CANCER GENETICS

original reports abstract

Cancer Risk Associated With *PTEN* Pathogenic Variants Identified Using Multigene Hereditary Cancer Panel Testing

Shelly Cummings, MS[1]; Andrew Alfonso, MS[1]; Elisha Hughes, PhD[1]; Matt Kucera, MStat[1]; Brent Mabey, MStat[1]; Nanda Singh, PhD[1]; and Charis Eng, MD, PhD[2,3,4,5]

PURPOSE *PTEN*-associated clinical syndromes such as Cowden syndrome (CS) increase cancer risk and have historically been diagnosed based upon phenotypic criteria. Because not all patients clinically diagnosed with CS have *PTEN* pathogenic variants (PVs), and not all patients with *PTEN* PVs have been clinically diagnosed with CS, the cancer risk conferred by *PTEN* PVs calculated from cohorts of patients with clinical diagnoses of CS/CS-like phenotypes may be inaccurate.

METHODS We assessed a consecutive cohort of 727,091 individuals tested clinically for hereditary cancer risk, with a multigene panel between September 2013 and February 2022. Multivariable logistic regression models accounting for personal and family cancer history, age, sex, and ancestry were used to quantify disease risks associated with *PTEN* PVs.

RESULTS *PTEN* PVs were detected in 0.027% (193/727,091) of the study population, and were associated with a high risk of female breast cancer (odds ratio [OR], 7.88; 95% CI, 5.57 to 11.16; $P = 2.3 \times 10^{-31}$), endometrial cancer (OR, 13.51; 95% CI, 8.77 to 20.83; $P = 4.2 \times 10^{-32}$), thyroid cancer (OR, 4.88; 95% CI, 2.64 to 9.01; $P = 4.0 \times 10^{-7}$), and colon polyposis (OR, 31.60; CI, 15.60 to 64.02; $P = 9.0 \times 10^{-22}$). We observed modest evidence suggesting that *PTEN* PVs may be associated with ovarian cancer risk (OR, 3.77; 95% CI, 1.71 to 8.32; $P = 9.9 \times 10^{-4}$). Among patients with similar personal/family history and ancestry, every 5-year increase in age of diagnosis decreased the likelihood of detecting a *PTEN* PV by roughly 60%.

CONCLUSION We demonstrate that *PTEN* PVs are associated with significantly increased risk for a range of cancers. Together with the observation that *PTEN* PV carriers had earlier disease onset relative to otherwise comparable noncarriers, our results may guide screening protocols, inform risk-management strategies, and warrant enhanced surveillance approaches that improve clinical outcomes for *PTEN* PV carriers, regardless of their clinical presentation.

JCO Precis Oncol 7:e2200415.

BACKGROUND

The tumor-suppressor phosphatase PTEN antagonizes the phosphatidylinositol 3-kinase/AKT signaling pathway that regulates apoptosis, cell-cycle arrest, and other cellular pathways.[1-3] Heterozygous germline pathogenic variants (PVs) in *PTEN* cause *PTEN* hamartoma tumor syndrome (PHTS). Multiple clinically diagnosed disorders, including Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), and Proteus-like syndrome, have been associated with germline PV in *PTEN*.[4-9] Individuals clinically diagnosed with CS may or may not have an underlying germline *PTEN* PV.[10-13] Indeed, *PTEN* PVs are only found in approximately 30%-35% of individuals with a clinical diagnosis of CS/CS-like and approximately 60% of individuals considered to have BRRS.[14] By definition, all *PTEN* PV carriers have a molecular diagnosis of PHTS, whether or not they meet diagnostic criteria for CS, BRRS, Proteus, or Proteus-like syndrome.

Patients molecularly diagnosed with PHTS have an elevated risk of benign and malignant tumors,[11] yet quantifying the cancer risk directly attributable to *PTEN* PVs has been challenging. Early analyses of *PTEN* cancer risk focused exclusively on patients with a clinical diagnosis of CS, but such evaluation does not fully reflect the cancer risk of a *PTEN* PV because many patients clinically diagnosed with CS do not have *PTEN* PVs.[4,9] Later analyses that assessed cancer risk in the subset of patients with CS known to harbor *PTEN* PVs also may not be accurate because not all patients with *PTEN* PVs are diagnosed with CS.[10,14]

With the widespread utilization of gene-panel sequencing in patients who have known cancer status, it

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on November 30, 2022 and published at ascopubs.org/journal/po on January 12, 2023: DOI https://doi.org/10.1200/PO.22.00415

CONTEXT

Key Objective

Cancer risks associated with germline pathogenic variants (PVs) in *PTEN* have been established from patients ascertained for Cowden or related syndromes, potentially resulting in inaccurate estimates of risk. This work describes cancer risks and cancer types associated with *PTEN* PVs in a large hereditary cancer screening cohort using a previously established methodology.

Knowledge Generated

PTEN PVs are associated with a high risk of female breast, endometrial, and thyroid cancers, as well as colon polyposis, and may be associated with ovarian cancer risk. *PTEN* PV carriers have an earlier age of onset than otherwise comparable individuals without *PTEN* PVs.

Relevance

These findings may help guide screening and risk-management strategies for individuals with *PTEN* PVs, regardless of clinical presentation.

is now possible to analyze the cancer risk associated with *PTEN* PVs irrespective of a patient's clinical presentation. Here, we assessed the cancer risk conferred by *PTEN* PVs in > 700,000 patients, who met broad criteria for clinical panel testing, by using a multivariable logistic regression framework that isolates the impact of *PTEN* PVs after accounting for clinical factors.

METHODS

Study Population Participants

We examined clinical and genetic records from a consecutive cohort of patients who underwent hereditary cancer testing between September 2013 and February 2022. Patients were eligible for inclusion if they were age 18 years or older at the time of testing and negative for PVs on cancer-associated genes other than *PTEN*. Patients were excluded from analysis if they were submitted from states that disallow use of genetic data after completion of genetic testing, if they submitted an incomplete test request form (TRF), or if they had multigene panel testing after receiving negative test results from a limited gene panel. Analyses were restricted to patients with full panel sequencing to ensure a homogenous study population. This study was conducted according to a study protocol that was approved by the Advarra Institutional Review Board (Pro00036775) with a waiver of informed consent. Further details are provided in Appendix 1.

Hereditary Cancer Testing

Genetic testing was performed in a Clinical Laboratory Improvement Amendments– and College of American Pathology–approved laboratory (Myriad Genetic Laboratories Inc, Salt Lake City, UT). The hereditary cancer panel was composed of 25-35 cancer-associated genes; the initial multigene panel test included 25 genes (*APC*, *ATM*, *BARD1*, *BMPR1A*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDK4*, *CHEK2*, *MLH2*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *P14ARF*, *P16*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *SMAD4*, *STK11*, and *TP53*). Subsequent additions to the panel test in 2016 and 2019 included *GREM1*, *HOXB13*, *POLD1*, *POLE*, *AXIN2*, *GALNT12*, *MSH3*, *NTHL1*, *RNF43*, and *RPS20*. This next-generation sequencing assay has been detailed previously.[15,16] Sequencing and large rearrangement analysis was performed for all genes evaluated except *HOXB13*, *POLD1*, and *POLE*, for which only sequencing is performed, and *EPCAM* and *GREM1*, in which only large rearrangement analysis is performed.

Variant classification was performed using the American College of Molecular Genetics and Genomics and Association for Molecular Pathology guidelines, as well as previously described statistical variant classification methods.[17-20] Variants with a laboratory classification of deleterious or suspected deleterious were considered pathogenic.

Statistical Methods

All analyses were conducted using R version 4.1 or higher.[21] CIs were calculated from Wald statistics. *P* values were derived from Wald statistics and reported as two-sided.

Association With Cancer Risk

We quantified disease risks associated with *PTEN* using a previously described multivariable logistic regression methodology.[22] Disease risks were estimated as adjusted odds ratios (ORs), with 95% CIs. These adjusted ORs represent the relative risks associated with *PTEN* PVs after accounting for other risk factors and may be interpreted as the fold-increase in risk for a *PTEN* PV carrier compared with a noncarrier who is identical with respect to age, personal/family cancer history, and ancestry. The adjusted ORs presented here should be consistent with the ORs that would be obtained from a population-based study using either multivariable regression or matched case-control analysis to adjust for age, personal/family cancer history, and ancestry.[22-24] As *PTEN* PVs are associated with childhood mortality,[25] the ORs in this study—where only

patients age ≥ 18 years were included—represent risks for *PTEN* carriers who survive to adulthood.

A threshold of five or more disease-affected *PTEN* PV carriers was prespecified as the minimum data required to investigate association with a specific disease using multivariable regression. We constructed a separate multivariable logistic regression model for each disease that met the minimum data threshold. Models testing association with female-specific cancers were restricted to female patients. For each disease, we coded disease status (affected or unaffected) as the dependent variable. Independent variables included *PTEN* PV status, age, sex (where applicable), ancestry, and personal and family histories associated with hereditary breast and ovarian cancer (HBOC), Lynch syndrome, and adenomatous polyposis colon cancer syndrome. Family history variables were coded as numeric counts of diagnoses, weighted according to degree of relatedness: we used a weight of 0.5 for each first-degree relative (FDR) and 0.25 for each second-degree relative (SDR). Further details regarding coding of variables are provided in Appendix 1.

Association With Age of Diagnosis

We investigated whether *PTEN* PV carriers tended to be diagnosed at earlier ages than noncarriers who were similar with respect to personal/family history and ancestry. For each phenotype that showed significant association with *PTEN*, we constructed a multivariable logistic regression model restricted to the subcohort of patients affected by that specific disease. *PTEN* PV status was the dependent variable in each disease model. Independent variables for age at diagnosis, ancestry, sex (where applicable), and personal/family history were coded as above for tests of association with disease risk.

Association With Familial Cancer

We tested increased rates of family cancer history among *PTEN* PV carriers compared with noncarriers with similar clinical features. For this analysis, we constructed a single multivariable logistic regression model on the basis of the entire study cohort. The dependent variable was *PTEN* PV status. Independent variables for age, ancestry, sex, and personal/family cancer history were coded as above for tests of association with cancer risk. ORs for association of *PTEN* PV status with familial disease were reported per one-half unit of the weighted relative count described above and represent the fold-increase in likelihood of detecting a *PTEN* PV because of one affected FDR or two affected SDRs. We examined all diseases for which five or more *PTEN* PV carriers reported at least one affected FDR or SDR.

RESULTS

We identified 727,091 patients who met study eligibility criteria. Clinical characteristics are detailed in Table 1. The study cohort was predominantly composed of female patients (699,209 [96.2%]) who were referred for genetic testing because of suspected HBOC syndrome. Nearly one third (226,120 [31.1%]) of patients reported a personal history of cancer. Most patients (689,692 [94.6%]) reported a FDR or SDR affected by a cancer associated with HBOC, Lynch syndrome, or adenomatous polyposis colon cancer syndrome.

Pathogenic *PTEN* variants were detected in 193 (0.027%) study subjects. Eight additional patients carried *PTEN* PVs but were excluded from the study cohort because they had a concurrent PV in a second hereditary cancer gene (Appendix Table A1). *PTEN* PV carriers were more frequently affected by benign or malignant neoplasms than noncarriers (69.4% *v* 31.1%) and tended to be younger (median age, 41 *v* 46 years) at the time of multigene panel testing (Table 1). Details regarding prevalence and types of cancers affecting study subjects and their families are tabulated by *PTEN* status in Appendix Table A2. Details regarding the distribution of age at diagnosis are provided in Appendix Table A3.

Association With Disease Risk

We had sufficient data to evaluate seven neoplastic phenotypes for association with *PTEN*: ductal invasive breast cancer, ductal carcinoma in situ (DCIS), endometrial cancer, thyroid cancer, colon polyposis (defined as ≥ 20 colon polyps), and nonpolyposis colorectal cancer (defined as colon or rectal cancer with < 20 colon polyps). We defined colon polyposis and nonpolyposis colorectal cancer as in previous work[22] on the basis of different genetic syndromes associated with colon polyposis versus hereditary nonpolyposis colon cancer. We further evaluated risk associated with overall female breast cancer, defined as any occurrence of ductal invasive breast cancer, lobular invasive breast cancer, or DCIS.

PVs in *PTEN* were associated with a high risk of overall female breast cancer (OR, 7.88; 95% CI, 5.57 to 11.16; $P = 2.3 \times 10^{-31}$), ductal invasive breast cancer (OR, 7.55; 95% CI, 5.24 to 10.88; $P = 1.7 \times 10^{-27}$), DCIS (OR, 11.56; 95% CI, 6.52 to 20.50; $P = 5.6 \times 10^{-17}$), endometrial cancer (OR, 13.51; 95% CI, 8.77 to 20.83; $P = 4.2 \times 10^{-32}$), thyroid cancer (OR, 4.88; 95% CI:, 2.64 to 9.01; $P = 4.0 \times 10^{-7}$), and colon polyposis (OR, 31.60; CI, 15.60 to 64.02; $P = 9.0 \times 10^{-22}$). We observed modest evidence suggesting association with risk of ovarian cancer (OR, 3.77; 95% CI, 1.71 to 8.32; $P = 9.9 \times 10^{-4}$). *PTEN* PVs were not associated with risk of nonpolyposis colorectal cancer (OR, 1.31; 95% CI, 0.48- to 3.59; $P = .60$). The results from multivariable regression models testing association of *PTEN* with disease risk are detailed in Table 2 and Appendix Table A4.

Association With Age of Diagnosis

For most *PTEN*-associated diseases, we found that PV carriers were diagnosed at earlier ages than noncarriers with similar clinical characteristics (Table 3 and Appendix Table A5). Among patients with breast cancer who were equivalent with respect to personal/family history and ancestry, every 5-year increase in age of diagnosis decreased the likelihood of

TABLE 1. Patient Characteristics

Characteristic	All, No. (%)	With *PTEN* PVs, No. (%)	Without *PTEN* PVs, No. (%)
Age at hereditary cancer testing, years			
Median	46	41	46
% ≤ 50	458,678 (63.1)	147 (76.2)	458,531 (63.1)
Cancer history			
Personal history of cancer	226,120 (31.1)	134 (69.4)	225,986 (31.1)
Family history of cancer	689,692 (94.6)	171 (88.6)	689,521 (94.6)
Sex			
Male	27,882 (3.8)	18 (9.3)	27,864 (3.8)
Female	699,209 (96.2)	175 (90.7)	699,034 (96.2)
Ancestry			
Ashkenazi Jewish	5,503 (0.8)	0 (0)	5,503 (0.8)
Asian	18,770 (2.6)	6 (3.1)	18,764 (2.6)
Black/African	77,969 (10.7)	23 (11.9)	77,946 (10.7)
Hispanic/Latino	71,598 (9.8)	31 (16.1)	71,567 (9.8)
Middle Eastern	4,469 (0.6)	2 (1.0)	4,467 (0.6)
Native American	5,380 (0.7)	2 (1.0)	5,378 (0.7)
Pacific Islander	893 (0.1)	0 (0.0)	893 (0.1)
White	474,118 (65.2)	115 (59.6)	474,003 (65.2)
Other	3,068 (0.4)	0 (0)	3,068 (0.4)
Multiple	65,323 (9.0)	14 (7.3)	65,309 (9.0)
Total	727,091	193 (0.03)	726,898 (99.97)

Abbreviation: PV, pathogenic variant.

detecting a *PTEN* PV by a factor of roughly 62% (OR per 5 years, 0.62; 95% CI, 0.56 to 0.70; $P = 1.5 \times 10^{-17}$). Similar associations between *PTEN* carrier status and age of diagnosis were observed for ductal invasive breast cancer, DCIS, endometrial cancer, thyroid cancer, and ovarian cancer (Table 3). Although *PTEN* PV carriers had substantially increased risk of colon polyposis, they did not have earlier onset than noncarriers (OR per 5 years, 1.01; 95% CI, 0.75 to 1.36; $P = .96$). Further details are provided in Table 3.

Association With Familial Cancer

We had sufficient data to evaluate whether *PTEN* carriers had stronger family histories than noncarriers with similar clinical characteristics for 11 different hereditary diseases (Table 4). For most hereditary diseases, we found that patients with PVs in *PTEN* did not have stronger family histories than noncarriers who were equivalent with respect to sex, personal history, age, and ancestry (Table 4 and Appendix Table A6). We observed marginal significance for association between *PTEN* PV status and familial kidney cancer: the likelihood of detecting a *PTEN* PV increased by roughly 2.28-fold because of each FDR or 2 SDRs affected by kidney cancer (OR, 2.28; 95% CI, 1.16 to 4.47; $P = .017$). No other familial diseases were more common among *PTEN* PV carriers than among noncarriers with similar clinical features.

Sex-Specific Sensitivity Analyses

For diseases that affect both males and females, and for which we had adequate data, we conducted sensitivity analyses by retesting associations within subcohorts defined by sex. We found no evidence of sex-specific differences in disease risk (Appendix Table A4), age of diagnosis (Appendix Table A5), or association with familial cancer (Appendix Table A6).

DISCUSSION

Here, we quantified the risk conferred by *PTEN* PVs on a range of cancers in a cohort containing > 700,000 patients screened with a clinical-grade multigene sequencing panel. To focus specifically on *PTEN* PV carriers, our analysis differed from preceding studies in two key aspects: first, patient inclusion in our cohort was independent of a clinical diagnosis for CS or CS-like phenotype or the *PTEN* risk calculator[11]; second, we used multivariable logistic regression analysis to isolate the impact of *PTEN* PVs after accounting for other clinical parameters. Our results suggest that, irrespective of clinical diagnosis, *PTEN* PVs significantly increase the risk for female breast cancer, endometrial cancer, thyroid cancer, and colon polyposis. After adjusting for other factors, we found that *PTEN* PVs were associated with earlier disease onset. Additionally, our observation of little to no association between *PTEN* PVs

TABLE 2. Association of *PTEN* Pathogenic Variant Status With Disease Risk

Characteristics	OR[a] (95% CI)	*P*
Overall female breast cancer[b]	7.88 (5.57 to 11.16)	2.3×10^{-31}
Ductal invasive breast cancer	7.55 (5.24 to 10.88)	1.7×10^{-27}
DCIS	11.56 (6.52 to 20.50)	5.6×10^{-17}
Endometrial cancer	13.51 (8.77 to 20.83)	4.2×10^{-32}
Thyroid cancer	4.88 (2.64 to 9.01)	4.0×10^{-7}
Colon polyposis	31.60 (15.60 to 64.02)	9.0×10^{-22}
Nonpolyposis colorectal cancer	1.31 (0.48 to 3.59)	.60
Ovarian cancer	3.77 (1.71 to 8.32)	9.9×10^{-4}

Abbreviations: DCIS, ductal carcinoma in situ; OR, odds ratio.
[a]ORs are adjusted for age, personal/family cancer history, and ancestry.
[b]Any diagnosis of ductal invasive breast cancer, lobular invasive breast cancer, or DCIS.

TABLE 3. Association of *PTEN* Pathogenic Variant Status With Age of Disease Onset

Characteristics	OR[a] per 5 Years (95%CI)	*P*
Overall female breast cancer[b]	0.62 (0.56 to 0.70)	1.5×10^{-17}
Ductal invasive breast cancer	0.63 (0.56 to 0.71)	8.8×10^{-14}
DCIS	0.55 (0.43 to 0.71)	6.4×10^{-6}
Endometrial cancer	0.76 (0.66 to 0.89)	4.0×10^{-4}
Thyroid cancer	0.61 (0.45 to 0.82)	1.2×10^{-3}
Colon polyposis	1.01 (0.75 to 1.36)	.960
Nonpolyposis colorectal cancer	0.62 (0.41 to 0.94)	.026
Ovarian cancer	0.62 (0.42 to 0.92)	.017

Abbreviations: DCIS, ductal carcinoma in situ; OR, odds ratio.
[a]ORs are adjusted for personal/family cancer history, and ancestry.
[b]Any diagnosis of ductal invasive breast cancer, lobular invasive breast cancer, or DCIS.

and familial cancer, despite being sufficiently powered to see such associations, suggests that many *PTEN* PVs arise de novo, consistent with previous reports.[26] Together, we expect these findings to better inform patients, their providers, and clinical-management guidelines about the impact of PVs in *PTEN* identified via sequencing.

Our observation of significantly increased risk of endometrial cancer associated with *PTEN* PVs (OR, 13.51; 95% CI, 8.77 to 20.83; $P = 4.2 \times 10^{-32}$) aligns with previous studies and underscores the importance of consideration of endometrial screening in PV carriers.[27-29] In an analysis of individuals who met one of several eligibility criteria for PHTS, the lifetime risk of developing endometrial cancer was 28%, and relative risk was observed to dramatically increase around age 25 years.[28] In our study, the median age of diagnosis was 37 years (range, 33-48.5 years). Currently, the National Comprehensive Cancer Network (NCCN) recommends individualized screening on the basis of personal and family history, as well as consideration of beginning endometrial screenings by age 35 years.[12] The significance of our regression results and the early age of onset observed herein suggest endometrial screening for all women with a PV in *PTEN* should be considered.

Our results in female breast cancer support current guidelines. We identified a significant female breast cancer OR of 7.88 (95% CI, 5.57 to 11.16; $P = 2.3 \times 10^{-31}$), which is consistent with previous reports in HBOC cohorts[22,30] and consistent in direction with *PTEN*-associated disease cohorts albeit smaller in size likely because of overestimation of risk on the basis of methodology in these individuals.[27,28] Additionally, the median age of onset of 40 years (range, 35-45 years) is consistent with previous reports.[22,30] Multiple studies have confirmed that CS-affected individuals are more likely to have malignant breast neoplasm if they also harbor a *PTEN* PV compared with those without *PTEN* PVs.[4] Furthermore, the previously reported average age of breast cancer diagnosis in individuals with a *PTEN* PV is 36-46 years.[30] Collectively, the results from our group and others support the current NCCN recommendations to start a screening regimen with clinical examinations at or before age 25 years, to conduct breast imaging at or before age 35 years, and to discuss prophylactic mastectomy with individuals harboring a germline PV in *PTEN*.

In our study, males were more highly represented in the *PTEN* PV cohort (9.3%; 18/193) than in the full testing cohort (3.8%; 27,931/727,962), yet too few had cancer diagnoses to fully assess PV-associated cancer risk. Notably, it was previously shown that males comprised 32.4%-44.6% of patients who met CS relaxed criteria (pathognomonic criteria, or at least two criteria, either major or minor) and had an underlying pathogenic *PTEN* variant.[11] Although male breast cancer in an individual with a germline *PTEN* PV has previously been reported,[31] there was no evidence for increased risk in a study of more than 3,000 probands with CS, and male breast cancer was not significantly associated with *PTEN* in that cohort.[11] Despite a significantly elevated lifetime risk of thyroid cancer, men with germline *PTEN* PVs continue to be difficult to ascertain because of a lower lifetime incidence of sentinel cancers typically associated with PHTS that would lead to molecular assessment (eg, breast cancer). When compared with females with germline *PTEN* PVs, males have propensity for developing cancers in sites not traditionally related to CS.[32]

We observed a significant thyroid cancer risk associated with *PTEN* PVs, consistent with previous findings in patients with CS, among whom 11% had epithelial cell thyroid carcinoma as their sentinel cancer.[33] In our study, the median age of diagnosis was 35 years (range, 18.5-39 years). However, studies have revealed earlier onset of thyroid cancer,[32,34] with one specifically finding increased risk in pediatric patients.[34] A prospective study of patients with CS (or Cowden-like syndrome) and a *PTEN*, *SDH*, or *KLLN* PV found that 16.7% presented with thyroid cancer before age 18 years.[34] Although the NCCN currently recommends screening starting at age 7 years, it is important

TABLE 4. Association of *PTEN* Pathogenic Variant Status With Familial Cancer

Characteristics	OR[a] (95% CI)	*P*
Ductal invasive breast cancer	1.07 (0.87 to 1.31)	.500
Endometrial cancer	1.28 (0.81 to 2.02)	.290
Thyroid cancer	1.39 (0.70 to 2.73)	.350
Colon polyposis	1.26 (0.77 to 2.04)	.360
Nonpolyposis colorectal cancer	0.75 (0.51 to 1.12)	.160
Kidney cancer	2.28 (1.16 to 4.47)	.017
Melanoma	0.64 (0.30 to 1.36)	.250
Gastric cancer	0.53 (0.17 to 1.54)	.240
Prostate cancer	1.11 (0.75 to 1.66)	.610
Pancreatic cancer	0.66 (0.30 to 1.42)	.290
Ovarian cancer	0.66 (0.39 to 1.11)	.120

Abbreviation: OR, odds ratio.

[a]Increased odds of detecting a *PTEN* pathogenic variant because of one first-degree or two second-degree relatives. ORs are adjusted for age, personal cancer history, and ancestry.

that individuals continue to follow these screening recommendations, as later ages of onset were observed in the cohort presented here than have been previously reported. It should be noted that the gene panel used in the current study does not include several well-characterized genes associated with a hereditary predisposition to medullary thyroid cancer, such as *RET* or *CDKN1B*. This might have led some patients with a personal or family history of undefined thyroid cancer to use a different diagnostic laboratory offering and could potentially result in an under-representation of patients with a personal and/or family history of thyroid cancer in our cohort.

Although our patient cohort was large and diverse in many respects (eg, ethnicity, cancer type, etc), it was also not a random cross-section of the population. For instance, most patients were tested because they met criteria for HBOC syndrome or Lynch syndrome. To isolate the impact of *PTEN* PV status, we used a multivariable logistic regression analysis that accounts for clinical factors. To ensure the statistical validity of these analyses, we only performed calculations for a given cancer if at least five patients were diagnosed with that cancer and carried *PTEN* PVs. Inaccuracies on the TRF could also influence our results: we typically cannot access health records that permit confirmation of the content reported on the TRF. A further limitation of the TRF is that it does not ascertain polyp histology, limiting the ability to characterize polyposis disease.

Our present work and previous research from ours and other groups elucidate a clear role for *PTEN* PVs in increasing cancer risk. Less clear is the precise way particular *PTEN* genotypes affect a patient's phenotype, and this is an important topic for future work. Thorough examination of risk modifiers (eg, family history of specific cancers), tumor histologic subtypes, and particular DNA variants (eg, single-nucleotide polymorphisms, indels, and copy-number variants)[35] will likely provide key insights into the highly variable clinical manifestation of *PTEN*-associated disorders.

AFFILIATIONS

[1]Myriad Genetics Inc, Salt Lake City, UT
[2]Genomic Medicine Institute, Cleveland Clinic, Cleveland, OH
[3]Center for Personalized Genetic Healthcare, Cleveland Clinic Community Care, Cleveland, OH
[4]Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH
[5]Department of Genetics and Genome Sciences, and CASE Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH

CORRESPONDING AUTHOR

Shelly Cummings, MS, 320 Wakara Way, Salt Lake City, UT 84108; e-mail: scumming@myriad.com.

EQUAL CONTRIBUTION

S.C., A.A., and E.H. contributed equally to this work as first authors.

AUTHOR CONTRIBUTIONS

Conception and design: Shelly Cummings, Elisha Hughes, Matt Kucera, Charis Eng
Collection and assembly of data: Shelly Cummings, Elisha Hughes, Matt Kucera, Brent Mabey, Nanda Singh
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Shelly Cummings
Employment: Myriad Genetics
Stock and Other Ownership Interests: Myriad Genetics
Travel, Accommodations, Expenses: Myriad Genetics

Andrew Alfonso
Employment: Myriad Genetics
Stock and Other Ownership Interests: Myriad Genetics

Elisha Hughes
Employment: Myriad Genetics
Stock and Other Ownership Interests: Myriad Genetics

Brent Mabey
Employment: Myriad Genetics
Stock and Other Ownership Interests: Myriad Genetics
Research Funding: Myriad Genetics

Nanda Singh
Employment: Myriad Genetics
Stock and Other Ownership Interests: Myriad Genetics
Patents, Royalties, Other Intellectual Property: Royalties for gene patents through the University of Utah, my former employer

Charis Eng
Stock and Other Ownership Interests: Family Care Path Inc
Patents, Royalties, Other Intellectual Property: I am the inventor of MyLegacy/MyFHH (a web-based, patient-entered family health history tool with risk algorithms and clinical decision support pushed to the point of care). This invention is utlized in Cleveland Clinic spin off company, Family Care Path Inc (see above)
Uncompensated Relationships: Family Care Path Inc

No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

The authors would like to thank Dale Muzzey, PhD, for assistance with manuscript editing, as well as Elizabeth Cogan, PhD, and Sarah Ratzel, PhD, for assistance with manuscript editing and preparation, and Nydia Santiago Neal for assistance with concept and design.

REFERENCES

1. Steck PA, Pershouse MA, Jasser SA, et al: Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 15:356-362, 1997
2. Maehama T, Dixon JE: The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3, 4, 5-trisphosphate. J Biol Chem 273:13375-13378, 1998
3. Stambolic V, Suzuki A, de la Pompa JL, et al: Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 95:29-39, 1998
4. Marsh DJ, Coulon V, Lunetta KL, et al: Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline PTEN mutation. Hum Mol Genet 7:507-515, 1998
5. Marsh DJ, Kum JB, Lunetta KL, et al: PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. Hum Mol Genet 8:1461-1472, 1999
6. Orloff MS, Eng C: Genetic and phenotypic heterogeneity in the PTEN hamartoma tumour syndrome. Oncogene 27:5387-5397, 2008
7. Yehia L, Eng C: PTEN hamartoma tumor syndrome, in Adam M, Ardinger H, Pagon R (eds): GeneReviews. Seattle, WA, University of Washington, 2021
8. Nelen MR, Padberg GW, Peeters EAJ, et al: Localization of the gene for Cowden disease to chromosome 10q22–23. Nat Genet 13:114-116, 1996
9. Liaw D, Marsh DJ, Li J, et al: Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 16:64-67, 1997
10. Pilarski R, Stephens JA, Noss R, et al: Predicting PTEN mutations: An evaluation of Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome clinical features. J Med Genet 48:505-512, 2011
11. Tan MH, Mester J, Peterson C, et al: A clinical scoring system for selection of patients for PTEN mutation testing is proposed on the basis of a prospective study of 3042 probands. Am J Hum Genet 88:42-56, 2011
12. Daly MB, Pilarski R, Berry M, et al: NCCN clinical practice guidelines in oncology, genetic/familial high-risk assessment: Breast, ovarian, and pancreatic (version 2.2022). NCCN clinical practice guidelines in oncology web site. 2022. https://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf
13. Eng C: Will the real Cowden syndrome please stand up: Revised diagnostic criteria. J Med Genet 37:828-830, 2000
14. Pilarski R: PTEN hamartoma tumor syndrome: A clinical overview. Cancers (Basel) 11:844, 2019
15. Yurgelun MB, Allen B, Kaldate RR, et al: Identification of a variety of mutations in cancer predisposition genes in patients with suspected Lynch syndrome. Gastroenterology 149:604-613.e20, 2015
16. Judkins T, Leclair B, Bowles K, et al: Development and analytical validation of a 25-gene next generation sequencing panel that includes the BRCA1 and BRCA2 genes to assess hereditary cancer risk. BMC Cancer 15:215, 2015
17. Richards S, Aziz N, Bale S, et al: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405-424, 2015
18. Bowles KR, Mancini-DiNardo D, Coffee B, et al: Hereditary cancer testing challenges: Assembling the analytical pieces to solve the patient clinical puzzle. Future Oncol 15:65-79, 2019
19. Eggington JM, Bowles KR, Moyes K, et al: A comprehensive laboratory-based program for classification of variants of uncertain significance in hereditary cancer genes. Clin Genet 86:229-237, 2014
20. Pruss D, Morris B, Hughes E, et al: Development and validation of a new algorithm for the reclassification of genetic variants identified in the BRCA1 and BRCA2 genes. Breast Cancer Res Treat 147:119-132, 2014
21. R Development Core Team: R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing, 2022
22. Kurian AW, Hughes E, Handorf EA, et al: Breast and ovarian cancer penetrance estimates derived from germline multiple-gene sequencing results in women. JCO Precis Oncol 10.1200/PO.16.00066, 2017
23. Rothman KJ, Greenland S, Lash TL: Modern Epidemiology, Volume 3. Philadelphia, PA, Wolters Kluwer Health/Lippincott Williams & Wilkins, 2008
24. Rajamani S: Eliminating Bias in Cancer Risk Estimates a Simulation Study [Dissertation]. University of Utah, Salt Lake City, UT, 2016
25. Plamper M, Gohlke B, Woelfle J: PTEN hamartoma tumor syndrome in childhood and adolescence—A comprehensive review and presentation of the German pediatric guideline. Mol Cell Pediatr 9:1-12, 2022
26. Mester J, Eng C: Estimate of de novo mutation frequency in probands with PTEN hamartoma tumor syndrome. Genet Med 14:819-822, 2012
27. Bubien V, Bonnet F, Brouste V, et al: High cumulative risks of cancer in patients with PTEN hamartoma tumour syndrome. J Med Genet 50:255-263, 2013
28. Tan MH, Mester JL, Ngeow J, et al: Lifetime cancer risks in individuals with germline PTEN mutations. Clin Cancer Res 18:400-407, 2012
29. Gammon A, Jasperson K, Champine M: Genetic basis of Cowden syndrome and its implications for clinical practice and risk management. Appl Clin Genet 9:83-92, 2016
30. Nusbaum R, Vogel KJ, Ready K: Susceptibility to breast cancer: Hereditary syndromes and low penetrance genes. Breast Dis 27:21-50, 2006

31. Fackenthal JD, Marsh DJ, Richardson AL, et al: Male breast cancer in Cowden syndrome patients with germline PTEN mutations. J Med Genet 38:159-164, 2001
32. Riegert-Johnson DL, Gleeson FC, Roberts M, et al: Cancer and Lhermitte-Duclos disease are common in Cowden syndrome patients. Hered Cancer Clin Pract 8:6, 2010
33. Ngeow J, Stanuch K, Mester JL, et al: Second malignant neoplasms in patients with Cowden syndrome with underlying germline PTEN mutations. J Clin Oncol 32:1818-1824, 2014
34. Ngeow J, Mester J, Rybicki LA, et al: Incidence and clinical characteristics of thyroid cancer in prospective series of individuals with Cowden and Cowden-like syndrome characterized by germline PTEN, SDH, or KLLN alterations. J Clin Endocrinol Metab 96:E2063-E2071, 2011
35. Yehia L, Seyfi M, Niestroj L-M, et al: Copy number variation and clinical outcomes in patients with germline PTEN mutations. JAMA Netw Open 3:e1920415, 2020

APPENDIX 1. SUPPLEMENTAL MATERIAL

Study Population

Patients were eligible for inclusion if they were age 18 years or older at the time of hereditary cancer testing and negative for pathogenic variants (PVs) in cancer-associated genes other than *PTEN*. Patients were excluded from analysis if they had variants of uncertain significance or low-penetrance variants in any cancer-associated genes, if test results indicated mosaicism in *PTEN*, if they submitted an incomplete test request form, if they had multigene panel testing after receiving negative test results from a limited gene panel, or if they submitted from states that disallow use of genetic data after completion of genetic testing (Alaska, Colorado, Florida, New York, New Hampshire, Oregon, Oklahoma, South Dakota, and Minnesota).

Coding of Variables in Logistic Regression Analysis

PTEN PV carrier status was coded as a binary variable. Patients with a *PTEN* variant classified as deleterious or suspected deleterious were coded as PV-positive. Patients were coded as PV-negative if only benign polymorphisms or no variants were detected.

Age was coded in years as a continuous variable. For each model, age at the time of genetic testing was used for patients unaffected by the cancer used as the outcome variable. Age at diagnosis of cancer was used for patients affected by the cancer used as the outcome variable.

Ancestries were coded as quantitative variables representing fractions of reported ancestries. For example, a patient who listed only Ashkenazi ancestry was coded with an Ashkenazi value of 1.0, and zero for all other ancestries. A patient who reported Asian and African ancestries was coded with Asian and African values of 0.5, and zero for all other ancestries. The ancestry White was used in place of ancestries listed as Central/Eastern Europe, Western/Northern Europe, and White/non-Hispanic. Ancestry variables included Ashkenazi Jewish, Asian, Black/African, Hispanic/Latino, Middle Eastern, Native American, White, and other.

Personal cancer variables were coded as binary (ever or never affected). Separate variables were coded for male breast cancer, ductal carcinoma in situ (DCIS), ductal invasive breast cancer, lobular invasive breast cancer, endometrial cancer, pancreatic cancer, gastric cancer, nonpolyposis colorectal cancer (defined as colon cancer or rectal cancer with < 20 colon polyps), colon polyposis (defined as ≥ 20 colon polyps), kidney cancer, melanoma, ovarian cancer, prostate cancer, and thyroid cancer. Patients with DCIS in addition to ductal invasive breast cancer were recoded to only be considered as having ductal invasive breast cancer. A personal history of overall female breast cancer was defined as an occurrence of ductal invasive breast cancer, DCIS, and/or lobular invasive breast cancer.

Familial cancers were coded as numeric counts of diagnoses, weighted according to degree of relatedness. We used a weight of 0.5 for each first-degree relative and 0.25 for each second-degree relative. All models included family history variables for each cancer type listed above.

TABLE A1. Genes With Concurrent Mutations in Patients With Germline *PTEN* Pathogenic Variants

Gene	No.
BRCA2	2
ATM	2
BRIP1	1
TP53	1
HOXB13	1
MYH	1
Total	8

TABLE A2. Prevalence of Personal and Familial Disease by *PTEN* PV Status

Characteristics	All No. (%)	With *PTEN* PVs No. (%)	Without *PTEN* PVs No. (%)
Personal history of disease			
Overall female breast cancer[a]	154,063 (21.2)	**92** (47.7)	153,971 (21.2)
Ductal invasive breast cancer	124,179 (17.1)	**75** (38.9)	124,104 (17.1)
DCIS	22,035 (3.0)	**17** (8.8)	22,018 (3.0)
Lobular invasive breast cancer	9,968 (1.4)	2 (1.0)	9,966 (1.4)
Male breast cancer	1,362 (0.2)	1 (0.5)	1,361 (0.2)
Endometrial cancer	12,547 (1.7)	**27** (14.0)	12,520 (1.7)
Thyroid cancer	4,751 (0.7)	**12** (6.2)	4,739 (0.7)
Colon polyposis	2,137 (0.3)	**15** (7.8)	2,122 (0.3)
Nonpolyposis colorectal cancer	16,026 (2.2)	**5** (2.6)	16,021 (2.2)
Kidney cancer	1,418 (0.2)	3 (1.6)	1,415 (0.2)
Melanoma	8,106 (1.1)	4 (2.1)	8,102 (1.1)
Gastric cancer	791 (0.1)	0 (0)	791 (0.1)
Prostate cancer	5,226 (0.7)	0 (0)	5,226 (0.7)
Pancreatic cancer	3,318 (0.5)	0 (0)	3,318 (0.5)
Ovarian cancer	17,281 (2.4)	**7** (3.6)	17,274 (2.4)
Family history of disease[b]			
Ductal invasive breast cancer	483,594 (66.5)	104 (53.9)	483,490 (66.5)
DCIS	2,595 (0.4)	2 (1.0)	2,593 (0.4)
Lobular invasive breast cancer	565 (0.1)	0 (0.0)	565 (0.1)
Male breast cancer	11,675 (1.6)	3 (1.6)	11,672 (1.6)
Endometrial cancer	63,729 (8.8)	20 (10.4)	63,709 (8.8)
Thyroid cancer	17,904 (2.5)	9 (4.7)	17,895 (2.5)
Colon polyposis	18,158 (2.5)	7 (3.6)	18,151 (2.5)
Nonpolyposis colorectal cancer	173,737 (23.9)	39 (20.2)	173,698 (23.9)
Kidney cancer	22,853 (3.1)	10 (5.2)	22,843 (3.1)
Melanoma cancer	45,739 (6.3)	9 (4.7)	45,730 (6.3)
Gastric cancer	43,706 (6.0)	8 (4.1)	43,698 (6.0)
Prostate cancer	107,202 (14.7)	30 (15.5)	107,172 (14.7)
Pancreatic cancer	78,605 (10.8)	12 (6.2)	78,593 (10.8)
Ovarian cancer	196,954 (27.1)	28 (14.5)	196,926 (27.1)

NOTE. Entries provided in bold reached significance and are provided in more detail in Table 2.
Abbreviations: DCIS, ductal carcinoma in situ; PV, pathogenic variant.
[a]Any diagnosis of ductal invasive breast cancer, lobular invasive breast cancer, or DCIS.
[b]Any first-degree or second-degree relative.

TABLE A3. Distribution of Age at Diagnosis by *PTEN* PV Status

Characteristics	All Median (IQR)	With *PTEN* PVs Median (IQR)	Without *PTEN* PVs Median (IQR)
Overall female breast cancer[a]	49 (43-58)	40.0 (35.00-45.00)	49 (43-58)
Ductal invasive breast cancer	49 (43-57)	40.0 (35.00-45.50)	49 (43-57)
DCIS	49 (43-57)	40.0 (34.75-43.25)	49 (43-57)
Lobular invasive breast cancer	53 (46-61)	43.0 (38.50-47.50)	53 (46-61)
Male breast cancer	62 (54-70)	—	62 (54-70)
Endometrial cancer	51 (40-60)	37.0 (33.00-48.50)	51 (40-60)
Thyroid cancer	40 (32-49)	35.0 (18.50-39.00)	40 (32-49)
Colon polyposis	50 (41-59)	52.5 (42.50-55.00)	50 (41-59)
Nonpolyposis colorectal cancer	49 (42-58)	45.0 (33.00-49.00)	49 (42-58)
Kidney cancer	52 (43-60)	54.0 (44.50-58.00)	52 (43-60)
Melanoma	43 (33-52)	33.5 (30.00-38.00)	43 (33-52)
Gastric cancer	49 (39-60)	—	49 (39-60)
Prostate cancer	60 (55-66)	—	60 (55-66)
Pancreatic cancer	61 (54-68)	—	61 (54-68)
Ovarian cancer	54 (43-63)	31.5 (16.50-45.75)	54 (43-63)

Abbreviations: DCIS, ductal carcinoma in situ; IQR, interquartile range; PV, pathogenic variant.
[a]Any diagnosis of ductal invasive breast cancer, lobular invasive breast cancer, or DCIS.

TABLE A4. Association of *PTEN* Pathogenic Variant Status With Disease Risk Within Each Sex[a]

Characteristics	Sex	OR[b] (95% CI)	*P*
Colon polyposis	Both	31.60 (15.60 to 64.02)	9.0×10^{-22}
Colon polyposis	Female	43.95 (19.89 to 97.13)	8.6×10^{-21}
Colon polyposis	Male	15.54 (4.68 to 51.58)	7.3×10^{-6}
Thyroid cancer	Both	4.88 (2.64 to 9.01)	4.0×10^{-7}
Thyroid cancer	Female	4.92 (2.60 to 9.29)	9.2×10^{-7}

Abbreviation: OR, odds ratio.
[a]Results shown are for cancers with at least five events in those with *PTEN* pathogenic variants.
[b]ORs are adjusted for age, personal/family cancer history, and ancestry.

TABLE A5. Association of *PTEN* Pathogenic Variant Status With Age of Disease Onset Within Each Sex[a]

Characteristics	Sex	OR[b] per 5 Years (95%CI)	*P*
Colon polyposis	Both	1.01 (0.75 to 1.36)	.96
Colon polyposis	Female	0.96 (0.60 to 1.53)	.86
Colon polyposis	Male	1.21 (0.70 to 2.10)	.49
Thyroid cancer	Both	0.61 (0.45 to 0.82)	1.2×10^{-3}
Thyroid cancer	Female	0.66 (0.48 to 0.89)	7.5×10^{-3}

Abbreviation: OR, odds ratio.

[a]Results shown are for cancers with at least five events in those with *PTEN* pathogenic variants.

[b]ORs are adjusted for personal/family cancer history, and ancestry.

TABLE A6. Association of *PTEN* Pathogenic Variant Status With Familial Cancer Within Each Sex

Characteristics	Sex	OR[a] (95% CI)	*P*
Ductal invasive breast cancer	Both	1.07 (0.87 to 1.31)	.500
Ductal invasive breast cancer	Female	1.08 (0.87 to 1.33)	.490
Ductal invasive breast cancer	Male	1.12 (0.52 to 2.43)	.770
Endometrial cancer	Both	1.28 (0.81 to 2.02)	.290
Endometrial cancer	Female	1.19 (0.73 to 1.94)	.490
Endometrial cancer	Male	2.50 (0.65 to 9.59)	.180
Thyroid cancer	Both	1.39 (0.70 to 2.73)	.350
Thyroid cancer	Female	1.38 (0.67 to 2.83)	.380
Thyroid cancer	Male	1.63 (0.31 to 8.65)	.570
Colon polyposis	Both	1.26 (0.77 to 2.04)	.360
Colon polyposis	Female	1.31 (0.75 to 2.28)	.340
Colon polyposis	Male	1.16 (0.41 to 3.29)	.780
Nonpolyposis colorectal cancer	Both	0.75 (0.51 to 1.12)	.160
Nonpolyposis colorectal cancer	Female	0.83 (0.54 to 1.27)	.400
Nonpolyposis colorectal cancer	Male	0.43 (0.13 to 1.42)	.170
Kidney cancer	Both	2.28 (1.16 to 4.47)	.017
Kidney cancer	Female	2.47 (1.24 to 4.92)	.010
Kidney cancer	Male	1.01 (0.05 to 19.35)	1.000
Melanoma	Both	0.64 (0.30 to 1.36)	.250
Melanoma	Female	0.51 (0.20 to 1.28)	.150
Melanoma	Male	1.39 (0.36 to 5.37)	.630
Gastric cancer	Both	0.53 (0.17 to 1.54)	.240
Gastric cancer	Female	0.61 (0.21 to 1.77)	.360
Gastric cancer	Male	—	—
Prostate cancer	Both	1.11 (0.75 to 1.66)	.610
Prostate cancer	Female	0.98 (0.62 to 1.56)	.930
Prostate cancer	Male	1.67 (0.81 to 3.43)	.160
Pancreatic cancer	Both	0.66 (0.30 to 1.42)	.290
Pancreatic cancer	Female	0.71 (0.31 to 1.63)	.410
Pancreatic cancer	Male	0.32 (0.03 to 3.16)	.330
Ovarian cancer	Both	0.66 (0.39 to 1.11)	.120
Ovarian cancer	Female	0.71 (0.42 to 1.21)	.200
Ovarian cancer	Male	0.15 (0.01 to 3.95)	.260

Abbreviation: OR, odds ratio.

[a]Increased odds of detecting a *PTEN* pathogenic variant because of one first-degree or two second-degree relatives. ORs are adjusted for age, personal cancer history, and ancestry.