Li-Fraumeni versus Pseudo-Li-Fraumeni Syndrome: Key Insights for Interpreting Next-Generation Sequencing Reports in Patients with Suspected Cancer Predisposition Syndromes

STEVEN SORSCHER,a RODWIGE DESNOYERS,a KAREN OUYANG,b SHAKTI RAMKISSOONc

aOncology Division, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA; bKaren Ouyang, Cancer Genetics and Hematology, Clinical Molecular Geneticist and Cytogeneticist Invitae Corporation, San Francisco, California, USA; cShakti Ramkissoon, Foundation Medicine, Inc., Cambridge, Massachusetts, USA

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INTRODUCTION

Next-generation sequencing (NGS) of tumors and now circulating cell-free DNA is increasingly used to identify “actionable” genetic abnormalities. Detecting abnormalities offers opportunities for physicians and patients to qualify for therapeutic strategies that target the oncogenic lesions identified by NGS. However, NGS tumor-testing companies are not currently licensed to report germline alterations because the assays are only validated to report somatic mutations.

Li-Fraumeni Syndrome (LFS) is an inherited cancer syndrome classically associated with germline TP53 mutations. The resulting cancers vary among patients but include early-onset colorectal cancer (CRC) [1, 2].

Here, we report a CRC patient with a TP53 germline mutation initially considered somatic because no TP53 germline mutation was noted in the liquid biopsy (cell-free DNA) NGS assay. Upon obtaining the unreported mutation allelic frequencies (MAFs) in the germline and liquid biopsy assays, it appeared that this patient harbors a germline TP53 mutation (LFS), rather than “Pseudo-Li-Fraumeni,” as a result of a hematopoietic progenitor cell somatic (acquired) mutation. Although MAF is routinely analyzed as part of NGS sequencing (germline or biopsies), such testing is not considered validated and other methods remain more definitive for distinguishing between germline and somatic mutations.

Case Report

A.W. is a 48-year-old man who underwent a low anterior resection for a colorectal adenocarcinoma in February 2016. Computed tomography (CT) scanning revealed liver metastases and mesenteric adenopathy. The patient’s peripheral blood smear was normal. The patient’s father had acute myelogenous leukemia at age 36 and his maternal grandmother’s brother had breast cancer (age unknown). A maternal aunt had ovarian cancer around age 45.

Because of A.W.’s personal and family cancer history, germline testing was done. Invitae Common Hereditary Cancer Panel Testing (Invitae Corporation, San Francisco, CA, https://www.invitae.com/en/) reported a TP53 c.731G>A (p.Gly244Asp) variant, which was designated a “likely pathogenic variant” and associated with autosomal dominant LFS. The Invitae report also raised the possibility that the TP53 mutation could instead “be somatic (acquired) in nature.” However, an unreported but later obtained MAF of 47% was far more consistent with a germline mutation (Invitae Corporation, San Francisco, California).

Later, after progression of his cancer on standard therapies, A.W. chose liquid biopsy NGS with the hope of identifying an actionable molecular abnormality that might suggest further therapeutic options. The Guardant360 liquid tumor biopsy testing (Guardant Health, Redwood City, CA, http://www.guar-danthealth.com/) reported an APC MAF of 8% but no somatic TP53 mutation (i.e., <0.2%); however, a germline plus somatic TP53 MAF of 53% was detected but not reported due to restrictions in germline variant reporting.

DISCUSSION

LFS is an inherited cancer syndrome classically caused by a germline TP53 mutation and subjects patients to a higher risk for developing brain tumors, breast cancers, leukemia, adrenocortical cancers, and, to a lesser extent, CRC. In one large reported cohort of patients with CRC, 1.3% harbored a TP53 germline mutation [1, 2]. TP53 loss of heterozygosity (LOH) in the cancer cells, resulting in no expression of functional TP53, is the presumed mechanism of transformation from normal to malignant cells.

However, somatic TP53 mutations in normal hematopoietic progenitor cells have been well described as well. For example, in a series of over 17,000 subjects who had no hematologic malignancies, 33 TP53 variants were identified. The authors concluded that the somatic mutations (including TP53) “markedly” increased the risk of developing hematologic malignancies, although the absolute risk remained quite small (0.5%...
per year) [3]. In a separate series of over 12,000 Swedish participants, the authors concluded “a subset of the genes that are mutated in patients with myeloid cancers is frequently mutated in apparently healthy persons. These mutations may represent characteristic early events in the development of hematologic cancers,” and their series again included subjects with mutated TP53 [4]. As expected in these healthy subjects, MAF was very low when a mutated TP53 was discovered [3, 4]. Remarkably, one subject with a particularly high TP53 MAF (24%) developed acute leukemia 34 months later and at that time had a TP53 MAF of 86% [4].

In our case presented here, the unreported 47% germline TP53 MAF together with the normal peripheral blood smear strongly suggest a germline mutation (LFS). The unreported 53% (germline plus somatic) TP53 MAF from the cell-free DNA likely represents a small percentage of cancer-derived DNA plus a far larger TP53 MAF from normal cell-derived cell-free DNA. This can be deduced from the 8% APC MAF presumably contributed only from the cell-free DNA of colon cancer cells. Guardant360 is not licensed or validated to report mutations considered to be germline, and therefore the report noted a TP53 MAF of ≤ 0.2%. Given the 53% TP53 MAF in the liquid biopsy (cell-free DNA), the 47% TP53 from germline testing, and APC 8% MAF, this patient’s colon cancer is less likely sporadic and more likely LFS-related. The accuracy of MAF could be affected by a variety of factors, including the use or absence of molecular barcodes or indexing [5]. Rather than using the non-validated MAFs, testing of nonhematopoietic normal tissue could be used to more definitively distinguish hematopoietic somatic from germline mutations. If the mutation in question is not identified, this result would support the diagnosis of a hematopoietic progenitor cell somatic mutation.

Because NGS tumor-testing companies are not validated or licensed to report germline mutations, there are profound clinical consequences. In some instances, MAF of 80% in BRCA1 from a liquid biopsy may not be reported, as it would be deemed to be germline. Without that unreported MAF physicians would be unaware of this potentially actionable abnormality (e.g., PARP inhibitor therapy might be effective for patients with BRCA1 LOH in tumor cells) and also unaware that such a patient almost certainly harbors a germline BRCA1 mutation. On the other hand, if the unreported MAFs suggest, for example, a somatic hematologic progenitor cell TP53 mutation, the patient could be presumed to be at increased risk of leukemia [3, 4].

While it remains possible that the observed TP53 mutation seen in the germline testing in A.W. is in fact somatic in the hematologic progenitor cells rather than germline, the unreported approximately 50% TP53 MAF seen in germline testing and the unreported 53% TP53 MAF in the liquid biopsy imply that the TP53 mutation in this case represents a germline mutation. Since next-generation sequencing of malignancies and germline testing for inherited cancer-causing genes have become nearly routine for patients with cancer, discovering this example of inconsistent results should raise the awareness and importance of considering obtaining unreported results to verify and understand the complete significance of all NGS results. If an LFS associated TP53 mutation is determined to be somatic, one must consider the patient at increased risk for the development of hematologic malignancy [3, 4].

**CONCLUSION**

In summary, this case raises key challenges in interpretation and limitations of currently U.S. Food and Drug Administration-approved and -validated germline and liquid biopsy assays. Our patient’s history raised the possibility of a hereditary cancer syndrome, but because of reports of somatic TP53 mutations, the company performing the germline testing also reported the possibility that this particular TP53 mutation could be somatic rather than germline.

Later, in order to identify potentially actionable molecular abnormalities, NGS of a liquid biopsy was obtained. The relatively high MAF in both the germline and liquid biopsy assays were consistent with the patient being of a Li-Fraumeni family and consistent with the CRC developing as a consequence of LFS. In this case, the MAF was helpful in clarifying the likely etiology of the TP53 mutation in the germline testing and why there was no reported TP53 mutation in the liquid biopsy. However, until MAF results are validated, we believe MAF results should not be routinely used to distinguish whether mutations identified with germline testing are germline or somatic in etiology. In determining whether a mutation is germline or somatic in hematopoietic cells, one could consider testing nonhematopoietic normal tissue.

**AUTHOR CONTRIBUTIONS**

Conception/Design: Steven Sorscher, Rodwige Desnoyers, Karen Ouyang, Shakti Ramkisson

Provision of study material or patients: Rodwige Desnoyers

Collection and/or assembly of data: Rodwige Desnoyers, Karen Ouyang

Data analysis and interpretation: Steven Sorscher, Shakti Ramkisson

Manuscript writing: Steven Sorscher, Karen Ouyang, Shakti Ramkisson

Final approval of manuscript: Steven Sorscher, Rodwige Desnoyers, Karen Ouyang, Shakti Ramkisson

**DISCLOSURES**

Karen Ouyang: Invitae Corporation (E, OI); Shakti Ramkisson: Foundation Medicine (E, OI). The other authors indicated no financial relationships.

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