

The differential diagnosis of a *TP53* genetic testing result

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The Li-Fraumeni cancer predisposition syndrome (LFS, MIM 151623) was originally described as an autosomal dominantly inherited syndrome characterized by childhood sarcomas and multiple other cancer types and is often due to germ-line mutations in the *TP53* gene.¹ Classic LFS diagnostic criteria based on the early-onset sarcoma phenotype are over 90% specific for *TP53* mutations; however, sensitivity is only 40%.² Therefore, more expansive criteria (Chompret criteria) were developed to predict the presence of a germ-line *TP53* mutation, including diagnosis of a “core cancer” (sarcoma, premenopausal breast, brain, leukemia, or adrenocortical carcinoma) and/or multiple primary malignancies and/or family members with early-onset cancer.³ These criteria are over 90% sensitive for *TP53* mutations, but specificity is low, ranging from 15 to 52%,² probably owing to locus heterogeneity, a broader phenotypic spectrum associated with *TP53* mutations than originally appreciated, and the existence of de novo mutations. In view of the phenotypic heterogeneity associated with germ-line *TP53* mutations, *TP53* is found on nearly all multiplex panels in current clinical use for cancer predisposition evaluation. There are many challenges in interpreting a *TP53* mutation in this current era of multiplex panel testing, given the low pretest probability of a mutation in the majority of individuals being tested, the preponderance of missense mutations in *TP53* with variable support of pathogenicity, and the potential for low or incompletely penetrant *TP53* mutations.

Recently, germ-line *TP53* mutation testing has become further complicated by the increasing recognition that massively parallel sequencing (MPS) detects mutations in *TP53*—among other genes—in circulating blood cells at allelic fractions inconsistent with a heterozygous or homozygous mutation.⁴ These so-called “mosaic mutations” have always existed;⁵ however, traditional Sanger sequencing-based genetic testing probably missed the vast majority of cases. Although rare, mosaic *TP53* results are creating significant clinical conundrums as the interpretation of the etiology of these results has differing clinical implications. In this issue of *Genetics in Medicine*, Weitzel and colleagues analyze data from a clinical testing laboratory and use ancillary testing to address the important clinical question of the frequency and possible etiologies of mosaic *TP53* results.⁶

In their study, Weitzel and colleagues show that of 353 *TP53* likely pathogenic or pathogenic mutation results identified by MPS-based multiplex panel or single-site testing, 72 results (20%) were found at less than 25% allelic fraction in peripheral blood samples. They went on to evaluate these 72 cases. Three cases were found in patients with overt hematological malignancy (myelodysplastic syndrome or chronic lymphocytic leukemia), an important reminder that blood is not an appropriate specimen for genetic testing in patients with hematological malignancies. Ancillary testing, defined as site-specific mutation testing of the identified *TP53* mutation in another tissue (skin fibroblasts, eyebrow pluck, or other tissue) or testing of a family member was performed for 35 of the remaining 69 cases. Three of these 35 mutations were reported to be “germ line” (two by positive mutation testing in family members and one by a positive fibroblast result). The other thirty-two cases with ancillary testing included 18 with negative tissue testing and 14 with negative testing of one or more family member(s). These cases were presumed by the authors to be due to an abnormal clonal expansion (ACE) in the blood compartment, which the authors also termed “somatic interference.” Because the remaining 34 cases were seen to have clinical characteristics similar to those of the 32 cases with ancillary testing, the authors concluded that these 34 cases also probably have an ACE. The authors propose that these ACEs are predominantly due to clonal hematopoiesis of indeterminate potential (CHIP), although this is not proven definitively, for example, by lymphocyte clonality analysis. While CHIP is a possible explanation for many of the mosaic *TP53* cases, the evidence is speculative, and other possible etiologies exist.

CHIP has gained recent attention as a marker of and perhaps etiological precursor to cardiovascular disease and hematological malignancy that increases with aging.^{4,7,8} CHIP, and other ACEs in the blood compartment, such as those resulting from the selective pressure of chemotherapy,⁹ represent only one of a number of possible etiologies of a variant in a blood sample at an allelic fraction other than 50% or 100%. As delineated in a recent review by Forsberg et al.,⁵ the genetic variation of any particular human soma at any

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particular point in time is the sum total of genetic variants inherited from the parental generation along with heritable variants that occur de novo in the zygote, potentially heritable postzygotic acquired variants in the cellular lineage giving rise to the soma being evaluated (somatic mosaicism), microchimerism (cells from another individual), and revertant mosaicism.⁵ While ACEs due to normal aging, hematological malignancy, or chemotherapy, indicate a nonheritable cause of a *TP53* mutation, de novo and somatic mosaic *TP53* mutations may be heritable if they occur in the gonad of the individual in addition to the soma that was evaluated by genetic testing. Furthermore, the extent of mosaicism of the individual may confer different cancer predisposition phenotypes, although this is less well understood. Therefore, the clinical implications of an ACE versus a somatic mosaic are significant. As opposed to an ACE mutation, a somatic mosaic mutation may affect radiosensitivity of tissues, leading to clinical recommendations of avoidance of radiation. In addition, a somatic mosaic mutation probably leads to an increased risk of malignant transformation of the mutated tissues. At this point in time, because of a lack of data to suggest alternatives, screening protocols similar to those employed for typical LFS patients are recommended clinically for somatic mosaic *TP53* carriers.

Recently, Renaux-Petel and colleagues¹⁰ sought to investigate the rate of de novo and somatic mosaic *TP53* mutations in LFS. Specifically relevant to the study by Weitzel et al., Renaux-Petel et al. performed MPS-based testing in 108 patients with phenotypes highly suggestive of LFS but for whom Sanger sequencing was negative. Somatic mosaic mutations were found in 2 of 49 individuals with adrenocortical carcinoma, 2 of 17 individuals with choroid plexus tumors, and 1 of 31 women with breast cancer under age 31; indicating a somatic mosaic rate of 5% in patients with an LFS phenotype. While these data indicate that somatic mosaicism in *TP53* is probably extremely rare, the entity exists.

Besides biological causes of a low allelic fraction mutation, such as ACEs or somatic mosaicism, the contribution of preferential identification of the wild-type allele due to technological issues with MPS testing cannot be ignored. MPS-based multiplex tests rely typically on either capture-based hybridization technologies or multiplex amplicon-based sequencing. Owing to preferential hybridization or amplification of the wild-type versus mutant allele, a truly heterozygous mutation with a low allelic fraction using MPS may appear mosaic.

The majority of cases in the study by Weitzel et al. were older patients, some having undergone genetic testing presumably after their cancer treatment. Therefore, it is likely that an ACE, due to CHIP or chemotherapy, is the etiology of the majority of mosaic *TP53* mutations in the study. However, preferential amplification of the wild-type allele may be the cause of the low allele frequencies of the mutations in the patient with breast cancer at age 34, whose twin also carried the mutation, and the patient with multiple primaries (rhabdomyosarcoma, myelodysplastic syndrome,

and leukemia) who had four positive family members. However, the patient with breast cancer at age 19 with positive fibroblast testing may represent a true somatic mosaic. It is important to point out that among the 14 patients with family-member-only ancillary testing and the 35 with no ancillary testing who are presumed to have an ACE, seven of these patients had breast cancer under age 31 and one additional proband's family fit Chompret criteria. These patients with phenotypes suggestive of LFS should not be assumed to have an ACE and ancillary tissue testing for the *TP53* mutation in other tissues and testing of offspring should be performed. However, if these criteria for ancillary testing were applied, the true germ-line mutation of the patient with breast cancer at age 34 would have been missed, suggesting that ancillary testing to rule out preferential identification of the wild-type allele or somatic mosaicism should be considered on an even broader scale than solely for patients with phenotypes suggestive of LFS.

The misinterpretation of a mosaic *TP53* finding has potential negative downstream consequences. If the *TP53* mutation is truly an ACE, treating that patient as LFS could subject a patient to unnecessary screening and downstream cancer-related stress. In addition, patients with ACE due to CHIP may be at risk for cardiovascular disease and secondary cancers, and screening protocols need to be developed to address these risks. On the flip side, missing true early postzygotic somatic mosaicism or actual heterozygous germ-line results through operating on the assumption of an ACE could lead to a lack of necessary screening, to the use of radiation when it should be avoided, and to a failure to test family members. As Weitzel and colleagues point out, ancillary testing of low-allele-frequency *TP53* mutations should therefore be performed. The challenge now is to create clinical practice guidelines for which patients should have ancillary testing in the face of a mosaic *TP53* result and the surveillance protocols to be used, depending on the deduced etiology.

DISCLOSURE

The authors declare no conflict of interest.

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