

PATIENT PRESENTATIONS WITH A LI-FRAUMENI SYNDROME DIAGNOSIS
THROUGH DIFFERENT ASCERTAINMENT METHODS: A CASE SERIES

By

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ABSTRACT OF THE THESIS

Patient Presentations with a Li-Fraumeni Syndrome Diagnosis through Different

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Li-Fraumeni syndrome is a well characterized cancer syndrome with high risk of multiple types of cancers. However, little is known about etiology of *TP53* variants when patients are ascertained through genomic screening versus cancer clinics. This case series analyzes the personal and family histories of patients diagnosed with a *TP53* pathogenic or likely pathogenic variant in order to add to the descriptive literature surrounding patients with *TP53* variants of suspected somatic etiology, likely due to clonal hematopoiesis of indeterminate potential (CHIP). Retrospective chart reviews were performed on twenty-two patients, ascertained through a cancer clinic and a genomic screening program. Results indicate patients over the age of sixty with limited personal and family history have variants suggestive of somatic etiology due to CHIP or mosaicism and may benefit from confirmatory or cascade genetic testing.

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Introduction

Li-Fraumeni syndrome (LFS) is a hereditary cancer syndrome characterized by young onset cancers including breast cancer, sarcomas, brain cancer, leukemia, and adrenal cortical carcinoma (ACC). Predisposition to LFS is caused by inheriting a pathogenic (P) or likely pathogenic (LP) variant in the *TP53* gene (Adema and Kasi, 2019). Based on data from individuals ascertained due to personal or family cancer history, the likelihood that men and women with a P/LP variant in the *TP53* gene will develop cancer by age 60 is 75% and nearly 100%, respectively. (Kratz et al, 2017).

Several criteria exist in order to define Li-Fraumeni patients based on their personal and family histories. The first two, used to define LFS patients, are Classic and Chompret criteria. These criteria take into account patients with sarcomas diagnosed <45 years and a history of multiple primary cancer diagnoses <45 years. Li-Fraumeni-like (LFL) is a term used to describe patients that do not meet classic LFS criteria. Patients with LFL can have a P/LP variant in the *TP53* gene; however, they do not have a personal or family history of cancer consistent with classic or Chompret LFS criteria (Fraumeni and Lufkin, 2017). Two criteria exist to better define LFL patients—Birch and Eeles criteria. These criteria focus mainly on family history of LFS cancers. Full definitions of all LFS and LFL criteria are listed in Table 3.

Because of the very high risk of cancer in many different organs, the recommended cancer surveillance for individuals with a P/LP *TP53* variant is intensive. According to the National Comprehensive Cancer Network (NCCN) guidelines, patients with LFS or LFL should have a breast MRI once a year and a clinical breast exam every year, alternating each every six months, with consideration of a risk-reducing bilateral mastectomy; an upper endoscopy and colonoscopy every 2-5 years; a full body MRI once a year; and an annual skin check. Surveillance should begin at 20 years old (National Comprehensive Cancer Network, V1 2020).

The clinical implications of a P/LP variant in *TP53* are further complicated by the possibility that the variant is in somatic rather than germline DNA. As patients age, hematopoietic stem cells are more susceptible to obtaining somatic variants in the *TP53* gene, a condition called clonal hematopoiesis of indeterminate potential (CHIP) (Weitzel et al, 2018). Further testing of different tissues can determine if a *TP53* variant is due to CHIP or if this variant is a germline change. Patients with CHIP tend to have little to no family history of LFS cancers and are not affected with cancer themselves until later ages. CHIP has been associated with increased risk for non-cancer conditions such as coronary artery disease (CAD) and ischemic stroke (Jaiswal S et al., 2014). Currently, no formal testing or surveillance guidelines exist for patients with LFS presentations consistent with CHIP, and little research has been done on patients' adherence to risk management recommendations when CHIP is suspected.

The goal of this study is to add to the descriptive literature of patients with germline *TP53* variants and patients with somatic *TP53* variants, with the hopes of an eventual consensus on the appropriate way to counsel and make further screening and confirmatory testing recommendations for these patients, depending on the method in which their P/LP *TP53* variant is ascertained. To that end, we attempted to determine the degree to which we see LFS cancers in patients identified through the genomic screening program versus in clinically ascertained patients. Case reviews were conducted to identify patients with suspected germline *TP53* variant etiology versus those with suspected somatic *TP53* etiology, potentially due to CHIP. Personal and family histories of cancers were evaluated for each patient.

Methods

Sample Population: The patients in this study were ascertained through two different groups—Geisinger’s MyCode project and Geisinger’s cancer genetics clinic. MyCode is a genomics project that includes a Genomic Screening and Counseling program (GSC) that reviews unselected participants’ exome data for P/LP variants in a list of clinically actionable genes, including *TP53* (Williams et al, 2018). Participants’ samples are stored in a biobank and can be used for future research projects. MyCode participants are notified if they test positive for

one of the conditions for which MyCode screens. The cancer genetics clinic at Geisinger sees patients referred for their personal or family cancer history. Only patients that tested positive for a P/LP variant in the *TP53* gene in both groups were included.

Inclusion criteria: Eligible participants included any patient with a P/LP variant found in the *TP53* gene through MyCode or the cancer genetics clinic.

Exclusion criteria: Patients under the age of 18 were excluded.

Study Design: This is a study is a retrospective chart review using data from Geisinger's electronic medical record (EMR) of individuals who have received results through both methods of ascertainment. The data on pedigrees and family history were ascertained through Geisinger's EMR.

Procedure: Eligible patient charts identified through MyCode or cancer genetics clinic program were examined via Geisinger's EMR. Each patient's chart was reviewed for the cancer- and cardio-associated findings listed below.

Materials: The patient data examined from the EMR were from May 2015-June 2019 from both the genomic screening program and a cancer genetics clinic, selecting patients with a P/LP variant in the *TP53* gene. Data examined included personal and family history of cancer, specific *TP53* variant, and personal and family history of CAD and strokes. Table 2 was used for each chart review to ensure consistency. Table 3 defines LFS and LFL criteria.

Analysis: EMR data were reviewed for family and personal history of participants to determine if there were differences between ascertainment groups. These data were then used to determine if patients have variants that appeared with suspected germline or somatic etiology. For the purpose of this review, patients were considered to have *TP53* variants with suspected germline etiology if the patient and/or their family had cancer histories of 1 or more component LFS cancers, at least one of four LFS or LFL criteria were met, and no mosaicism was detected via genetic testing. Patients were considered to have *TP53* variants with suspected somatic etiology due to CHIP if the patient and/or their family did not have a cancer history of any LFS component cancer; if only Birch or Eeles LFL criteria were met or no criteria were met; they had a personal history of CAD and/or family history of stroke; or if mosaicism was detected. Patients' personal and family histories were analyzed in order to place each patient in a suspected etiology group.

Results

Nine patients have tested positive for a P/LP variant in *TP53* through the cancer genetics clinic. Thirteen patients have tested positive for a P/LP variant in *TP53* through the genomic screening program. Children (<18 years of age) were excluded from the study. The ratio of males to females was 4:9 for the MyCode participants and 2:7 for the cancer genetics clinic participants. Across both

groups, the ratio of males to females is 3:8. Table 1 below gives demographic information on both ascertainment groups. Table 2 below shows each patient's individual chart review.

Table 1

Patient Demographics

	<i>Cancer Clinic</i>	<i>GSC</i>	<i>Totals</i>
<i>N</i>	9	13	22
<i>Mean age (range)</i>	52 (22-73)	76 (62-86)	66 (22-86)
<i>Median age</i>	55	75	72
<i>Male</i>	2	4	6
<i>Female</i>	7	9	16
<i>Race (white)</i>	9	13	22
<i>Ethnicity (non-Hispanic/Latino)</i>	8	13	21
<i>Ethnicity (Hispanic/Latino)</i>	1	0	1

Table 2
Case Review Chart

Case #	Cohort	Variant	Age	Sex	Personal hx cancer (age at dx)	Family history cancer (age at dx)	LFS criteria met	LFL criteria met	Personal hx CAD	Family hx stroke	Suspected Etiology
1	Cancer	c.584T>C, p.Ile195Thr	65	F	Breast dx 62	Mat uncle 1-4, CRC dx unknown	No	No	No	No	Somatic
2	Cancer	c.473G>A, p.Arg158His (LP)	42	F	N/A	MGM panc, dx unknown Mother, multiple myeloma, dx unknown	No	No	No	No	Somatic
3	Cancer	c.542G>A, p.Arg181His	46	M	N/A	Mother, brain dx 64, CRC dx 50 PGF CRC dx 75, prostate dx 82 PGM, mat aunt breast dx unknown	No	Yes	No	No	Germline
4	Cancer	c.374C>T, p.Thr125Met	68	F	Bladder dx 65, CRC dx 67	Father, brain dx 49 PGF, pat aunt, pat uncle sarcoma dx unknown Pat aunt, uterine dx unknown	No	Yes	No	No	Germline

						MGM, CRC dx unknown Brother, bladder dx 74 Pat aunt, mat aunt, niece, breast cancer dx unknown					
5	Cancer	c.659A>C, p.Tyr220Ser Mosaic, 10-12%	69	F	Ovarian dx 67, melanoma dx unknown	Niece, ovarian dx 45 Mother, breast dx 56, cervix dx 32 Two brothers, daughter, cousin, melanoma dx unknown	No	No	No	No	Somatic
6	Cancer	c.818G>A, p.Arg273His	73	F	Breast dx 66, bladder dx 69	Sister, brain dx 68 Two sisters, breast dx 62 and 68 Niece, breast dx 40	No	Yes	No	No	Germline
7	Cancer *Son of patient #9	c.742C>T, p.Arg248Trp	22	M	N/A	Mother, breast dx 41, panc dx 42, thyroid dx 42, lung dx 43	No	Yes	No	No	Germline

						MGF leukemia dx unknown Pat uncle stomach and lung dx unknown					
8	Cancer	c.422G>A, p.Cys141Tyr (LP)	28	F	Breast dx 27	Father, sarcoma dx 41 PGM, sarcoma dx 35	Yes	Yes	No	No	Germline
9	Cancer *Mother of patient #7	c.742C>T, p.Arg248Trp	55	F	Breast dx 41 Panc dx 42 Thyroid dx 42 Lung dx 43 L adrenal mass, dx 51	PGM, pat cousin, breast dx unknown Pat uncle, sarcoma, lung, stomach, and brain dx unknown PGM, MGM, father, leukemia dx unknown	Yes	Yes	No	No	Germline
10	GSC	c.733G>A, p.Gly245Ser	72	F	Breast dx 52	Sister breast dx 45, multiple myeloma dx 70's Pat uncle brain dx 50's	No	Yes	No	No	Germline

						PGF ocular melanoma dx unknown Mother and father, CRC dx 57 and 82 MGM, stomach dx 70's Daughter and niece, cervix dx unknown					
11	GSC	c.638G>A, p.Arg213Gln	69	F	N/A	Mat aunt melanoma dx 40, CRC dx 78 Father, lung/throat dx 55 MGM, CRC dx unknown	No	No	No	No	Somatic
12	GSC	c.949C>T, p.Gln317X	82	F	Breast dx 77, CRC dx 78	Brother, breast dx 76 Niecesx2, breast dx 30's Niece, ovarian dx 27	No	Yes	No	No	Germline
13	GSC	c.524G>A, p.Arg175His	75	F	Breast dx 71 and 75 Skin dx unknown	Mother, breast dx 72 Mat aunt, CRC dx unknown	No	No	No	No	Somatic

						Daughter, skin dx unknown MGM, 3 mat uncles; unknown cancers					
14	GSC	c.542G>A, p.Arg181His	82	F	N/A	Father and sister, lung dx unknown	No	No	Yes	No	Somatic
15	GSC	c.742C>T, p.Arg248Trp	72	M	Hepatocellular, dx 72	MGF, unknown cancer Mat uncle, possible sarcoma	No	No	Yes	No	Somatic
16	GSC	c.473G>A, p.Arg158His (LP)	d. 85	F	Breast, dx 76	N/A	No	No	No	No	Somatic
17	GSC	c.672+2T>A (LP)	d. 74	M	Lymphoma, dx 70	Mother and sister, unknown cancers	No	No	Yes	No	Somatic
18	GSC	c.783-7_799 del24 (LP)	86	F	N/A	Mother, Hodgkin's lymphoma dx unknown	No	No	No	No	Somatic
19	GSC	c.818G>A, p.Arg273His Mosaic	74	M	N/A	Mother, cervix, Father, lip, dx unknown	No	No	No	No	Somatic
20	GSC	c.473G>A, p.Arg158His	79	F	Uterine, dx 45 Papillary thyroid, dx 66	Mother, breast dx 70, thyroid dx 70 Father, prostate dx 68	No	No	No	Yes	Somatic

21	GSC	c.542G>A, p.Arg181His	62	M	N/A	Father, lung dx 60	No	No	Yes	No	Somatic
22	GSC	c.329G>T, p.Arg110Leu	79	F	Leukemia dx 73 Skin dx 76	Mother, liver dx 70	No	No	No	No	Somatic

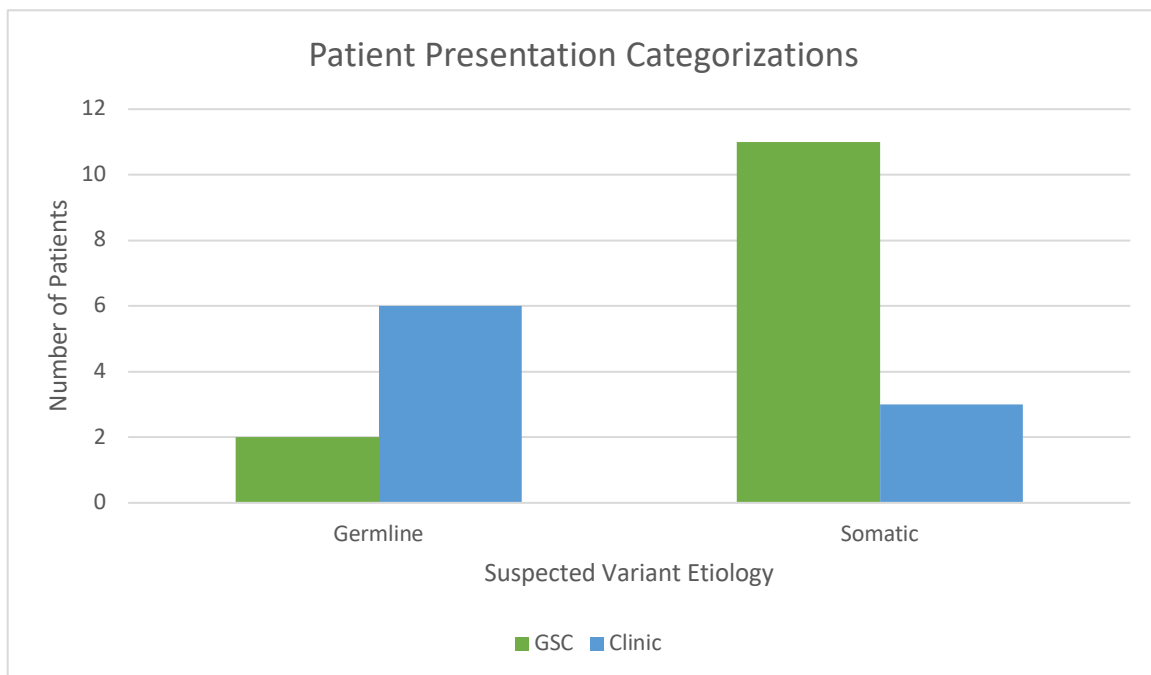
To characterize the degree to which typical LFS cancers are seen in the personal and family histories of both ascertainment groups, case reviews were performed via electronic medical records. Results indicate that most patients across both ascertainment groups identified over the age of sixty have personal and family histories that are suspected somatic etiology. These patients were most often identified through the genomic screening program as opposed to the cancer clinic. Across both groups, eight patients were consistent with germline etiology and 14 patients were consistent with somatic etiology.

In the GSC group, two out of thirteen patients had histories of suspected germline etiology and 11 had histories of suspected somatic etiology. Of the 11 patients suspected to have somatic etiology, four had personal history of CAD and one had a family history of ischemic strokes.

Six out of nine cancer clinic patients had histories of suspected germline etiology and three had histories of suspected somatic etiology. Two clinic patients with suspected somatic etiology had neither personal history of CAD nor family histories of ischemic strokes; however, both patients had variants that were mosaic. Figure 1 below gives a graphic representation of each category.

Out of all 14 patients in both groups with histories consistent of somatic etiology, none met either LFS or LFL criteria (Table 2), as defined in Table 3. These patients consistent with somatic etiology did not have further testing performed, such as a skin-punch biopsy, to further determine the etiology of their individual variants.

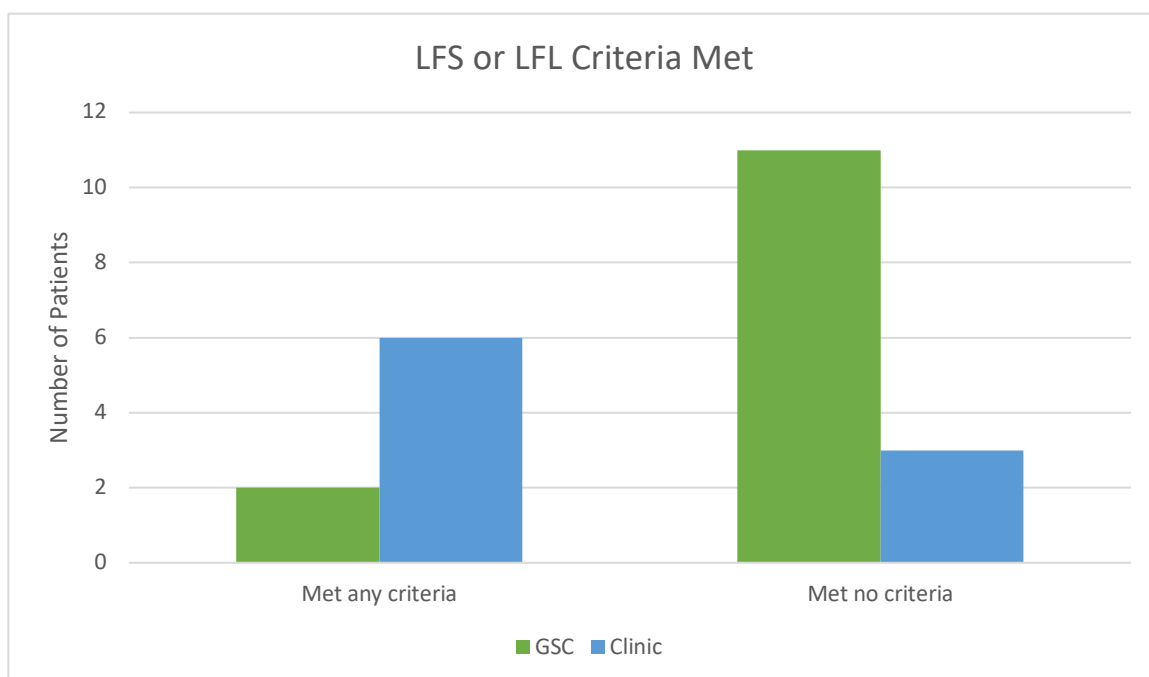
Figure 1
Patient Presentation Categorizations



Two Chi square analyses were performed in order to compare ascertainment groups. The first Chi square analysis was performed to compare the number of patients in each ascertainment group with suspected somatic versus suspected germline etiology. A higher rate of cancer clinic patients were placed in the suspected germline category when compared to the genomic screening group. This is a statistically significant difference, $\chi^2 (1, N = 22) = 6.044, p = .0139$. The second Chi square analysis was performed to compare the number of patients meeting LFS/LFL criteria in each ascertainment group. A higher rate of cancer clinic patients met LFS/LFL criteria when compared to the genomic screening program patients. This is a statistically significant difference, $\chi^2 (1, N = 22) = 8.526, p = .004$. Out of all patients in both ascertainment groups,

Chompret criteria were met in two cases and Eeles criteria were met in eight cases. No patient met Classic LFS criteria or Birch criteria. Figure 2 below illustrates the breakdown of criteria met by patients in each ascertainment group.

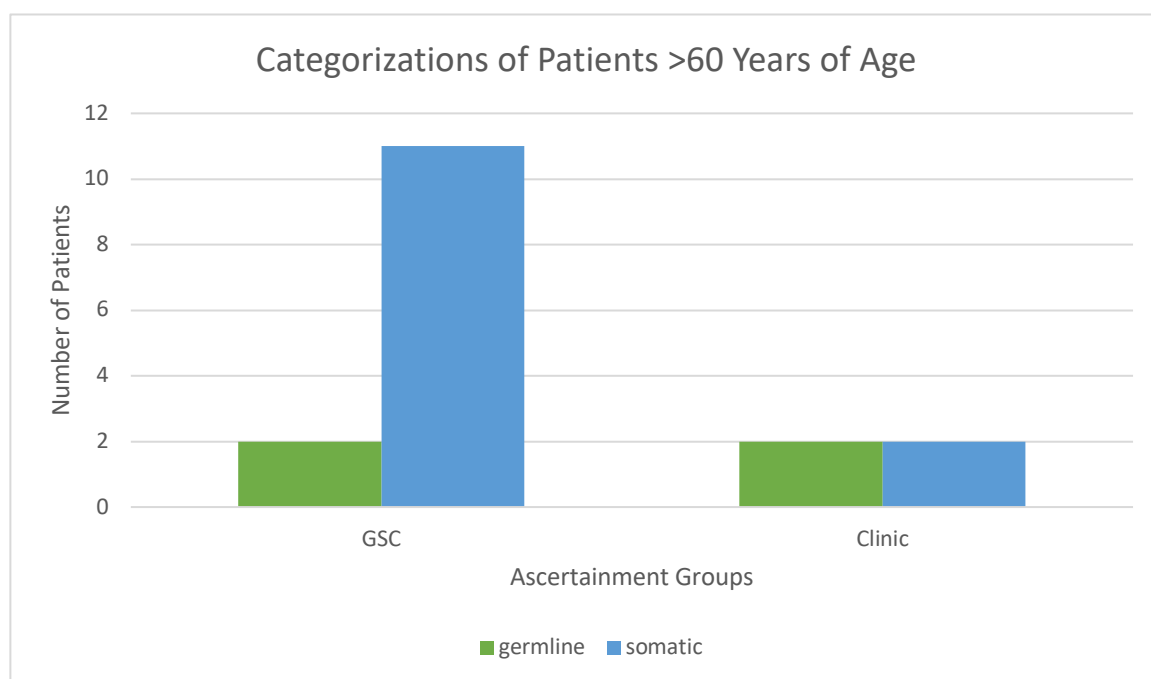
Figure 2
LFS or LFL Criteria Met



Seventeen out of 22 patients were over the age of 60 at the time of this case review. Four of these patients had suspected germline etiology and thirteen had suspected somatic etiology. Of the remaining 5 patients that were <60, four were in the suspected germline category and one was placed in the suspected somatic category. This demonstrates a higher proportion of patients identified >60 years of age to be in the suspected somatic category, as illustrated in Figure 3 below. Of all patients across both groups >60, only four met LFL-

Eeles criteria. No other criteria were met by patients >60. We report a higher likelihood of suspected somatic etiology when a variant is identified over the age of 60, and LFL-Eeles criteria as the most commonly met criteria among these patients.

Figure 3
Categorizations of Patients >60 Years of Age



Personal history of component LFS cancers was present in four out of nine cancer clinic patients and five out of twelve genomic screening patients. Four cancer clinic patients and six genomic screening patients had personal histories of non-component LFS cancers (Table 2). No patients in either ascertainment group had a first-degree relative (FDR) or second-degree relative (SDR) with a diagnosis of ACC, one of the LFS component cancers. In both

groups, the most common component cancer diagnosis in FDR and SDR was breast cancer. Within the FDR's, the second most common component cancer diagnosis was brain cancer, followed by leukemia and sarcomas. Leukemia and sarcoma history were only seen in the cancer clinic group; there was no FDR history of leukemia or sarcoma in the genomic screening group. Within the SDR's, the second most common component cancer diagnoses were sarcomas, followed by brain and leukemia. Several patients in both groups had family histories of non-component cancers, the most common being colon, melanoma, and stomach cancers. For the purpose of this case review, the focus remained on the known component cancers associated with LFS; non-component cancers were not used to classify individuals' suspected etiology.

Discussion

This case review describes patient presentations and family histories in individuals with a P/LP variant in the *TP53* gene. These patients underwent testing between May 2015-June 2019, and were ascertained through Geisinger's cancer genetics clinic and Geisinger's MyCode Genomic Screening Program. There is little performed research related to LFS/LFL patients with a variant potentially explained by CHIP. Given the uptake in panel testing in a cancer setting (Neben et al, 2019), this study has important clinical implications for patients over the age of 60 with limited relevant family history.

Case Review Findings

All patients in the cancer clinic group met NCCN testing criteria for breast and/or ovarian cancers, colon cancers, or Li-Fraumeni syndrome based on personal and family histories. While no patients in this group met classic criteria and only two met Chompret criteria, six out of nine did meet LFL-2 Eeles criteria, demonstrating that LFL criteria is important for identifying these families. All six patients that met Eeles criteria were placed in the suspected germline category.

Patients in the genomic screening program group did not need to meet any testing criteria to participate in MyCode. Only two patients in this group met LFL-2 Eeles criteria; each was placed in the suspected germline category. The difference between cancer clinic patients and genomic screening program patients meeting LFS/LFL criteria is statistically significant, with more patients in the cancer clinic group meeting criteria than genomic screening patients.

Data from the genomic screening program cohort suggests significantly lower cancer risks than previously reported in clinically ascertained patients with a P/LP *TP53* variant. All patients in this group were at an age at which cancers caused by *TP53* variants would have been expected to have appeared. Limited personal and family histories of cancers and the number of patients with CAD and/or mosaicism further suggest somatic etiology due to CHIP in this cohort. It is also possible that *TP53* variants ascertained via a genomic screening program

have lower penetrance than previously reported. Either explanation for the differences in the presentations of patients ascertained via the genomic screening program vs. cancer genetics clinic – somatic etiology or lower than anticipated cancer penetrance – has implications for genetic counseling of these individuals.

Genetic Counseling Considerations

This case series provides important descriptive literature in regard to LFS patients with suspected somatic etiology. Fourteen out of seventeen patients over the age of 60 were categorized as suspected somatic etiology, while the remaining three were categorized as suspected germline; therefore, in the absence of large personal and family histories, it is reasonable to consider confirmatory testing by skin-punch biopsy. This may be beneficial to patients over the age of 60 at the time of their variant identification because the likelihood of having no cancer diagnoses up until this age is slim. By offering confirmatory testing, genetic counselors may be able to better define the cancer risks to their patients and their patient's family. Offering familial cascade testing in addition to confirmatory testing of the patient would further solidify the patient's risks.

In the era of panel testing, patients may meet NCCN testing criteria for breast or ovarian cancer testing, but do not meet testing criteria for every gene included on a panel. In light of an unexpected *TP53* variant identified through

panel testing, it is important for genetic counselors to be aware of and consider CHIP as a potential cause for this variant. It may be useful to consider further confirmatory testing. Genetic counselors should also be aware of comorbidities associated with CHIP. Personal and family histories of CAD and strokes are common complications associated with CHIP. Patients must be made aware of these comorbidities and referred to the correct physicians to be treated accordingly.

Future Directions

Future directions for this research include other facilities performing similar chart reviews in order to ascertain a larger cohort of patients with presentations suggestive of somatic etiology. Offering confirmatory testing to a cohort of patients with likely somatic *TP53* variants in order to understand risk to patients and their families, and creating a guideline for handling such patients, are important next steps.

This possibility of somatic variants due to CHIP can also extend to other genes, including *CHEK2* and *ATM* (Slavin et al, 2019), where further research is needed in order to better care for these patients. These patients are also at risk for the comorbidities associated with CHIP; therefore, identifying these patients with likely somatic variants in different genes is of great importance as well.

Limitations

This case series focused on a small sample size of patients with a rare genetic cancer syndrome (N = 22). Given the small numbers in each ascertainment group and associated concerns about insufficient power to detect between-group differences, we limited the statistical comparisons of the two ascertainment groups. This review also is limited by the fact that no patients with suspected somatic etiology had confirmatory testing at the time of chart review. Another limitation is that only one patient pursued confirmatory testing in her son, confirming the variant to be of germline origin. No confirmatory testing of the patient or cascade testing of family members was performed on any other patients, making it difficult to ascertain the specific risks to these patients and their families and true variant etiology. Due to the fact that programs such as Geisinger's MyCode project do not exist in large numbers, it may be difficult to ascertain another patient population such as this one. However, the presence of the cancer clinic as a comparison group to MyCode is a strength of the study due to the ability to compare patients with the same variant findings in different ascertainment groups.

While it is not possible to draw large conclusions from this study, this will add to the descriptive literature surrounding LFS patients with variants of suspected somatic etiology due to CHIP.

Conclusions

In summary, we report a correlation between older ages of *TP53* variant identification with suspected somatic etiology consistent of CHIP. In the absence of extensive family histories and LFS/LFL criteria met, it may be valuable for patients over the age of 60 to have confirmatory testing, such as a skin-punch biopsy. While a negative skin punch biopsy would not rule out germline etiology entirely, it would provide a lower likelihood that the identified variant was germline. Cascade testing of potentially at-risk family members would prove beneficial in order to further determine risks to relatives. In order to avoid unnecessarily screening patients for LFS cancers, confirmatory testing would be beneficial for patients over the age of 60 with a P/LP variant in the *TP53* gene. This information is important for genetic counselors to be aware of when testing patients > 60 years old with limited relevant cancer family history.

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Appendix

Table 3
LFS and LFL Criteria Definitions

Criteria	Definition
Classic LFS criteria	“Combination of individual diagnosed at age <45y with a sarcoma, <i>AND</i> a first degree relative diagnosed at age <45y with cancer <i>AND</i> an additional first- or second-degree relative in the same lineage with cancer diagnosed at age <45y, or a sarcoma at any age” (NCCN, V1 2020).
LFS Chompret criteria	“Individual with a tumor from the LFS spectrum (soft tissue sarcoma, osteosarcoma, CNS tumor, breast cancer, adrenocortical carcinoma), before 46y of age, <i>AND</i> at least one first- or second-degree relative with any of the aforementioned cancers (other than breast cancer if the proband has breast cancer) before the age of 56y or with multiple primaries at any age <i>OR</i> individual with multiple tumors (except multiple breast tumors), two of which belong to the LFS tumor spectrum with the initial cancer occurring before the age of 46y <i>OR</i> individual with adrenocortical carcinoma, or choroid plexus carcinoma or rhabdomyosarcoma of embryonal anaplastic subtype, at any age of onset, regardless of family history <i>OR</i> breast cancer before 31y of age” (NCCN, V1 2020).
LFL-1: Birch definition	“A person diagnosed with any childhood cancer, sarcoma, brain tumor, or adrenocortical tumor before age 45 <i>AND</i> A first-degree or second-degree relative diagnosed with a typical LFS cancer, such as sarcoma, breast cancer, brain cancer, adrenocortical tumor, or leukemia, at any age <i>AND</i> A first-degree or second-degree relative diagnosed with any cancer before age 60” (Fraumeni and Lufkin, 2017).
LFL-2: Eeles definition	“2 first-degree or second-degree relatives diagnosed with a typical LFS cancer, such as sarcoma, breast cancer, brain cancer, adrenocortical tumor, or leukemia, at any age” (Fraumeni and Lufkin, 2017).