient’s bone marrow RNA (FIGURE 7). The functional significance of this translocation is under investigation. This translocation was not detected in an additional 20 t-AML samples, as assessed by RT-PCR or fluorescence in situ hybridization (eFigure 3). Of note, it is likely that only a minority of the somatic mutations detected in this leukemia genome contributed to transformation. The identification of the “driver” mutations will require sequencing of a much larger set of t-AML genomes to look for recurrent mutations.

**COMMENT**

This case highlights the utility of whole-genome sequencing to identify clinically relevant germline genetic abnormalities contributing to cancer susceptibility. In this patient with early-onset breast and ovarian cancer, commercial testing for BRCA1 and BRCA2 mutations had negative results. Although germline TP53 mutations are associated with early-onset breast cancer, they account for only about 5% of such cases, and the family history in our patient did not meet either classic or Chompret-modified criteria for Li-Fraumeni syndrome. This syndrome (LFS; Online Mendelian Inheritance in Man [OMIM] #151623) is characterized by early-onset sarcomas; adrenocortical carcinoma; brain tumors; breast cancer; and, less commonly, leukemia. However, t-AML is a rare but well-described complication of chemotherapy. Thus, clinical suspicion for
Li-Fraumeni syndrome was low in this case, and commercial testing for TP53 mutations was not performed. Whole-genome sequencing has at least 2 key advantages over candidate gene sequencing to identify mutations of cancer susceptibility genes. First, it provides a comprehensive and nonbiased approach to mutation detection. A review of the literature identified genetic variants in nearly 100 genes that are associated with cancer susceptibility.19 Conventional sequencing of each of these genes is cost-prohibitive and would not identify mutations in novel cancer susceptibility genes. For example, the current cost to comprehensively sequence BRCA1 and BRCA2 alone is approximately $4000. On the other hand, the cost to sequence a human genome is rapidly falling, with a current “fully loaded” cost estimate of $20 000 per genome. Second, whole-genome sequencing using paired-end reads is able to detect structural variants (eg, deletions, amplification, inversions, and translocations) that are typically missed by conventional sequencing. For example, in families at high risk of breast cancer, sophisticated molecular analyses were able to detect genomic rearrangements of BRCA1 or BRCA2 in 12% of cases that tested negative (wild type) for mutations by conventional sequencing.20

Based on the family history and genotyping of the patient’s mother, we suspect that the TP53 deletion arose spontaneously in our patient. Although reports of de novo TP53 mutations are uncommon, a recent study found de novo TP53 mutations in at least 7% of 341 patients with early-onset cancer, suggesting they may be more common than previously thought.21 Furthermore, while other germline cancer susceptibility mutations may have been present in this patient, the TP53 mutation, which lacks DNA binding ability, very likely contributed to her cancer susceptibility. Indeed, mutations that disrupt the TP53 DNA binding domain are common in Li-Fraumeni syndrome.6 We do not currently know the significance of the majority of the normal skin genome alterations identified through whole-genome sequencing, which include approximately 10 000 nonsynonymous SNPs per genome. However, as additional genomes are sequenced, the database of rare germline SNPs will expand. This should facilitate the identification of novel cancer susceptibility gene mutations.

The finding of the germline TP53 mutation has important clinical implications for the patient’s 3 children. Carriers of a deleterious TP53 germline mutation have an approximately 90% lifetime risk of developing cancer, with about 50% of such individuals presenting before the age of 40 years.22 Furthermore, a recent study shows that magnetic resonance image screening of individuals with Li-Fraumeni syndrome identifies asymptomatic and potentially treatable cancers.23 Anticipating that clinically relevant information would be obtained by whole-genome sequencing, we built into our informed-consent document a method to communicate clinically relevant information to family members. Specifically, the researchers, in consultation with a clinical geneticist, determined that the TP53 mutation was of potential clinical importance to the patient’s surviving family members. The treating physician then contacted the next of kin to inform them that a clinically significant mutation was identified and encouraged a meeting with a genetic counselor for education and consideration of genetic testing for at-risk family members.

In summary, there is a need for a comprehensive, accurate, and cost-effective method to identify genetic variants contributing to cancer susceptibility in individuals with a suggestive history, because this information has important potential implications for the prevention and early diagnosis of associated cancers. Whole-genome sequencing circumvents the limitations of conventional candidate gene testing by providing an unbiased survey of the genome and the ability to detect structural variants that are often missed with conventional assays. As the cost of whole-genome sequencing decreases, it may become a preferred method to identify variants in cancer susceptibility genes.

Author Contributions: Dr Link had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Dr Link, Schueettelz, and Shen contributed equally to this work.

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The eFigures, eTables and eMethods are available at http://www.jama.com.

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