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PTEN Germline Mutations in Patients Initially Tested for Other Hereditary Cancer Syndromes: Would Use of Risk Assessment Tools Reduce Genetic Testing?

JESSICA L. MESTER,[a,b] REBEKAH A. MOORE,[a,d] CHARIS ENG[a,b,c,d,e]

[a]Genomic Medicine Institute, [b]Taussig Cancer Institute, and [c]Stanley Shalom Zielony Institute for Nursing Excellence, Cleveland Clinic, Cleveland, Ohio, USA; [d]Department of Genetics and Genome Sciences and [e]CASE Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

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Key Words. Risk assessment • Genetic testing • PTEN hamartoma tumor syndrome • Cowden syndrome • Hereditary cancer syndromes

Learning Objectives

Cite the risk assessment tools available for several hereditary cancer predisposition syndromes.

Describe ways in which use of these risk assessment tools can lead to cost savings and decreased time to correct diagnosis.

Explain the impact of correct genetic diagnosis on the patient's medical management and on predictive testing for family members.

ABSTRACT

Purpose. PTEN Hamartoma Tumor syndrome (PHTS) includes patients with Cowden syndrome or other syndromes with germline mutation of the *PTEN* tumor suppressor gene. The risk for breast, colorectal, and endometrial cancer and polyposis is increased, creating clinical overlap with hereditary breast and ovarian cancer (HBOC), Lynch syndrome (LS), and adenomatous polyposis syndromes (APS). We reviewed our series of patients with PHTS to determine how often testing criteria for these syndromes were met and how often other-gene testing was ordered before testing *PTEN*.

Patients and Methods. Patients were prospectively recruited by relaxed International Cowden Consortium criteria or presence of known germline *PTEN* mutation. Mutations were identified by mutation scanning/multiplex ligation-dependent probe amplification analysis and confirmed by sequencing/quantitative polymerase chain reaction. Patients were excluded if they were adopted, were <18 years of age, or if they were diagnosed with Cowden syndrome before 1998. Standard risk-assessment models were applied to determine whether patients met HBOC testing criteria, LS-relevant Amsterdam II/Bethesda 2004 criteria, or had adenomatous polyps. Prior probability of *PTEN* mutation was estimated with the Cleveland Clinic *PTEN* risk calculator.

Results. Of 137 *PTEN* mutation-positive adult probands, 59 (43.1%) met testing criteria for HBOC or LS. Of these, 45 (32.8%) were first offered HBOC, LS, or APS testing. Of those who underwent APS testing, none of the six patients met criteria. Initial risk assessment by a genetics specialist was significantly associated with immediate *PTEN* testing in patients also meeting HBOC testing criteria. Using this *PTEN* risk assessment tool could have spared gene testing for 22 unlikely syndromes, at a total cost of $66,080.

Conclusion. PHTS is an important differential diagnosis for patients referred for HBOC, LS, or APS. Risk assessment tools may help focus genetic analysis and aid in the interpretation of multiplex testing. ***The Oncologist*** 2013;18:1083–1090

Implications for Practice: Whereas hereditary breast and ovarian cancer syndrome, Lynch syndrome, and familial adenomatous polyposis are some of the most common causes of hereditary cancer predisposition, other conditions with overlapping clinical spectra exist. Timely identification of the right syndrome is critical for patient management and testing at-risk relatives. When multiple conditions are possible, risk assessment tools help clinicians judge which condition is most likely, allowing genetic testing to proceed in a stepwise and cost-effective manner. Here we present a series of patients with germline mutations of *PTEN*, a gene causing predisposition to breast, uterine, colorectal, and other cancers as well as to gastrointestinal polyposis. Many patients were tested for another syndrome prior to *PTEN* testing. Had now-existent risk assessment tools been used, elevated *PTEN* mutation risk in these patients might have been recognized immediately, leading to health care savings and shortened time to diagnosis. The article also discusses how the use of risk assessment tools will remain important as genetic testing shifts from a single-gene to a multiplex approach.

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Correspondence: Charis Eng, M.D., 9500 Euclid Avenue, NE50, Cleveland, Ohio 44195, USA; Telephone: 216-444-3440; Fax: 216-636-0655; E-Mail: engc@ccf.org Received May 14, 2013; accepted for publication August 13, 2013; first published online in ***The Oncologist*** *Express* on September 13, 2013. ©AlphaMed Press 1083-7159/2013/$20.00/0 http://dx.doi.org/10.1634/theoncologist.2013-0174

Introduction

On average, 10% of all malignancies have a Mendelian genetic cause. Inherited cancer syndromes confer risks for cancers far higher than sporadic counterparts. As knowledge accumulates, the genetic differential diagnosis for any single cancer type grows, and clinicians are faced with determining which inherited cancer syndrome is most likely for any given presentation and family history. Most single-gene tests cost at least $1,000, and genetic testing is most cost-effective for both the patient and the health care system when performed in a sequential manner, where testing for the most-suspected condition is done first. Often, several inherited cancer syndromes are considered in the differential diagnosis for a given patient, and the task of prioritization of gene testing, often resulting in multiple rounds of gene testing, becomes a challenge. Multiplex testing, where several genes are tested at once as part of a panel, is emerging as an alternative. However, such testing is not as sensitive as gene-specific sequencing and only detects small genetic alterations as opposed to partial or whole gene deletions and duplications, which require different testing methodologies for detection [1]. In this situation, understanding when a priori risk for a particular syndrome is elevated helps the testing laboratory ensure quality coverage of the genomic regions of most interest and enables the clinician to understand when additional testing to check for large rearrangements should be pursued.

PTEN Hamartoma Tumor Syndrome (PHTS) is a molecularly defined umbrella term used to describe individuals with Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome, and germline mutation in the *PTEN* tumor suppressor gene [2, 3]. Patients with PHTS have increased risks for several cancer types, with recent work suggesting higher lifetime risks for breast, thyroid, endometrial, renal, and colorectal carcinomas as well as for melanoma than previously reported in patients with CS studied prior to the discovery of *PTEN* [4]. The lifetime risk for breast cancer for these individuals is now projected to be up to 85%, which is equivalent to the highest risk estimate for patients with Hereditary Breast and Ovarian Cancer syndrome (HBOC). In addition, lifetime endometrial cancer (EC) risk is projected to be up to 30%, which is within the 25%–60% EC risk for patients with Lynch syndrome (LS) [4–7]. The PHTS-related colorectal cancer risk is twofold elevated above the incidence in the general population, with polyposis of several histologic subtypes, including adenomas, developing in the majority of patients [8]. Given the autosomal-dominant inheritance pattern of PHTS and its overlapping clinical presentation with HBOC, LS, and the adenomatous polyposis syndromes (APS) [9] (Table 1), the clinician is challenged to develop an informed differential diagnosis to understand which syndrome should be considered first so that genetic testing can proceed in a manner that is both health care cost-effective and provides a timely diagnosis for the patient and the patient's family members.

To investigate the clinical overlap of PHTS and HBOC, LS, and APS, we reviewed our series of patients with PHTS to determine how many also met testing or diagnostic criteria for any of the latter three syndromes. We also explored how often testing for one of these syndromes was conducted before consideration of the eventual PHTS diagnosis. Finally, we used the Cleveland Clinic PTEN risk calculator to compare patients' a priori probability of *PTEN* mutation with their a priori probability of carrying mutations in *BRCA1* or *BRCA2* (referred to together as *BRCA1/2*) or *MLH1/MSH2/MSH6* using a variety of risk models.

Methods

Patient Eligibility and Recruitment

Patients were prospectively recruited after providing informed consent for Cleveland Clinic IRB# 8458-PTEN substudy, which opened in October 2005 and continues to recruit participants. Inclusion criteria were as follows: minimally meeting relaxed International Cowden Consortium criteria (meaning full diagnostic criteria [10] minus one feature); macrocephaly plus autism/developmental delay/mental retardation; penile freckling; or presence of known germline *PTEN* mutation. Germline *PTEN* mutation analysis was performed per protocols described elsewhere [11]. Only patients with pathogenic germline mutations were included in this study. Patients were excluded if a clinical diagnosis of CS or Bannayan-Riley-Ruvalcaba syndrome was made in the patient or family before 1998 (when publications describing *PTEN* as the causative gene for CS and Bannayan-Riley-Ruvalcaba syndrome were released), if family history was unavailable, or if the proband was less than 18 years old. Clinical data including pedigree, genetic testing reports, and medical records were requested for all patients.

Risk Assessment

To determine clinical overlap with HBOC, each patient's medical and family histories were reviewed to determine whether the National Comprehensive Cancer Network's (NCCN) HBOC testing criteria in effect on the date of the patient's initial cancer genetics risk assessment were met. Additionally, risk for *BRCA1/2* mutation was assessed by means of the following risk prediction models: BRCAPRO (BayesMendel Lab http://bcb.dfci.harvard.edu/bayesmendel/software.php) [12], Myriad II (Myriad BRCA Risk Calculator, https://www.myriadpro.com/brca-risk-calculator) [13], and Penn II (University of Pennsylvania Abramson Cancer Center http://www.afcri.upenn.edu/itacc/penn2/). These models were selected because of their utility for patients with and without a breast cancer diagnosis. Clinical overlap with LS was evaluated by review of patients' personal and family history to determine whether Amsterdam II [14] and/or Bethesda 2004 criteria [15] were met. Additionally, *MLH1, MSH2,* and *MSH6* mutation risks were calculated using the PREMM1,2,6 (Dana-Farber Cancer Institute http://premm.dfci.harvard.edu/) [16] and MMRpro (BayesMendel Lab, http://bcb.dfci.harvard.edu/bayesmendel/software.php) [17] models, which were selected because they incorporated uterine cancer diagnosis in the risk assessment and are valid for patients of all ages. Medical history, including pathology reports, were reviewed to determine whether 10 or more adenomatous polyps were identified; this is the established threshold for consideration of APS [9]. Clinic notes were also reviewed to determine whether testing had been considered but not pursued. Risk for *PTEN* mutation was calculated by means of the Cleveland Clinic *PTEN* risk calculator (Cleveland Clinic Genomic Medicine Institute http://www.lerner.ccf.org/gmi/ccscore/), which is

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Table 1. Clinical descriptors of syndromes included in this study

Syndrome	Associated neoplasias	Gene(s)	Inheritance
PHTS	Breast, non-medullary thyroid, endometrial, renal, colorectal, melanoma, GI polyposis, benign skin lesions, dysplastic cerebellar gangliocytoma (Lhermitte–Duclos disease)	*PTEN*	Autosomal dominant
HBOC	Breast, ovarian, pancreatic, prostate, melanoma	*BRCA1, BRCA2*	Autosomal dominant
LS	Colorectal, endometrial, stomach, ovarian, small bowel, hepatobiliary tract, urinary tract, brain/CNS, sebaceous neoplasms	*MLH1, MSH2, MSH6, PMS2, EPCAM*	Autosomal dominant
APS (includes FAP and MAP)	Colorectal, small bowel, GI polyposis, papillary thyroid (FAP)	*APC, MUTYH*	FAP: Autosomal dominant MAP: Autosomal recessive

Abbreviations: APS, adenomatous polyposis syndromes; CNS, central nervous system; FAP, familial adenomatous polyposis; GI, gastrointestinal; HBOC, hereditary breast and ovarian cancer syndrome; LS, Lynch syndrome; MAP, MUTYH-associated polyposis; PHTS, PTEN Hamartoma Tumor Syndrome.

based on multiple logistic regression approaches, taking into account age of tumor onset and relative prevalence of the component phenotype in the general population [11].

Cost Data and Statistical Analysis

Testing costs for HBOC, LS, and APS were estimated using the Myriad Genetics services and price list for May 2011 (Myriad Genetics, Salt Lake City, UT). At that time, test costs for *BRCA1/2*, LS, and APS were $3,340, $4,480, and $2,050, respectively. Cost of *APC* analysis alone was conservatively estimated by subtracting the cost of the *MYH* mutation panel ($400) from the comprehensive APS analysis cost. Comparative analyses were performed by unpaired *t*-test and Fisher's exact test.

Results

Among the 4,030 individuals accrued for the main 8458-PTEN study, 267 individuals were found to have clearly pathogenic germline *PTEN* mutations. Of these, 137 were adult probands meeting this study's eligibility guidelines as described previously. Table 2 summarizes patient demographics and pertinent patient history. Age at pedigree generation was comparable between females and males ($p = .47$).

Overlap With HBOC

Among the 96 females, 41 (42.7%) were diagnosed with invasive breast cancers (4 with bilateral tumors) at an average age at first diagnosis of 46.2 years, and 2 women (2.1%) were diagnosed with ovarian cancer at age 33 and 62 years. One man had ductal carcinoma in situ (DCIS) diagnosed at age 46 and nine women also had DCIS; two had bilateral DCIS. Family history of breast cancer was common; 59 of 137 (43.1%) patients had at least one affected first- or second-degree relative. Among the 30 (21.9%) patients with an affected first-degree relative, the relative's average age at diagnosis was 54.5 years, significantly older than probands' age at diagnosis ($p = .0015$). Twelve patients had a first- or second-degree relative with ovarian cancer, with an average age at diagnosis of 54.4 years.

Forty-nine (47.9%) women met the NCCN testing criteria for HBOC. Of these 49, 30 were first offered *BRCA1/2* analysis and 19 were first tested for *PTEN* mutations. Two patients first offered *BRCA1/2* analysis were found to have a *BRCA2* mutation and were then tested for *PTEN* because of personal or family history features not fully explained by the *BRCA2* result; these two patients were excluded from the following analyses. Frequency of and age at breast cancer diagnosis were similar between patients first tested for *BRCA1/2* and those tested for *PTEN*, as were a priori probabilities of *PTEN* and *BRCA1/2* mutations across all risk models (Table 3). Thus, we sought to understand what motivated providers to pursue *PTEN* analysis before *BRCA1/2* despite the lack of a *PTEN* risk calculation tool to assist in their decision-making process. None of the characteristics associated with PHTS were significantly more prevalent in patients first offered testing for either *PTEN* or *BRCA1/2*. However, patients with a personal and/or family history possibly consistent with LS were more likely to first be offered *PTEN* analysis ($p = .02$). Those whose initial risk assessment was provided by a genetics specialist (counselor or genetics specialist physician) as opposed to a non-genetics specialist (nurse or non-genetics specialist physician) were also more likely to be offered *PTEN* analysis initially ($p = .03$). Four men, including the one with DCIS, also met NCCN HBOC testing criteria; however, none was offered *BRCA1/2* analysis. Four women offered *BRCA1/2* analysis first were ultimately not tested because of insurance coverage issues or patient choice.

Table 2. Patient demographics and personal history information

Characteristic	n (%)	Median/mean age, years (range)
Gender		
Female	96 (70.1%)	46.0/44.7 (19–74)
Male	41 (29.9%)	45.0/43.0 (18–73)
Race		
White	103 (75.2%)	–
Asian	7 (5.1%)	–
Black	3 (2.2%)	–
Native Hawaiian/other Pacific Islander	1 (0.7%)	–
Two or more groups	13 (9.5%)	–
Unknown	10 (7.3%)	–
Personal history		
Breast cancer (females)	41 (42.7%)	47.0/46.2 (29–66)
Ovarian cancer (females)	2 (2.1%)	47.5/47.5 (33–62)
Endometrial cancer (females)	22 (22.9%)	48.0/45.6 (21–68)
Colorectal cancer	9 (6.6%)	43.0/41.4 (21–53)
≥10 polyps, any histology	30 (21.9%)	N/A

Abbreviation: N/A, not applicable.

Table 3. Comparisons between women meeting the NCCN HBOC testing criteria first offered either *BRCA1/2* or *PTEN* analysis

Characteristic	*BRCA1/2* tested or offered first ($n = 28$)	*PTEN* tested or offered first ($n = 19$)	*p* value
BRCAPro model *BRCA1/2* mutation risk	11.6%	10.8%	0.84
Penn II model *BRCA1/2* mutation risk	16.4%	13.9%	0.50
Myriad II model *BRCA1/2* mutation risk	7.2%	5.3%	0.10
Cleveland Clinic model *PTEN* mutation risk	35.6%	47.6%	0.28
History suspicious for LS (met Amsterdam II or Bethesda criteria or endometrial cancer diagnosed <50 years of age)	**3 (10.7%)**	**8 (42.1%)**	**0.02**
Initial risk assessment by genetics specialist[a]	**19 (76%)**	**18 (100%)**	**0.03**
Early-onset invasive breast cancer (<50 years)	10 (35.7%)	8 (42.1%)	0.76
Invasive breast cancer diagnosed at any age	21 (75%)	10 (52.6%)	0.13
Ovarian cancer	1 (3.6%)	1 (5.3%)	1.00
Thyroid cancer	4 (14.3%)	3 (15.8%)	1.00
Renal cancer	1 (3.6%)	2 (10.5%)	0.56
Endometrial cancer	3 (10.7%)	6 (31.6%)	0.13
Colorectal cancer	0 (0%)	3 (15.8%)	0.06
Melanoma	1 (3.6%)	1 (5.e%)	1.00
Macrocephaly, any degree[a]	21 (84%)	17 (94.1%)	1.00
Extreme macrocephaly (≥60 cm)[a]	9 (39.1%)	10 (58.8%)	0.23
Lhermitte-Duclos disease	1 (3.6%)	1 (5.3%)	1.00
Intellectual disability/autism spectrum	0 (0%)	1 (5.3%)	0.40
Oral papillomatosis	7 (25%)	7 (36.8%)	0.52
Trichilemmoma	3 (10.7%)	2 (10.5%)	1.00
Acral keratoses	7 (25%)	4 (21.1%)	1.00
Lipoma	7 (25%)	7 (36.8%)	0.52
≥1 hamartomatous or ganglioneuromatous GI polyp	5 (17.9%)	5 (26.3%)	0.50
Any histology/number of GI polyps	16 (57.1%)	11 (57.9%)	1.00
≥5 GI polyps, any histology	8 (28.6%)	5 (26.3%)	1.00
≥10 GI polyps, any histology	4 (14.3%)	1 (5.3%)	0.63
Goiter	18 (60%)	16 (84.2%)	0.19
Hashimoto's thyroiditis	7 (25%)	4 (21.1%)	1.00
Benign breast disease	22 (78.6%)	13 (68.4%)	0.51
Arteriovenous malformation/vascular neoplasm	6 (21.4%)	3 (15.8%)	0.72

[a]Provider type performing initial risk assessment and exact head circumference measurement unknown for some patients.
Abbreviations: GI, gastrointestinal; HBOC, hereditary breast and ovarian cancer; LS, Lynch syndrome; NCCN, National Comprehensive Cancer Network.

Three additional female patients underwent *BRCA1/2* analysis despite not meeting NCCN testing criteria.

Overlap With LS

Colorectal cancer was diagnosed in nine (6.6%) patients with PHTS at an average age of 41.4 years. Average age at diagnosis was 45.6 years among the 22 of 96 (22.9%) women with EC. Two probands had both colorectal cancer and EC diagnoses. Family history (including first- and second-degree relatives) was positive for colorectal cancer and EC diagnoses in 30 (21.9%) and 20 (14.6%) families, respectively.

Amsterdam II or Bethesda 2004 criteria were met by 11 (8.0%) patients with PHTS (Table 4), 3 of whom underwent tumor or germline testing for LS with normal results. Three additional patients who did not meet either criterion also had normal results on LS testing. Two of these patients underwent germline testing of all five LS-related genes (patients 6821 and 6975). The third (patient 4755) underwent MSI testing of a uterine tumor per the protocol of the treating hospital's pathology department because of her young age at diagnosis. Among the 11 patients meeting either Amsterdam II or Bethesda 2004 criteria, the average risk for *MLH1, MSH2,* and *MSH6* mutation ranged from 19.9% (PREMM1,2,6) to 35.0% (MMRPro). In these same patients, the average a priori risk for *PTEN* mutation was 55.5%.

Overlap With APS

Gastrointestinal polyps were identified in most (89/137; 65.0%) patients, and 30 patients (21.9%) had at least 10 confirmed polyps of diverse histologic subtypes. However, no patient had 10 or more confirmed adenomas. Although 36 probands (26.3%) reported that a first- or second-degree relative had one or more polyps, patient knowledge of the histologic subtype or approximate number of polyps that relatives had was poor.

Six patients were tested for mutations in *APC* and/or *MUTYH* (Table 5). Although four had at least 10 colorectal pol-

Table 4. Patients tested for LS and/or meeting LS testing criteria

Patient ID	Sex	Cancer type: age at diagnosis	Other PHTS features	PTEN mutation risk	Family history suspicious for LS	PREMM1,2,6 mutation risk	MMRPro mutation risk	Meets BC	Meets AC	MSI/IHC Testing	Lynch germline testing
4565	F	Breast: 48 EC: 56	FCBD, fibroma, MNG, L, Ma, OMP	41%	Mother EC at age 77; maternal aunt stomach at age 40s	16.8%	4.4%	No	Yes	MSI, IHC of MLH1/MSH2/MSH6/PMS2	No
2986[a]	F	CRC: 35 DCIS: 47 EC: 53 Breast: 56	FCBD, GP, Ma, MNG, L, OMP, T	>99%	Father CRC at age 50; paternal uncle CRC at age 40; paternal grandfather CRC at unknown age	76.2%	99.9%	Yes	Yes	Not analyzed	*MLH1/MSH2* sequencing/rearrangement; *MSH6* sequencing
4755	F	Thyroid: 17 EC: 21	MNG, Ma	>99%	None	6.3%	19.9%	No	No	MSI	No
6821	F	EC: 68 Breast: 66	FCBD, UF, Ma, MNG	7%	Brother CRC at age 62; both grandfathers CRC at age 70s	13.3%	1.3%	No	No	Not analyzed	*MLH1/MSH2/MSH6/PMS2* normal sequencing/rearrangement; *EPCAM* normal rearrangement
6975	F	EC: 29	MNG, L, Ma, OMP	72%	Mother EC at age 59	14.9%	18.8%	No	No	Not analyzed	*MLH1, MSH2, MSH6, PMS2* normal sequencing/rearrangement; *EPCAM* normal rearrangement
7018	F	CRC: 31	Ma, L, MNG	41%	None	7.3%	3.4%	Yes	No	MSI, IHC of MLH1/MSH2/MSH6/PMS2	No
2466	F	DCIS: 31 CRC: 47 Breast: 48	FCBD, MNG, L, Ma	7%	None	<5%	5.80%	Yes	No	Not analyzed	No
2736	F	Ovarian: 63	GP, UF, MNG, FCBD, Ma	10%	Daughter CRC at age 38; Father CRC at age 50s	28.1%	63.3%	No	Yes	Not analyzed	No
2127	F	EC: 41 CRC: 48	GP, MNG	12%	Maternal aunt CRC at age 50s	21.1%	86.9%	Yes	No	Not analyzed	No
47	M	CRC: 39	AK, L, Ma, OMP, T	54%	None	8.8%	3.6%	Yes	No	Not analyzed	No
4886[a]	F	CRC: 21	MNG, L, Ma	72%	None	12%	8.6%	Yes	No	Not analyzed	No
3935	M	CRC: 43	AK, PF, MNG, T, Ma, OMP	97%	Mother EC at age 60	16.6%	79.1%	Yes	No	Not analyzed	No
6092	M	Renal: 49 CRC: 53 CRC: 53	MNG, GP, Ma	88%	None	13.2%	26.8%	Yes	No	Not analyzed	No
5183	F	Melanoma: 32 Melanoma: 36 Breast: 50 EC: 49	Ma, AK, MNG, L, FCBD, OMP, UF	88%	Mother ovarian at age 87; maternal grandmother EC at age 70s	15.2%	3.4%	No	Yes	Not analyzed	No

[a]Patients also in Table 5.

Abbreviations: AC, Amsterdam II criteria; AK, acral keratoses; BC, Bethesda 2004 criteria; CRC, colorectal cancer; DCIS, ductal carcinoma in situ; EC, endometrial cancer; F, female; FCBD, fibrocystic breast disease; GI, gastrointestinal; GP, GI polyposis; ID, identification; IHC, immunohistochemistry; L, lipoma; LS, Lynch syndrome; M, male; Ma, macrocephaly; MNG, multinodular goiter; MSI, microsatellite instability; OMP, oral mucosa papillomatosis; PF, penile freckling; PHTS, PTEN Hamartoma Tumor Syndrome; T, trichilemmoma; UF, uterine fibroids.

yps, none had more than 5 confirmed adenomas. One of the two patients with a personal history of colorectal cancer also underwent germline LS testing. Patients initially tested for APS were the group with highest a priori *PTEN* mutation probability, averaging 73%. As in those patients tested for LS, *APC* and/or *MUTYH* testing yielded normal results in all patients.

Discussion

In nearly half (67/137; 48.9%) of all probands ultimately discovered to have germline *PTEN* mutations, personal or family history findings led to the patient meeting testing criteria for HBOC and/or LS. A total of 45 patients (32.8%) were offered or underwent germline or tumor testing for HBOC, LS, and/or APS, and a total of 46 tests were ordered for other conditions prior to *PTEN* analysis. While this is reasonable for patients meeting standard testing criteria for the syndrome in question, for others, this testing may have been avoided had the *PTEN* risk assessment tool existed when the patient's initial clinic visit occurred. Subtracting the two PHTS patients who also had *BRCA2* mutations as well as those patients for whom risk for the other syndrome was greater than or equivalent to the patient's a priori *PTEN* mutation risk, testing of 22 conditions at a total cost of $66,080 could have been avoided. Testing of 22 conditions at a total cost of $66,080 could have been avoided. This testing included 15 tests for HBOC, 6 tests for APS, and 1 test of all LS-related genes.

Of the patients with PHTS for whom *BRCA1/2* analysis could have been justified based on the patient meeting NCCN testing criteria for HBOC, the only significant differences between those first testing negative for *BRCA1/2* mutation and those for whom *PTEN* was prioritized were findings that would also raise concern for LS as well as initial risk analysis performed by a genetics specialist. This speaks to two important points. For one, we found characteristics reminiscent of both HBOC and LS in 8% (11/137) of patients with germline *PTEN* mutations. This could muddy the risk assessment waters for a provider unfamiliar with less popular genetic syndromes such

Table 5. Patients tested for adenomatous polyposis syndromes

Patient ID	Sex	Cancer type: age at diagnosis	Confirmed adenomas (n)	Histology: number of other polyps	Other PHTS features	*PTEN* mutation risk	Family history of GI, other *APC*-suspicious cancers	Family history of GI polyps	*APC/MUTYH* testing
5270	F	N/A	"At least" 5	HP: >6 L: 1	AVM, Ma, FCBD, MNG, L	97%	Father stomach at age 75	None noted	*APC* sequencing/rearrangement *MUTYH* - G382D/Y165C
3818	M	LDD: 49	0	HP: 5 NB: ~100	L, Ma	12%	None	Brother, but number and type unknown	*APC* sequencing/rearrangement *MUTYH* - G382D/Y165C
5021	M	LDD: 49	1	FG: 25 Gang: 1 NB: 20	Ma	91%	Maternal grandfather stomach at unknown age	None noted	*APC* sequencing/rearrangement *MUTYH* - G382D/Y165C
4886[a]	F	Rectal: 22	0	Gang: 1 L: 3 NB: 7	MNG, L, Ma	72%	Maternal uncle pancreas at age 60s	Maternal grandmother and paternal grandfather had "several," but type unknown	*APC* sequencing/rearrangement *MUTYH* - G382D/Y165C
3577	M	None	2	FG: 1 HP: 1 Gang: 1	L, Ma	98%	Maternal great-uncle osteosarcoma at age 76; nephew sarcoma at age 21; paternal uncle pancreas at age 51; paternal grandmother colon at unknown age	Cousin had "multiple" adenomas at age 47	*APC* sequencing
2986[a]	F	Colon: 35 DCIS: 47 EC: 53 Breast: 56	"At least" 5	Hamartoma: 3	AK, Ma, T, FCBD, MNG, L, OMP	>99%	Father colon at age 50; paternal uncle colon at age 40; paternal grandfather colon at unknown age	Father had "several," but type unknown	*APC* sequencing/rearrangement

[a]Patients also in Table 4.

Abbreviations: AK, acral keratoses; AVM, arteriovenous malformation; DCIS, ductal carcinoma in situ; EC, endometrial cancer; F, female; FCBD, fibrocystic breast disease; FG, fundic gland; Gang, ganglioneuroma; GI, gastrointestinal; HP, hyperplastic; ID, identification; L, lipoma; LDD, Lhermitte-Duclos disease; M, male; Ma, macrocephaly; MNG, multinodular goiter; N/A, not applicable; NB, not biopsied; OMP, oral mucosa papillomatosis; PHTS, PTEN Hamartoma Tumor Syndrome; T, trichilemmoma.

as PHTS. Direct-to-consumer marketing has increased physician awareness of HBOC [18], creating concern that widespread marketing of a test appropriate for only a small proportion of the population may lead to overconsideration of this syndrome for persons more likely to have sporadic cancer diagnoses or another inherited cancer syndrome. In addition, when genetics specialists (most of whom were genetic counselors) were involved in the patient's initial risk assessment, the underlying PHTS diagnosis was significantly more likely to be recognized and tested for immediately as opposed to first ruling out HBOC, ultimately leading to cost savings. Although it was reassuring to know that those with PHTS who first tested negative for another syndrome were ultimately referred to a genetics specialist for further assessment, how many others with PHTS or another less common cancer predisposition syndrome such as Li-Fraumeni or Hereditary Diffuse Gastric Cancer remain undiagnosed? Given the cancer burden associated with PHTS, timely diagnosis is critical so that patients can begin receiving appropriate screening for the cancers associated with this syndrome and site-specific mutation analysis can be performed on at-risk family members.

Although polyps of diverse histologic subtypes and variable numbers were reported, no patient had 10 or more adenomas, the accepted threshold for offering genetic testing for APS [19]. Six patients in this series were tested for mutations in *APC* and/or *MUTYH* prior to *PTEN* analysis, despite having no more than five adenomas. Given that the diversity of gastrointestinal polyps associated with PHTS has only recently been elucidated [8], we are hopeful that, prospectively, these data will help providers recognize the benefits gained from the time and effort invested in gathering and reviewing polyp pathology reports. Patients may not be aware that polyps were found, and may not be reliable reporters of the histologic subtype [20].

Genetic testing technology is advancing at a rapid pace. Presently, panels that test for multiple genes associated with a particular phenotype exist, but each panel's cost is still greater than one individual gene test, and the status of insurance coverage for these new tests is still in question, creating a possible financial burden for patients. Additionally, when multiple genes are screened at once, the likelihood for several variants of uncertain significance to be identified increases. Whereas such testing may make sense for syndromes caused by multiple genes or for syndromes with nondescript or few features, PHTS stands apart in that only one gene is involved and adult patients have multiple dermatologic and physical findings in addition to multisystem disease involvement. While it may seem easier to order a test than to evaluate head size, study pathology reports, and take a full patient medical history, identifying when PHTS is highly suspect can result in cost savings to both the patient and the healthcare system. This clinical information also serves as a useful corollary for multigene panels. Such testing can identify multiple variants of uncertain significance for which clinical and family history information are needed to help the testing laboratory better interpret patient results [21]. The laboratory would also be able to check the read quality of the highest-risk genes to determine whether the coverage of those areas was sufficient; if not, gene-specific testing would then be called for. If read coverage

is sufficient but results are uninformative, the clinician would then consider additional testing to detect large deletions or duplications (disease-causing alterations not detected by multigene testing technologies) in the genes where a priori mutation risk was greatest.

As health care systems attempt to be appropriately cost-effective by minimizing expenditures, the use of tools that assist clinicians in the risk assessment process becomes even more important. Until recently, risk prediction models did not exist to help clinicians gauge the probability for a particular patient to carry a germline *PTEN* mutation in a manner similar to those created for HBOC and LS. The Cleveland Clinic *PTEN* risk calculator is based on the patient's personal medical history and physical findings and has been shown to outperform the NCCN CS testing criteria [11]. This tool was developed from a prospective series of 3,042 probands at minimum meeting relaxed International Cowden Consortium diagnostic criteria. For each studied phenotype, a weighted score was awarded after comparing age-related prevalence within mutation positive and negative research participants to expected community frequencies as derived from published literature and the Surveillance Epidemiology and End Results database. The weight was adjusted for phenotypes where referral bias was evident, mostly for cancer diagnoses, and then totaled to calculate a total PTEN risk score, which then correlates with percent risk (a priori probability) for finding a germline *PTEN* mutation. Using the tool also provides clinicians with a ready-made checklist of the most relevant medical history questions to explore with the patient, which can be especially helpful for topics unrelated to the patient's initial reason for referral (i.e., inquiring about thyroid nodules in patients with breast cancer or measuring head circumference in patients with endometrial cancer). Head circumference measurement has previously been suggested as a helpful screen for PHTS in patients being seen at breast and thyroid cancer clinics [22, 23].

We acknowledge the limitations inherent in this study, the first being possible ascertainment bias. Average age at diagnosis of breast, colon, and uterine cancers was younger for probands compared with their close relatives, who may or may not have also shared a PHTS diagnosis. Given that personal and family history of early-onset cancers is a key indicator for cancer genetics referral, it is possible that our data set includes an overrepresentation of patients with these characteristics, albeit these patients and families are typical of those presenting to cancer genetics and various high-risk cancer clinics. This enrichment may have been a factor in identifying two probands with both germline *PTEN* and *BRCA2* mutations. However, it also leads one to consider whether patients in whom *PTEN* alone was tested may have a second mutation in another cancer predisposition gene that might contribute to their presentation. With the recent Supreme Court decision now enabling all testing companies to include *BRCA1/2* in their panels, we predict that multiplex panels will be both clinically useful and cost-effective for patients with near-equivalent risks for HBOC and PHTS or other conditions. Given the relatively low a priori *BRCA1/2* mutation probability ($<$10%) in 14 of the 19 patients meeting NCCN HBOC testing criteria but not offered this analysis, it seems unlikely that a second mutation is present in many other patients. However, risk for mutation in one of the LS-related genes was still considerable for half of the patients meeting Amsterdam II or Bethesda 2004 criteria for whom germline or tumor testing was not pursued. Because the PREMM1,2,6 and MMRpro tools do not calculate *PMS2* or *EPCAM* mutation risks, these models may underestimate a patient's risk for having a gene mutation causative of LS. Knowing that most patients with LS have mutations in *MLH* or *MSH2* [24], this underestimation is likely small.

Conclusion

In summary, when patients are referred for consideration of HBOC, LS, or APS, PHTS should also be considered and investigated for. In patients with gastrointestinal polyposis, confirming polyp pathology prior to pursuing genetic testing is a critical first step to develop an informed differential diagnosis. Using the Cleveland Clinic *PTEN* risk assessment tool can help clinicians prioritize testing, providing a numerical risk percentage for *PTEN* mutation to compare with current and future risk assessment models for other syndromes. Our observations based on these specific examples of common heritable cancer syndromes underscore the challenge of overlapping phenotypes confounding genetic differential diagnoses and the possibility of serial gene testing delaying appropriate diagnosis and wasting health care dollars. Our data suggest that one solution may be the judicious use of risk assessment tools. Importantly, our observations here are germane to the fast-approaching whole genome sequencing for clinical application. Until every single variant is associated with clear clinical outcomes, focusing analysis on the clinically relevant portions of a whole genome sequence will likely be a reasonable strategy, and family history and risk assessment tools will help guide the focus [21].

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Author Contributions

Conception/Design: Jessica L. Mester, Charis Eng
Provision of study material or patients: Charis Eng
Collection and/or assembly of data: Jessica L. Mester, Rebekah A. Moore, Charis Eng
Data analysis and interpretation: Jessica L. Mester, Rebekah A. Moore, Charis Eng
Manuscript writing: Jessica L. Mester, Charis Eng
Final approval of manuscript: Jessica L. Mester, Rebekah A. Moore, Charis Eng

Disclosures

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