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Germline PTEN, SDHB-D and KLLN Alterations in Endometrial Cancer Patients with Cowden and Cowden-Like Syndrome: an International Multi-Center Prospective Study

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Abstract

Background—Endometrial cancer (EC) has only been recently recognized as a major component in Cowden syndrome (CS). Germline *PTEN* (*PTEN_mut+*), *SDHB-D* (*SDHx_var+*) and *KLLN* (*KLLN_Me+*) alterations cause CS and CS-like (CSL) phenotypes. This study aims to identify prevalence and clinico-pathologic predictors of germline *PTEN_mut+*, *SDHx_var+* or *KLLN_Me+* in CS/CSL patients presenting with EC.

Methods—*PTEN* and *SDHB-D* mutation and *KLLN* promoter methylation analyses were performed on 371 prospectively enrolled (2005–2011) patients. PTEN protein was analyzed from patient-derived lymphoblast lines. *PTEN* Cleveland Clinic score (CC score) is a weighted regression-based risk calculator that gives a priori risk of *PTEN*_mut+. Demographic and clinicopathologic features were correlated with specific gene.

Results—Germline *PTEN_mut+*, *SDH*x_var+ and *KLLN*_Me+ were found in 7%, 9.8% and 10.5% of informative samples, respectively. Predictors of *PTEN*_mut+ included age≤50 (OR6.1, p=0.015 for age<30, OR4.4, p=0.001 for age 30–50), macrocephaly (OR14.4, p<0.001), higher CC score (OR1.35 for 1 unit increment, p<0.001), PTEN protein level at the lowest quartile (OR5.1, p=0.039) and coexisting renal cancer (OR5.7, p=0.002). *KLLN*_Me+ patients were a mean 8 years younger than *KLLN*_Me− ones (44 vs. 52, p=0.018). Predictors of *KLLN*_Me+ were younger age and higher CC score. On the other hand, no clinical predictors of *SDH*_var+ were found.

Conclusions—We identified clinical predictors of *PTEN* and *KLLN* alterations, but not *SDHx_var+*. Having these predictors should alert the treating physician to potential heritable risk for referral to genetic professionals. High-risk cancer surveillance and prophylactic surgery of the uterus may be considered for *KLLN*_Me+ similar to *PTEN*_mut+ patients.

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Conflicts of Interest
The authors declare no relevant conflicts of interest.

Keywords

Cowden syndrome; Cowden-like syndrome; endometrial cancer; *PTEN*; *SDHB-D*; *KLLN*

Introduction

Endometrial cancer is the fourth most common cancer and the seventh leading cause of death in women in the United States. It is estimated that 49,560 will be diagnosed with endometrial cancer and 8,190 will die of the disease in 2013[1]. Most cases of endometrial carcinoma are sporadic. However, heritable endometrial cancer has been associated with two inherited cancer syndromes, Lynch and Cowden syndromes. Little is known about the latter in endometrial cancer presentations.

Cowden syndrome (CS) is an autosomal dominant disorder characterized by multiple hamartomas and high risks of breast, thyroid, renal and endometrial carcinomas[2,3]. Germline *PTEN* mutation has been reported in 77–81% of classic CS cases[4,5]. However, we recently demonstrated that germine *PTEN* mutation was found in only 25% of classic CS patients accrued from the community[3].

Endometrial cancer has been recently recognized as a major component of Cowden syndrome. Individuals with germline *PTEN* mutations have a 28% lifetime endometrial cancer risk[2]. Identifying *PTEN*-related endometrial cancers is important because of the increased risks of other cancers and implications for family members, with the potential for gene-directed cancer prevention. Therefore, it is important to identify clinical predictors, which can serve as a tool for oncologists to identify those at high-risk of germline *PTEN* mutation for referral to genetics specialists for gene testing and gene-informed medical management and high risk surveillance.

Subsets of CS/CSL individuals with no germline *PTEN* mutations were found to have germline *SDHB/C/D* variation and *KLLN* promoter methylation[6,7]. Succinate dehydrogenase (SDH) belongs to mitochondrial complex II that participates in both the electron transport chain and Krebs cycle. Heterozygous germline mutations in *SDHB/C/D* cause pheochromocytoma and/or paraganglioma[8–11]. Germline *SDHB/C/D* variation was found in 8% of *PTEN* mutation negative CS/CSL individuals[7]. Individuals with *SDHB/C/D* variants have higher risks of breast and thyroid cancers compared to those with germline *PTEN* mutation[7]. Among 123 CS/CSL individuals with no germline *PTEN* or *SDH* alteration, 37% were found to have germline *KLLN* promoter methylation with higher prevalence of breast and renal cancers compared to those with germline *PTEN* mutations[6]. *KLLN* is a p53-regulated gene located upstream of *PTEN* and shares a bidirectional promoter region.

In CS/CSL individuals with endometrial cancer, information on the prevalence of germline *SDHB/C/D* variation and *KLLN* promoter methylation is absent. Further, it is unknown whether germline *SDH* or *KLLN* alterations have similar clinical features compared to those with germline *PTEN* mutation or not. These data are important when counseling patients about gene-specific risks of endometrial cancer, and hence, affects their clinical management and subsequent follow-up. Therefore, the objectives of this study are to

determine the prevalence of germline *PTEN*, *SDHB/C/D* and *KLLN* alterations among CS/CSL individuals with endometrial cancer. Furthermore, we sought to investigate clinical features predictive of germline *PTEN*, *SDHB/C/D* and *KLLN* alterations among CS/CSL individuals with endometrial cancer, to help identify those in general oncologic practices for referral to genetics professionals.

Materials and Methods

Research participants

We included CS and CSL patients with EC who were prospectively enrolled from 10/1/2005 to 12/31/2011 in accordance with our research protocol IRB8458 (sub-study PTEN), which was approved by the Cleveland Clinic and respective Institutional Review Boards. Probands who met at least the relaxed International Cowden Consortium operational criteria for CS were eligible. Relaxed criteria are defined as full criteria (Table 1) minus one criterion, and such individuals are referred to have CSL. These patients were recruited from both community and academic medical centers throughout North America, Europe, and Asia. Cancer genetics professionals reviewed all medical records, and if necessary, further primary documentation of medical records/pathology reports were obtained with the patients' consent. *PTEN* Cleveland Clinic scoring system (CC score) is derived as described in our prior studies[3].

PTEN mutation and deletion analysis

All patients underwent *PTEN* (NM_000314.4) mutation analysis. Genomic DNA was extracted from peripheral blood leukocytes using standard methods[12]. Scanning of genomic DNA samples for *PTEN* mutations was performed with a combination of denaturing gradient gel electrophoresis, high-resolution melting curve analysis (Idaho Technology, Salt Lake City, UT) and direct Sanger sequencing (ABI 3730xl; Applied Biosystems, Foster City, CA) as previously described[13]. Deletion analysis using the multiplex ligation-dependent probe amplification (MLPA) assay[14] was performed with the P158 MLPA kit (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's protocol. All patients underwent PCR-based Sanger sequencing of the *PTEN* promoter region as previously described[15]. Promoter mutations/variations were defined as previously reported[15,16], except for −1084T>C; this variant has been rarely reported in population controls of European descent (two of 150)[17] and further work is required to characterize its functionality, thus, we consider it as a variant of unknown clinical significance (VUS) at this time.

SDHB/C/D mutation and deletion analysis

Germline genomic DNA from 367 eligible patients was analyzed for *SDHB/C/D* mutations/variations as previously reported by our laboratory[7,18,19].

KLLN promoter methylation analysis

KLLN promoter methylation analysis was performed on 228 patients using Sequenom MassArray Assay. Sodium bisulfite treatment of genomic DNA was performed following manufacturer's protocol of EZ DNA Methylation Kit (Zymo Research, Orange, CA, USA)

using 750 ng in 50 μl of distilled water and M-Dilution Buffer. The treated samples were re-suspended in 30 μl of M-Elution Buffer and stored at −20°C. Bisulfite treated genomic DNA then subjected to PCR amplication, in vitro transcription and analyzed using Homogeneous MassCLEAVE (hMC) base-specific cleavage and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS as previously described[20–22]. Positive (very high methylation) and negative (0 to very low methylation) controls are run with each batch for Sequenom analysis. We also QC by selecting random samples for bisulfite sequencing. The resultant methylation calls were performed by the EpiTyper software v1.0 (Sequenom) to generate quantitative results for each CpG site. We analysed methylation levels at 9 informative CpGs amongst the 33 existing CpGs within the region from −1577 to −1907. The average methylation was calculated as a mean value of the CpGs' methylation value for the 9 CpGs and expressed as percent methylation. Only those with complete methylation information at all 9 CpGs were included in the analysis. *KLLN* methylation status was expressed as a categorical variable (either positive or negative for methylation). Those with average methylation ≥ 90th percentile were considered as having positive *KLLN* promoter methylation. In this study, average methylation of 25% or more met the above criteria and chosen as a cutoff to call positive *KLLN* promoter methylation.

Analysis of PTEN protein by immunoblotting

Human immortalized lymphoblast-derived cell lines were obtained from each patient and cultured in RPMI 1640 supplemented with 20% fetal bovine serum. All cell lines were cultured at 37 C and 5% CO2. Whole-cell lysates were prepared with mammalian protein extraction reagent (ThermoFisher Scientific, Waltham, MA) supplemented with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Protein lysates were separated by SDS-PAGE and transferred onto nitrocellulose. Antibodies used included anti-PTEN mouse monoclonal (Cascade Biosciences, Portland, OR) at 1:5000, anti-glyceraldehyde-3-phosphate dehydrogenase rabbit monoclonal (Cell Signaling) at 1:20,000, and anti-actin mouse monoclonal (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:20,000. The blots were scanned digitally with the Odyssey imaging system (Li-Cor Biotechnology, Lincoln, NE). Detected fluorescence intensities for protein bands were background adjusted and normalized between gels with the median expression of individual proteins on each blot. Blood PTEN protein levels were classified as PTENQ1-4. PTENQ1 corresponds to the lowest quartile of PTEN expression seen and PTENQ4 being the highest.

Statistical analysis

Associations between categorical and continuous covariates were assessed using Chi Square test, Welch t-test and ANOVA test. Univariate logistic regression analyses were used to calculate odds ratio (OR) and 95% CI. All tests of significance were at the $p<0.05$ level, and p-values were two-tailed. R-Studio, integrated development environment for R (version 0.97.551, Boston, MA) was used for the statistical analysis.

Results

Demographic and clinical characteristics

Three hundred and seventy-one CS/CSL patients with endometrial cancer (EC) were eligible. Median age was 54 years (21–88; Table 2). Histologies include endometrioid in 156 (42%), serous/clear cell 19 (5%), mucinous 1 (0.3%), sarcoma 10 (2.7%) and not otherwise specified/unknown 185 (50%). At least one uterine fibroid was present in 37.5% of the entire cohort (139/371). More than half of our cohort (226/371) had prevalent breast cancer. Mean CC score was 9.3 (range 1–69).

Frequency of germline PTEN mutation or variation and associated clinical features

Of 371 patients, 26 (7%) have germline pathogenic *PTEN* mutation (*PTEN*_mut+), 19 (5%) have VUS (*PTEN*_VUS), and the remaining 326 (88%) have no mutation or VUS (*PTEN*_mut−). Clinical characteristics of CS/CSL patients with EC stratified by germline *PTEN* mutation status are listed in table 3. *PTEN*_mut+ participants were younger than those with *PTEN*_VUS and *PTEN*_mut− (mean age 44 vs. 52 vs. 54 years, p<0.001) with almost ¾ of *PTEN*_mut+ presenting at age ≤ 50 years compared to *PTEN*_VUS and *PTEN*_mut− patients (p=0.006, Table 3). *PTEN*_mut+ individuals had higher mean CC score compared to *PTEN*_VUS and *PTEN*_mut− patients (30 vs. 6.7 vs. 7.7, p<0.001).

The blood PTEN protein expression score was analyzed. 62.5% of *PTEN*_mut+ individuals had PTENQ1 compared to ~25% each for *PTEN*_VUS and *PTEN*_mut− patients (p=0.01).

*PTEN*_mut+ individuals were more likely to have macrocephaly than *PTEN*_VUS and *PTEN*_mut− patients (77% vs. 16% vs. 19%, p<0.001). There was no difference in distribution of uterine fibroids, or breast and thyroid cancers when stratified by germline *PTEN* mutation status (Table 3). Interestingly, the proportion of *PTEN*_mut+ individuals who also had renal cancer was higher than *PTEN*_VUS and *PTEN*_mut− patients (19% vs. 0% vs. 4% respectively, p=0.001).

Significant clinical predictors for germline *PTEN* mutation included age ≤ 50 years (OR 6.1, 95% CI 1.4–26.2, p=0.015 for age <30 years and OR 4.4, 95% CI 1.7–11.2, p=0.001 for age 30–50 years), having macrocephaly (OR 14.4, 95% CI 5.6–37.6, p<0.001), higher CC score (OR 1.35 for 1 unit increment in CC score, 95% CI 1.2–1.5, p<0.001), PTENQ1 compared to PTENQ4 (OR 5.1, 95% CI 1.1–4.6, p=0.039) and having renal cancer (OR 5.7, 95% CI 1.86–17.55, p=0.002).

Germline SDHB/C/D variation and associated clinical features

Of 367 patients analyzed for *SDHx* variation, 36 (9.8%) were found to have germline *SDHB/C/D* variation. No difference in the frequency of germline *SDHB/C/D* variation was found between *PTEN*_mut+ and *PTEN*_mut− patients (9.1% vs. 9.2%, p=0.9). Six variants were identified (Table 4). Of these, 5 have been reported in our prior studies[7] and a new *SDHD* variant (c.278A>G, p.Tyr93Cys) was detected in two patients with no germline *PTEN* mutation. Both patients were African American and diagnosed with breast and

endometrial cancers. There was no difference in clinical characteristics when stratified by germline *SDHB/C/D* variation status (Table 5).

Germline KLLN promoter methylation and associated clinical features

Of the 371 eligible patients, 228 were completely informative at the analyzed CpGs. Among them, 24 (10.5%) were found to have germline *KLLN* promoter methylation. The prevalence of germline *KLLN* promoter methylation among *PTEN*_mut+ was higher than that in *PTEN*_mut− patients (13% vs. 9.0%). However, the difference was not statistically significant (p=0.16). Patients with germline *KLLN* promoter methylation were a mean 8 years younger than those with no germline *KLLN* promoter methylation (mean age 44 vs. 52, p=0.018). Further, patients with germline *KLLN* promoter methylation had significantly higher mean CC score compared to those with no germline *KLLN* promoter methylation (14 vs. 10.6, p=0.01). There was no difference in other clinical characteristics when stratified by germline *KLLN* promoter methylation status (Table 5).

Clinical predictors of germline *KLLN* promoter methylation are younger age (OR 1.25 for each 5 years younger, 95% CI 1.04–1.50, p=0.015) and higher CC score (OR 1.03 for 1 unit increment in CC score, 95% CI 0.99–1.07, p=0.09).

Discussion

Cowden syndrome has only recently joined Lynch syndrome whereby endometrial cancer is recognized as an important component. However, this cancer has not been systematically studied in patients with Cowden and Cowden-Like syndromes. Our study represents the first and largest investigating the prevalence of germline *PTEN*, *SDHB-D* and *KLLN* alterations and associated demographic and clinical characteristics in CS/CSL patients with endometrial cancer. It is clinically important to identify which subset(s) of endometrial cancer patients actually have CS/CSL defined by each of these genes and to determine gene-specific other cancer risks to inform risk sub-stratification and referral to high risk professionals.

We recently have shown that patients with germline *PTEN* mutations have an increased lifetime risk of endometrial cancer[2]. Here, we sought to determine whether demographic or clinical features in endometrial cancer presentations with CSL features can predict a priori a higher likelihood of harboring a *PTEN* mutation, to signal referral for genetic evaluation. Here, we show that age<50, macrocephaly, high CC score and/or prevalent or synchronous renal cell carcinoma could predict for germline *PTEN* mutation. Similar to our observations in CS-associated thyroid cancer[23], we were able to associate low blood PTEN protein levels with a higher likelihood of harboring germline *PTEN* mutation. The odds of someone with PTENQ1 expression having an underlying germline *PTEN* mutation is 5.1-fold greater than in those who had PTENQ4 expression. Arguably, this might be a straightforward screening assay in the clinical setting. However, one limitation of this study is the absence of a normal range for blood PTEN protein expression levels. Nonetheless, any one or more of these five factors in a patient with endometrial cancer should prompt a healthcare provider to refer her for cancer genetics evaluation for consideration of *PTEN*-related CS. We also found that the mean age of endometrial cancer diagnosis in those with *PTEN* mutations was 44 years, with

three-quarters diagnosed under 50. This observation may guide age-range for consideration of surveillance or prophylactic surgery.

In our previous small highly selected pilot series identifying germline *KLLN* methylation in CS/CSL susceptibility, we could not detect an increased snap-shot prevalence of endometrial cancer in those with *KLLN* methylation compared to *PTEN*-related CS/CSL[6]. We now show in this large prospective unselected series that the average age at diagnosis with endometrial cancer in both *PTEN*-mutation and *KLLN* promoter methylation carriers are similar, compared to *SDHx* where their average age is close to that of sporadic cases. Until further data, the surveillance recommendations for the uterus in *KLLN*-associated CS/CSL should be similar to those of *PTEN*-associated CS/CSL. Similar to those with *PTEN* germline mutations, younger age at endometrial cancer diagnosis and high CC score help to a priori predict the presence of germline *KLLN* methylation.

The PTEN Cleveland Clinic (CC) scoring system was created as a semi-quantitative weighted risk calculator derived by logistic regression approach taking into account presence or absence of a series of CS type features and ages of onset in our prospective CSL cohort compared to the features in the general community[3]. The CC score was validated in an independent series and outperformed the NCCN criteria. Strictly speaking, the CC score gives a priori probability of finding a germline *PTEN* mutation given the presence or absence of certain clinical features. As such, it could be surprising that a high CC score predicted for *KLLN* methylation. Notwithstanding, the CC score also reflects CS-type phenotypic load and earlier age of onset of the phenotype. In contrast to *PTEN* and *KLLN*, none of the demographic or clinical features or CC score was particularly associated with germline *SDHx* variation. These observations together may suggest that while *PTEN* and *KLLN* associate with similar phenotypes, *SDHx* does not, or may be more of a modifier[7,24]. In fact, *SDHx* variation has an important modifying effect on *PTEN* mutation, associated with increased prevalence of breast and thyroid carcinomas, compared to *PTEN* mutation alone[7,24].

Endometrioid endometrial cancer is the most prevalent histologic type in the general population. Our data in this study confirm that endometrioid histology is also the most prevalent histologic type in CS/CSL patients with endometrial cancer including *PTEN* mutation positive individuals. Similarly, histological distribution of endometrial cancer in CS/CSL patients with germline *SDHB-D* or *KLLN* alterations were similar to that of the general population.

Our study is limited by lack of central pathology review of all endometrial cancer cases and lack of information on histology in a significant fraction of our cohort. However, it has several strengths including its prospective nature, large sample size, and cases originating from both academic and community settings. All cases were reviewed by a specialized cancer genetic specialist and well trained cancer-specialist genetic counselors. Further, all genetic testing was performed in one laboratory using standardized protocols. This study represents the first and largest study to date investigating the spectra of germline alteration of these five genes in CS/CSL patients with endometrial cancer.

In conclusion, identifying CS-related endometrial cancer is important at both individual and family levels in terms of future cancer screening and prevention. However, predicting such patients is often difficult and can be easily missed by the treating oncologists. Our study provides information about gene-specific CS-related endometrial cancer that can be utilized by all oncologists or other clinicians who provide care to patients with endometrial cancer. We identified clinical features predictive of germline *PTEN* mutation among patients diagnosed with endometrial cancer. Presence of one or more of these clinical features should alert the treating physician to potential heritable risk for referral to genetic counseling and cancer risk management. Further, endometrial cancer patients with germline *KLLN* promoter methylation are likely to have increased phenotypic load and present at younger ages, akin to those of germline *PTEN* mutation. Thus, high-risk cancer surveillance and prophylactic surgery of the uterus may be considered for *KLLN*-Me+ patients similar to those with *PTEN* mutations. Based on our data, given the later age of onset in CS and CSL patients with *SDHB-D* variations, the recommendation for these patients might be different and warrant further investigation.

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Table 1

Clinical operational diagnostic criteria for CS based on International Cowden Consortium, which is same as National Comprehensive Cancer Network 2006.

Pathognomonic criteria	Adult Lhermitte-Duclos disease (LDD) (cerebellar tumors) Mucocutaneous lesions Trichilemmomas, facial Acral keratoses Papillomatous papules Mucosal lesions
Major criteria	Breast cancer Thyroid cancer (nonmedullary) Macrocephaly (megalocephaly) (i.e. 97th percentile and above) Endometrial cancer
Minor criteria	Other thyroid lesions (e.g. adenoma, multinodular goiter) Mental retardation (i.e. IQ of 75 and below) Gastrointestinal hamartomas Fibrocystic disease of the breast Lipomas Fibromas Genitourinary tumors (especially renal cell carcinoma) Genitourinary malformations Uterine fibroids
Operational diagnosis in an individual (any of the following)	Mucocutaneous lesions alone if there are: Six or more facial papules of which three must be trichilemmomas, or Cutaneous facial papules and oral mucosal papillomatosis, or Oral mucosal papillomatosis and acral keratoses, or Six or more palmoplantar keratoses, or Two or more major criteria of which one must be macrocephaly or LDD, or One major and at least three minor criteria, or Four or more minor criteria
Operational diagnosis in a family where one individual is diagnostic for Cowden Syndrome	Any one pathognomonic criterion Any one major criteria with or without minor criteria Two minor criteria History of Bannayan-Riley-Ruvalcaba syndrome
Cowden-Like Syndrome	Individuals meeting above criteria minus one criterion

Table 2

Demographic and clinical characteristics of Cowden syndrome and Cowden syndrome-like patients with endometrial cancer (n=371).

Variable		N (%)
Age	Mean Median Range <30 years 30–50 years >50 years	53 years 54 years (21–88) 16 (5%) 110 (33%) 204 (62%)
Histology	Endometrioid Serous/clear cell Mucinous Sarcoma NOS*/Unknown	156 (42%) 19 (5%) 1 (0.3%) 10 (2.7%) 185 (50%)
Race	White African American Asian Others Unknown	218 (59%) 9 (2.4%) 8 (2.2%) 21 (5.7%) 115 (31%)
Uterine fibroid	Yes No	139 (37.5%) 232 (62.5%)
Breast Cancer	Yes No	226 (61%) 145 (39%)
Renal cancer	Yes No	18 (5%) 352 (95%)
Thyroid cancer	Yes No	51 (13.7%) 320 (86.3%)
Macrocephaly	Yes No	84 (22.6%) 287 (77.4%)
Cleveland Clinic Score	Mean	9.3 (1–69)
PTEN protein level	1 2 3 4	69 (27.7%) 59 (23.7%) 60 (24.1%) 61 (24.5%)

* Not otherwise specified

Table 3

Demographic and clinical characteristics of Cowden syndrome and Cowden syndrome-like patients with endometrial cancer stratified by germline *PTEN* mutation status (n=371).

Variable		*PTEN* mut+ EC n=26	*PTEN* mut− EC n=326	*PTEN_VUS EC*[#] n=19	Global p-value	Pairwise p-value[*]
Age	Mean <30 year old 30–50 years > 50 years	44.2 (21–69) 3 (12%) 15 (60%) 7 (28%)	53.9 (15.6–88) 13 (5%) 89 (31%) 184 (64%)	52 (30.5–68.7) 0 6 (32%) 13 (68%)	<0.001 0.006	<0.001 0.001
Histology	Endometrioid Serous/clear cell Mucinous Sarcoma NOS[**]/Unknown	17 (65%) 0 (0%) 1 (4%) 0 (0%) 8 (31%)	130 (40%) 18 (6%) 0 (0%) 10 (3%) 168 (51%)	44 (47%) 1 (5%) 0 0 35 (47%)	<0.001	<0.001
Race	White African American Asian Others Unknown	19 (73%) 0 1 (4%) 2 (8%) 4 (15%)	191 (59%) 8 (3%) 7 (2%) 18 (6%) 102 (31%)	8 (90%) 1 (5%) 0 1 (5%) 9 (47%)	0.35	
Uterine Fibroid	Yes No/ unknown	12 (46%) 14 (54%)	118 (36%) 208 (64%)	9 (47%) 10 (53%)	0.39	
Breast Cancer	Yes No/ unknown	14 (54%) 12 (46%)	201 (62%) 125 (38%)	11 (58%) 32 (42%)	0.70	
Renal cancer	Yes No/ unknown	5 (19%) 21 (81%)	13 (4%) 312 (96%)	0 19 (100%)	0.001	0.006
Thyroid cancer	Yes No/ unknown	4 (15%) 22 (85%)	44 (14%) 282 (86%)	3 (16%) 16 (84%)	0.93	
Macrocephaly	YES No/Unknown	20 (77%) 6 (23%)	61 (19%) 265 (81%)	3 (16%) 16 (84%)	<0.001	<0.001
Cleveland Clinic Score	Mean Median	30 29	7.7 7	6.7 7	<0.001	<0.001
PTEN protein level	PTEN Q1 PTEN Q2 PTEN Q3 PTEN Q4	10 (62.5%) 4 (25%) 0 (0%) 2 (12.5%)	57 (25%) 53 (24%) 56 (25%) 59 (26%)	2 (25%) 2 (25%) 4 (50%) 0 (0%)	0.01	0.005

[*] Pairwise p-value comparing only those with germline *PTEN* mutation and those with no mutations or VUS. This test was done only in situations when global p-value was significant (<0.05).

[#] VUS: variants of unknown significance

[**] Not otherwise specified

Table 4

SDHB/C/D variants in Cowden syndrome and Cowden syndrome-like patients with endometrial cancer.

		Variant	N
PTEN mut+ N=26 (2/22=9%)	*SDHB*	c.487T>C, p.Ser163Pro	2
PTEN mut- or VUS n=345 (34/345=10%)	*SDHB*	c.487T>C, p.Ser163Pro c.8C>G, p.Ala3Gly	16 1
	SDHC	c.430G>C, p.Glu144Gln	5
	SDHD	c.278A>G, p.Tyr93Cys c.149A>G, p.His50Arg c.34G>A, p.Gly12Ser	**2** 6 6

Table 5

Demographic and clinical characteristics of Cowden syndrome and Cowden syndrome-like patients with endometrial cancer stratified by germline *SDHB/C/D* variation status (n=367).

Variable		SDHx variant Negative N=331	SDHx variant Positive N=36	p-value
Age	Mean Range	53.0 21–88	55.0 21–86	0.36
Histology	Endometrioid Serous/clear cell Mucinous Sarcoma NOS*/Unknown	139 (42%) 15 (5%) 1 (0.3%) 9 (3%) 167 (51%)	14 (39%) 4 (11%) 0 1 (3%) 17 (47%)	0.69
Race	White African American Asian Other Unknown	195 (59%) 7 (2%) 8 (2%) 19 (6%) 102 (31%)	22 (61%) 2 (6%) 0 2 (6%) 10 (28%)	0.63
Uterine Fibroid	Yes No/unknown	121 (37%) 210 (63%)	16 (44%) 20 (56%)	0.45
Breast Cancer	Yes No/unknown	202 (61%) 129 (39%)	23 (64%) 13 (36%)	0.73
Renal cancer	Yes No/unknown	15 (5%) 315 (95%)	2 (6%) 34 (94%)	0.73
Thyroid cancer	Yes No Unknown	46 (14%) 285 (86%)	5 (14%) 31 (86%)	0.99
Macrocephaly	Yes No	75 (23%) 256 (77%)	5 (14%) 31 (86%)	0.31
Cleveland Clinic Score	Mean Median	9.1 7	7.8 6	0.38
PTEN protein level	PTEN Q1 PTEN Q2 PTEN Q3 PTEN Q4	59 (26%) 55 (25%) 54 (23%) 56 (25%)	8 (36%) 4 (18%) 6 (27%) 4 (18%)	0.67

*Not otherwise specified

Table 6

Demographic and clinical characteristics of Cowden syndrome and Cowden syndrome-like patients with endometrial cancer stratified by germline *KLLN* promoter methylation status (n=228).

Variable		***KLLN_Me+ N=24***	***KLLN_Me− N=204***	**p-value**
Age	Mean Range	44 21–66	52 21–86	0.018
Histology	Endometrioid Serous/clear cell Mucinous Sarcoma NOS*/Unknown	6 (25%) 1 (4%) 0 0 17 (71%)	87 (17%) 8 (4%) 0 1 (1%) 105 (51%)	0.35
Race	White African American Asian Other Unknown	12 (50%) 0 0 2 (8%) 10 (42%)	106 (52%) 3 (2%) 5 (3%) 13 (6%) 77 (38%)	0.88
Uterine Fibroid	Yes No/unknown	8 (33%) 16 (67%)	73 (36%) 131 (64%)	0.8
Breast Cancer	Yes No/unknown	13 (54%) 11 (46%)	122 (60%) 82 (40%)	0.75
Renal cancer	Yes No/unknown	3 (12.5%) 21 (87.5%)	10 (5%) 194 (95%)	0.12
Thyroid cancer	Yes No/ Unknown	5 (21%) 19 (79%)	28 (14%) 176 (86%)	0.52
Macrocephaly	Yes No	10 (42%) 14 (58%)	53 (26%) 151 (74%)	0.10
Cleveland Clinic score	Mean Median	14 16	10.5 8	0.01
PTEN protein level	PTEN Q1 PTEN Q2 PTEN Q3 PTEN Q4	6 (33%) 3 (17%) 6 (33%) 3 (17%)	47 (31%) 35 (23%) 39 (26%) 31 (20%)	0.85

*Not otherwise specified