Identification of *TP53* as an Acute Lymphocytic Leukemia Susceptibility Gene Through Exome Sequencing

Bradford C. Powell, MD, PhD[1], Lichun Jiang, PhD[2], Donna M. Muzny[2], Lisa R. Treviño, PhD[2], ZoAnn E. Dreyer, MD[3,4], Louise C. Strong, MD[5], David A. Wheeler, PhD[2], Richard A. Gibbs, PhD[1,2], and Sharon E. Plon, MD, PhD[1,2,3,4]

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX

[2]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX

[3]Department of Pediatrics, Baylor College of Medicine, Houston, TX

[4]Texas Children's Cancer Center, Texas Children's Hospital, Houston, TX

[5]Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, TX

Abstract

Although acute lymphocytic leukemia (ALL) is the most common childhood cancer, genetic predisposition to ALL remains poorly understood. Whole-exome sequencing was performed in an extended kindred in which five individuals had been diagnosed with leukemia. Analysis revealed a nonsense variant of *TP53* which has been previously reported in families with sarcomas and other typical Li Fraumeni syndrome-associated cancers but never in a familial leukemia kindred. This unexpected finding enabled identification of an appropriate sibling bone marrow donor and illustrates that exome sequencing will reveal atypical clinical presentations of even well-studied genes.

Keywords

exome sequencing; acute lymphocytic leukemia; genetic predisposition to disease; genetic testing

INTRODUCTION

Molecular diagnosis for cancer susceptibility typically proceeds in a sequential process starting with gene or genes most consistent with the clinical presentation. This process is effective if there is a small set of implicated genes, but is expensive and time consuming for families with atypical presentation or a large number of possible causative genes. The cost of genome-scale sequencing has decreased so that it can be effectively used in clinical practice and potentially obviate these problems.

A Hispanic kindred (pedigree in Fig. 1) was first referred for genetic evaluation when patient 1 (III-7) was diagnosed with B cell ALL as a teenager. Family history included a prior sibling who died from childhood ALL (individual III-11) and her deceased father with

Corresponding Author: Sharon E. Plon (splon@bcm.edu), Feigin Center, Suite 1200, 1102 Bates Street, Houston, TX 77030-2399, Tel: (832) 824-4539, Fax: (832) 825-4276.

leukemia diagnosed at age 51. Patient 1's older brother had recently been diagnosed with a thoracic mass subsequently reported to be an esophageal carcinoma. Patient 1's leukemia was refractory to chemotherapy, and she died from recurrent disease 51 days after peripheral blood stem cell transplant (PBSCT). Eight years later, patient 2 (individual IV-2), a 13 year old paternal cousin once removed of patient 1, presented with a diagnosis of pre-B cell ALL. Bone marrow cytogenetics revealed multiple hyperdiploid clones apparently secondary to doubling of a hypodiploid clone which is highly associated with early treatment failure [1]. PBSCT was recommended. The family contains five individuals with leukemia (three documented to be childhood ALL) with only one solid tumor reported, consistent with autosomal dominant familial leukemia. Thus, it was recommended not to use a sibling donor unless testing for the familial ALL mutation was possible.

Li Fraumeni syndrome (OMIM: 151623) was considered, but *TP53* testing was not pursued because of the absence of LFS associated cancers (sarcomas, breast cancer, brain tumors or adrenal tumors) as well as prior studies suggesting that *TP53* mutations did not cause familial ALL or lymphoma [2–4]. A search for an unrelated donor was unsuccessful, making identification of the specific familial mutation critical to further therapy.

METHODS

All subjects were enrolled under a protocol approved by Institutional Review Board of the Baylor College of Medicine which includes specific language permitting whole exome sequencing and reporting of clinically-relevant results. Whole exome sequencing was performed on (1) DNA from remission blood or lymphoblastoid cell lines from patient 1 and 2 and Patient 1's mother (a married-in familial control) and (2) leukemia bone marrow diagnostic sample from patient 2.

Exome sequencing was performed using a capture platform, Vcrome 2.1, comprising 42 megabases of target sequences including coding sequences from VEGA [5], CCDS [6] and RefSeq [7] genesets, followed by sequencing on Illumina HiSeq with 100 bp paired-end reads. Reads were aligned to the hg19 human reference genome using BWA 0.5.9 [8] with realignment around indel sites using the GATK 1.0.5336 [9]. Variants were called using Atlas2 [10]. Analysis focused on rare or novel sequence variants (with population minor allele frequency undefined or less than or equal to 1% in both Thousand Genomes whole-genome phase 1 data and the NIEHS Environmental Genome Project).

RESULTS

Analysis of each individual demonstrated approximately 2400 rare variant alleles of which 700 were predicted to affect protein sequence through missense, frameshift, nonsense, or codon insertion/deletion events (Fig. 2A). The union of rare variants affecting protein sequence in the two affected individuals comprised 201 variants. Subtraction of the rare variants from the unaffected familial control resulted in a final set of 72 heterozygous rare variants for further consideration. Strikingly, this process identified (Fig. 2B) a nonsense variant of *TP53* (c.916C>T; p.R306X) which was confirmed in patient 2 from an independent remission blood sample by Sanger sequencing in a clinical molecular diagnostic laboratory. This information was then used to determine that his HLA matched brother was negative for the *TP53* mutation. A bone marrow transplant with his brother as donor was initiated. Another brother, age 5, was positive for the mutation and, along with his mother who is an obligate carrier, is undergoing cancer surveillance [11]. Exome sequencing of Patient 2's leukemic bone marrow revealed the p.R306X germline mutation and a second somatic p.T118A *TP53* mutation which is a rare variant in a conserved DNA binding domain consistent with a second hit in this tumor suppressor gene [12].

DISCUSSION

The highly aneuploid nature of the leukemias seen in this family as well as the high mortality in this kindred (four of the five individuals have died and patient 2 has an extremely high-risk form of childhood ALL) is consistent with that reported for leukemia containing somatic *TP53* mutations [13]. *TP53* mutations may be a rare cause of familial leukemia. In addition to prior negative *TP53* studies in familial leukemia/lymphoma [2–4], our own exome and deletion analysis of five other small familial ALL kindreds did not demonstrate other *TP53* constitutional mutations.

Conversely, the family's pattern of cancer does not include any of the characteristic LFS tumors (sarcomas, brain tumors, breast cancers and adrenocortical carcinomas) and does not meet the Chompret or other criteria used to decide on *TP53* testing [14, 15]. Review of a large cohort of *TP53* mutation positive extended kindreds (n=107) revealed only a single family with multiple cases of ALL (two sisters from a kindred with other typical LFS tumors [16]), further demonstrating that familial ALL is not typically associated with *TP53* mutations. The predisposition to leukemia does not appear to be related to this specific p.R306X *TP53* mutation. In the International Agency for Research on Cancer's locus-specific database of *TP53* mutations (version R15, November 2010 [17]), this mutation has been described in both germline and somatic samples. Among the five p.R306X germline families with 11 affected individuals, there is the typical LFS pattern with breast (4), soft-tissue (3), nasopharynx (1), brain (1), unknown site (1) malignancies and only one hematopoetic cancer. There are 150 somatic p.R306X mutation reports, again with a typical tumor pattern and only one somatic mutation each of acute myelogenous leukemia, precursor cell leukemia NOS and mantle cell lymphoma. Therefore, in addition to the known *MDM2* [18] promoter SNP309T>G (rs2279744) and *TP53* R72P (rs1042522) polymorphisms [19] associated with age of tumor onset in *TP53* mutation carriers, the atypical pattern of leukemia in this family suggests the possibility of a rare leukemia modifier cosegregating with the *TP53* mutation. Supplementary Table 1 provides the other rare sequence variants that cosegregate with leukemia, although none obviously participate in the p53 pathway. However, verification of a leukemia modifier will require analysis of other rare *TP53* mutation kindreds with this unusual ALL predisposition. We also cannot exclude that the leukemia phenotype could result from interactions with epigenetic or environmental exposures. In summary, identification of the *TP53* mutation in this kindred through exome sequencing provided clinically actionable information including identification of (1) the appropriate sibling donor for transplant, (2) a young sibling at risk for cancer requiring cancer surveillance, (3) the mother of patient 2, an obligate *TP53* mutation carrier who requires early, intensive breast cancer surveillance and (4) two other mutation negative siblings not at increased cancer risk. As exome analysis enters the clinical arena we can anticipate similar unexpected findings to broaden our understanding of the clinical spectrum of mutations in many previously well-studied genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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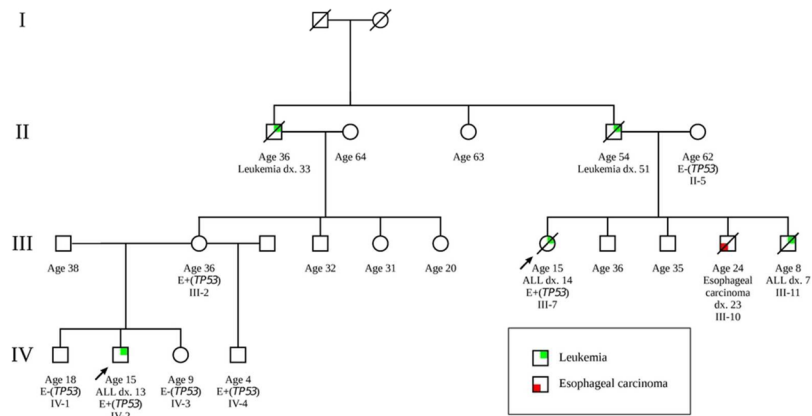
Figure 1.
Clinical pedigree of the individuals involved in this study. Arrows indicate consultands who presented for genetic evaluation and "dx." indicates age at cancer diagnosis. This kindred includes five individuals who had developed leukemia with only one other individual being affected with esophageal carcinoma. Exome sequence analysis was performed the affected individuals III-7 (patient 1) and IV-2 (patient 2) as well as the familial control (mother of patient 1; individual II-5) who did not transmit the cancer risk. Targeted analysis for the *TP53* familial mutation by Sanger sequencing subsequently revealed that individual IV-4 carries the mutation (E+(*TP53*)) but individuals IV-1 and IV-3 do not (E−(*TP53*)).
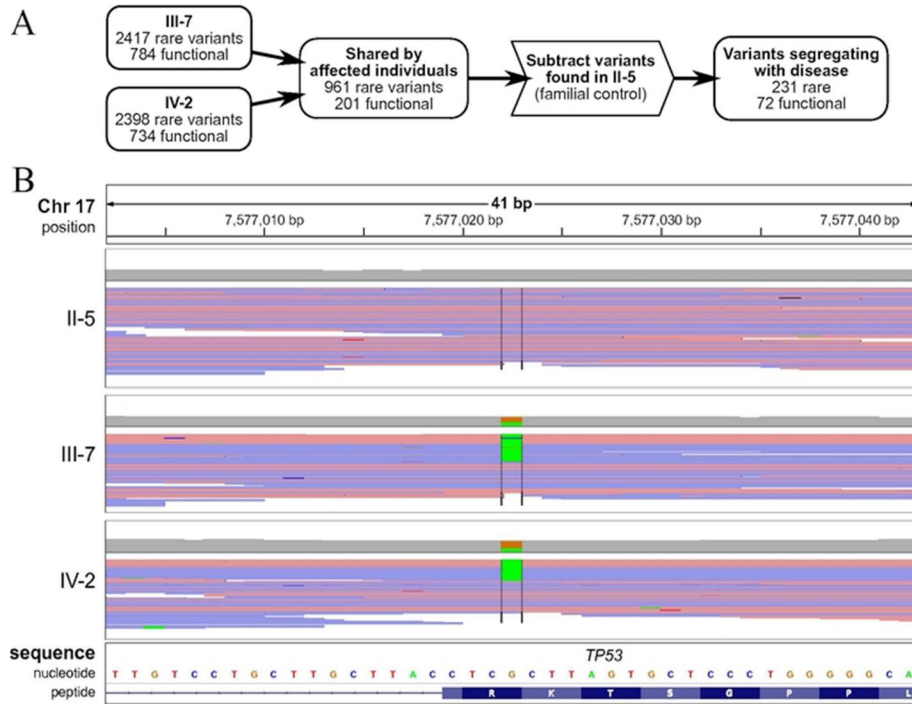
Figure 2.
The heterozygous *TP53* mutation c.916C>T was present in the two affected individuals but not in the nontransmitting parent. (A) Germline variants were filtered by familial relationships, rarity (allele frequency <= 0.01 in 1000 Genomes Project data and NIEHS Environmental Genome Project) and potential for functional change to protein sequence (missense, nonsense, frameshift, codon insertion/deletion or splice-altering). 231 rare variants (72 predicted to change protein sequence) were present in both affected individuals but not in the familial control. Further details regarding these variants are given in Supplementary Table 1. (B) The variant c.916C>T was present in two patients but not in the familial control through whole exome sequencing. Sequence reads near this mutation are displayed using the Integrative Genomics Viewer, with reads colored by strand (red and blue for positive and negative strand, respectively). The view is centered at cDNA position 916 of *TP53* and variant (non-reference) base calls are highlighted for each subject.