Predicting *PTEN* mutations: an evaluation of Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome clinical features

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ABSTRACT

Background Cowden syndrome (CS) is associated with benign hamartomatous lesions and risks for thyroid, breast and endometrial cancers. Bannayan–Riley–Ruvalcaba syndrome is an allelic disorder characterised by macrocephaly, intestinal polyps, lipomas, and pigmented penile macules. Diagnostic criteria for CS are based on the presence of a range of clinical features. However, prior data on the component clinical features have been based primarily on compilations of cases reported before development of consensus diagnostic criteria.

Objective This study sought to determine the clinical features most predictive of a mutation in the largest single cohort of patients with clinical testing for *PTEN* mutations reported to date.

Methods Molecular and clinical data were reviewed on 802 patients referred for *PTEN* analysis by a single laboratory.

Results Deleterious mutations were found in 172 (21.4%) subjects. Among mutation carriers significant differences from previous reports were found for the frequencies of several clinical features, including macrocephaly, uterine fibroids, benign breast disease, and endometrial cancer. Logistic regression analyses indicated that female mutation carriers were best identified by the presence of macrocephaly, endometrial cancer, trichilemmomas, papillomatous papules, breast cancer, benign thyroid disease, and benign gastrointestinal (GI) lesions. For males, the most discriminating features were macrocephaly, lipomas, papillomatous papules, penile freckling, benign GI lesions, and benign thyroid disease. Age related differences were also identified.

Conclusion The mutation frequency in patients meeting CS diagnostic criteria (34%) was significantly lower than previously reported, suggesting a need for reevaluation of these criteria. A mutation prediction model has been developed which can help identify patients appropriate for *PTEN* testing in clinical practice.

INTRODUCTION

The *PTEN* ('*p*hosphatase and *ten*sin homologue on chromosome 10') tumour suppressor gene on chromosome 10q23 is a dual specificity phosphatase with multiple and as yet incompletely understood roles in cellular regulation. Germline mutations in *PTEN* cause a spectrum of clinical syndromes, including Cowden syndrome (CS), Bannayan–Riley–Ruvalcaba syndrome (BRRS), and autism spectrum disorders with macrocephaly.

CS is a multisystem disorder involving increased risks for malignancy and hamartomatous tissue overgrowth. Diagnostic criteria for CS were initially established in 1996[1] and subsequent modifications have been proposed, including the addition of endometrial cancer[2] and renal cell carcinoma[3] and moving adult Lhermitte–Duclos disease (LDD) into the pathognomonic category.[4]

PTEN mutations were first reported in individuals with CS in 1997.[5 6] It was subsequently suggested that approximately 80% (30 of 37) of individuals with a clinical diagnosis of CS have a detectable germline coding sequence mutation in *PTEN*.[7] However, variable mutation detection rates have been found in other studies, including three of 27 (11%),[8] nine of 19 (47%),[5] and eight of 13 (61%) patients.[9] A small but as yet undefined proportion of CS patients have deletions or large rearrangements of the *PTEN* gene. A recent report found such changes in three of 15 patients who were negative for mutations using denaturing gel gradient electrophoresis (DGGE).[10] Another study found no deletions, but approximately 10% of DGGE mutation negative patients had variants in the *PTEN* promoter.[11]

BRRS is a congenital disorder whose hallmark features are macrocephaly, hamartomatous intestinal polyps, lipomas, and pigmented macules on the penis. Additional features include developmental delay, large birth weight, and joint hyperextensibility.[12] Consensus diagnostic criteria have not been established but diagnosis is based upon presence of the hallmark features. Initially thought to be a separate disorder, BRRS was subsequently shown to be allelic to CS with approximately 60% of patients with BRRS having detectable coding sequence mutations in *PTEN*.[13]

Most previous reports on the prevalence of the malignant and non-malignant features of CS/BRRS have been based upon data compiled from individual case reports and studies of small cohorts. More importantly, the majority of these reports were published before the adoption of the Consortium diagnostic criteria for CS in 1996. Thus, the true frequencies of the clinical features in CS and BRRS are not clearly known (see Pilarski[14] for review). The commonly reported frequencies for CS are shown in Box 1.

In an effort to obtain better data on these features and their correlation with *PTEN* mutations, we report here our experience with 172 patients with *PTEN* mutations, the largest single cohort with clinical testing reported to date. In

Box 1 Commonly reported frequencies of Cowden syndrome clinical features

- Mucocutaneous lesions (90–100%)
  - Trichilemmomas
  - Acral keratoses
  - Verucoid or papillomatous papules
- Thyroid abnormalities (50–67%)
  - Goitre
  - Adenoma
  - Cancer (3–10%)
- Breast lesions
  - Fibroadenomas/fibrocystic disease (76% of affected females)
  - Adenocarcinoma (25–50% of affected females)
- Gastrointestinal lesions (40%)
  - Hamartomatous polyps
- Macrocephaly (38%)
- Genitourinary abnormalities (44% of females)
- Uterine leiomyoma (multiple, early onset) (see Eng[15])

addition, we have assessed which clinical features are most predictive of a mutation, and have developed a mutation-prediction model which should be useful in clinical practice.

METHODS

Study subjects consisted of patients whose samples were sent for clinical *PTEN* testing to the Molecular Pathology Laboratory at The Ohio State University between 1 October 2000 and 31 October 2008. Samples were sent from clinical centres throughout the USA based on the clinical judgement of the referring provider; testing criteria were not set by our laboratory. Approximately 90% of referrals came from genetics and cancer genetics programmes, but they were accepted from all medical specialities. The study was approved by the Institutional Review Board of The Ohio State University.

Samples on 1132 subjects were submitted for analysis during the study period. Of these, 302 subjects (27 with mutations and 275 without) were not included because of incomplete clinical information provided by the ordering healthcare provider. Another 23 subjects were found to have genetic variants of uncertain significance (G44D, C71Y, T78T, A126T, 141P, E157G, R173H, T202I, L345R, IVS1-9C→G, IVS1+7A→G, IVS3 -7delCTTTT, IVS5-11 to 20insT, and IVS6+5G→C) and were also excluded, based on benign or discrepant predictions from the Polyphen (http://genetics.bwh.harvard.edu/pph/) and/or SIFT (http://sift.jcvi.org/www/SIFT_BLink_submit.html) prediction models. Although the number of patients with an uncertain variant was small, there were no apparent differences in their clinical features compared to the whole cohort. Five subjects were excluded because of unknown gender. Thus, a total of 802 subjects (535 female; 67%) were included, 172 with germline *PTEN* mutations and 630 without. Sixty-seven mutations were detected through full gene sequencing, 75 through confirmation of a mutation previously identified through research testing of a proband, and 30 through testing for a mutation that had been previously identified in an affected relative.

Peripheral blood samples were obtained from all patients. For patients undergoing full gene analysis, mutation analysis of the entire coding sequence, exon–intron boundaries, and flanking sequences of the *PTEN* gene was performed using DNA sequencing. Primer sequences and PCR conditions have been previously described.[16 17]

The clinical history on each subject was obtained through use of a 'clinical features checklist' (table 1) which ascertained for the presence or absence of each of the component features of CS/BRRS at the time of testing. Cancer diagnoses were specifically ascertained for brain, breast, endometrial, gastrointestinal (GI), renal, skin, and thyroid cancers. Simple tabulation counts were performed for all clinical features. The categories of benign breast, GI, and thyroid diseases referred specifically to structural lesions (eg, cysts, fibroids, nodules, polyps) in these systems, rather than functional disorders (eg, hypo/hyperthyroidism, coeliac disease).

All subjects were classified as to whether they met the revised International Cowden Consortium Diagnostic criteria (box 2), BRRS criteria (defined as having any three of these features: macrocephaly, lipomas, developmental delay/mental retardation or penile freckling in males), CS/BRRS overlap (meeting diagnostic criteria for both disorders) or none.

Statistical analyses

The comparison of age by gender was done using a two sample t test. We developed predictive models for mutation detection using logistic regression methods on the following subsets of subjects: males with macrocephaly, overall and by age group (≤18 years and >18 years); and females, overall and by age group (≤18 years and >18 years). We chose to do gender and age subgroup analyses because we felt, based on clinical experience, that the characteristics found in these groups (both gender and childhood vs adult onset of disease) were different enough to warrant separate models. Because all males age 18 and under (and the majority of males over 18) with a mutation had macrocephaly, this was not a usable variable in the modelling. Given the high clinical significance of macrocephaly, however, we restricted the modelling in males to only those with macrocephaly rather than drop the variable entirely. Therefore these

Table 1 Clinical features checklist used in this study

CLINICAL FEATURE	Present	Absent	Unknown
CNS			
Macrocephaly			
Lhermitte-Duclos disease			
Benign Tumors			
Malignant Tumors			
MR/DD			
THYROID			
Benign (eg. Multinodular goiter)			
Malignant			
BREASTS			
Benign (e.g., fibrocystic)			
Malignant			
SKIN/MUCOSA			
Trichilemmoma			
Papillomatous Papules			
Pigmented Macules on Penis			
Lipomas			
Malignant Tumor			
GASTROINTESTINAL TRACT			
Glycogenic Acanthosis			
Benign (e.g., hamartomas)			
Malignant			
GENITOURINARY			
Endometrial fibroids			
Endometrial cancer			
Renal cell cancer			
GU developmental anomalies			

Box 2 Revised Cowden syndrome Consortium diagnostic criteria[3]

Pathognomonic criteria
- Lhermitte–Duclos disease (LDD)—adult
- Mucocutaneous lesions:
  - Trichilemmomas, facial
  - Acral keratoses
  - Papillomatous lesions

Major criteria
- Breast cancer
- Thyroid cancer (papillary or follicular)
- Macrocephaly (≥97%ile)
- Endometrial cancer

Minor criteria
- Other structural thyroid lesions (eg, adenoma, multinodular goitre)
- Mental retardation (ie, IQ≤75)
- Gastrointestinal hamartomas
- Fibrocystic disease of the breast
- Lipomas
- Fibromas
- Genitourinary tumours (eg, uterine fibroids, renal cell carcinoma) or
- Genitourinary structural malformations
- Uterine fibroids

Operational diagnosis in an individual (any of the following):
1. Mucocutaneous lesions alone if:
   a) there are six or more facial papules, of which three or more must be trichilemmoma, or
   b) cutaneous facial papules and oral mucosal papillomatosis, or
   c) oral mucosal papillomatosis and acral keratoses, or
   d) palmoplantar keratoses, six or more
2. Two or more major criteria, but one must include macrocephaly or LDD; or
3. One major and three minor criteria; or
4. Four minor criteria

models are generalisable to males who present with macrocephaly only. Stepwise modelling methods were then used to develop the most parsimonious model for each of these subgroups.

We also compared the observed cancer incidence in this cohort to that expected, based on lifetime invasive cancer risk from birth to the age of *PTEN* mutation detection using the National Cancer Institute's DevCan software,[18] which is based on the sex and age conditional probability of developing cancer.[19 20] Because there were too few cancers within specific sites and types of cancer to examine each site/type, we compared the observed to expected number of cases for the combined group of the three cancers for which this group should be at greater risk (breast, thyroid, and endometrial cancers).

RESULTS

Genotypes

Of the 802 usable subjects, germline *PTEN* mutations were found in 172 (21.4%). Of these, 90 were female and 82 were male. One hundred and three different mutations were identified including two small deletions, 30 frameshift, 39 missense, 20 nonsense, and 11 splice-site mutations. Table 2 lists the distinct mutations identified in this cohort. The vast majority of mutations were seen in only one to three families each.

Of the 39 missense mutations identified, 17 (A34D, N48K, H61R, Y68H, P95L, P96R, A120E, G129E, R130G, R130L, R130Q, C136R, C136Y, Y155H, S170R, Y176C, and F347L) have been previously reported to be deleterious.[15 21–27] Seven other missense changes (C124W, G129V, G132V, I135K, G165R, S170I, and L345P) involved the same codons but different amino acid substitutions from other previously identified mutations,[15 22 23] and three are in codons adjacent to previously reported mutations (D22G near D24Y, and I32N and G36R next to A34D and M35T).[15 22] None of these changes has been reported in the National Center for Biotechnology Information's dbSNP database of genetic polymorphisms (http://www.ncbi.nlm.nih.gov/snp, accessed November 2010).

Clinical features

Table 3 lists the frequencies of the various clinical features for the mutation positive cohort as a whole, and broken down by gender and age. The mean age at the time of *PTEN* mutation diagnosis was 29.8 years for the cohort as a whole, with a significant difference between the genders (females: 34.4 years, males: 24.7 years; p=0.002). This is likely because macrocephaly, developmental delay, and penile freckling are common in males and are easily recognised at young ages, leading to testing at an earlier age. This may also explain the finding that although males accounted for only 33% of subjects who were tested they made up almost 48% of those with a mutation.

The presence of mental retardation or developmental delay (MR/DD) was not ascertained until midway through the time period of this study. Of 110 subjects with mutations for whom the information was ascertained, MR/DD was reported in 10% of females and 26% of males overall, but in 18% of females and 46% of males who tested positive under age 18.

Six of the nine patients in this study who were diagnosed with LDD had a mutation. The negative patients were 1, 6, and 12 years old at the time of testing (with the 1-year-old child meeting CS diagnostic criteria), while those with mutations ranged in age from 19–64 years old. This is consistent with a prior report suggesting that adult onset LDD, but not childhood onset, is strongly linked with *PTEN* mutations.[28]

Phenotypes

Two hundred and thirty of the 802 subjects met CS diagnostic criteria. Of these, 79 were found to have a *PTEN* mutation, for a detection rate of 34%. Of subjects meeting BRRS diagnostic criteria, 23 of 42 (55%) had a mutation, and seven of nine (78%) 'overlap' patients meeting diagnostic criteria for both CS and BRRS had a mutation. Of the 172 patients with *PTEN* mutations, 63 (37%) did not meet diagnostic criteria for either CS or BRRS. Compared to the mutation positive patients who met CS/BRRS diagnostic criteria, these patients had similar frequencies of macrocephaly (77%), MR/DD (22.6%), and benign skin lesions (37%), but all other clinical features were seen at half the frequency or less (data not shown).

Observed versus expected cancers

Because the age at diagnosis was not ascertained for the subjects in this study, we were unable to do a typical comparison of observed to expected numbers of cancers compared to the general population. However, as a surrogate we assessed cancer rates at the time of molecular diagnosis for the entire cohort, and separately for female versus male subjects over age 18 years.

Table 2 103 germline *PTEN* mutations found in 172 patients, categorised by mutation type; the number of patients affected and the exon involved

Nucleotide change	Protein change	EXON	Mutation type	N
c.94_96delATT		2	Deletion	2
c.597_599delGTT		6	Deletion	2
c.18_22delAGAGA		1	Frameshift	1
c.21_22delGA		1	Frameshift	1
c.92delA		2	Frameshift	1
c.287delC		5	Frameshift	1
c.309dupC		5	Frameshift	1
c.339_343delTGAAG		5	Frameshift	1
c.370delT		5	Frameshift	1
c.405dupA		5	Frameshift	2
c.420dupA		5	Frameshift	1
c.445_446insA		5	Frameshift	1
c.461delT		5	Frameshift	1
c.514dupA		6	Frameshift	4
c.548_551delAGAA		6	Frameshift	2
c.548dupA		6	Frameshift	1
c.593_602delTGATGTTTGA		6	Frameshift	2
c.604_delACTATTC		6	Frameshift	1
c.611dupC		6	Frameshift	1
c.731delC		7	Frameshift	1
c.740_741insCG		7	Frameshift	1
c.757dupA		7	Frameshift	1
c.833dupT		8	Frameshift	1
c.846_847delACinsT		8	Frameshift	1
c.849_858delAGAGGAAACC		8	Frameshift	1
C.870delA		8	Frameshift	1
c.875dupA		8	Frameshift	1
c.900delC		8	Frameshift	3
c.956dupA		8	Frameshift	1
c.968delAAinsG		8	Frameshift	1
c.972delT		8	Frameshift	1
	p.Arg15Ser (R15S)	1	Missense	1
	p.Asp22Gly (D22G)	2	Missense	1
	p.Thr26Pro (T26P)	1	Missense	1
	p.Ile32Asn (I32N)	2	Missense	1
	p.Ala34Asp (A34D)	2	Missense	1
	p.Gly36Arg (G36R)	2	Missense	1
	p.Asn48Lys (N48K)	2	Missense	3
	p.His61Arg (H61R)	3	Missense	3
	p.Tyr68His (Y68H)	3	Missense	1
	p.Ala79Thr (A79T)	4	Missense	1
	p.Tyr88His (Y88H)	5	Missense	2
	p.Pro95Leu (P95L)	5	Missense	2
	p.Pro96Arg (P96R)	5	Missense	1
	p.Ile101Thr (I101T)	5	Missense	1
	p.Ala120Glu (A120E)	5	Missense	2
	p.Cys124Trp (C124W)	5	Missense	1
	p.Gly129Glu (G129E)	5	Missense	1
	p.Gly129Val (G129V)	5	Missense	1
	p.Arg130Gly (R130G)	5	Missense	1
	p.Arg130Leu (R130L)	5	Missense	1
	p.Arg130Gln (R130Q)	5	Missense	4
	p.Gly132Va (G132V)l	5	Missense	2
	p.Ile135Lys (I135K)	5	Missense	1
	p.Cys136Arg (C136R)	5	Missense	4
	p.Cys136Tyr (C136Y)	5	Missense	4
	p.Leu152Pro (L152P)	5	Missense	1
	p.Tyr155His (Y155H)	5	Missense	1
	p.Gly165Arg (G165R)	6	Missense	1
	p.Ser170Ile (S170I)	6	Missense	1
	p.Ser170Arg (S170R)	6	Missense	2
	p.Tyr176Cys (Y176C)	6	Missense	1
	p.Leu181Prp (L181P)	6	Missense	1
	p.Phe200Ser (F200S)	6	Missense	1
	p.Asp252Gly (D252G)	7	Missense	1
	p.Lys260Arg (K260R)	7	Missense	1
	p.Leu345Pro (L345P)	9	Missense	1
	p.Leu345Va (L345V)l	9	Missense	1
	p.Phe347Leu (F347L)	9	Missense	1
	p.Pro354Gln (P354Q)	9	Missense	1
	p.Tyr16X (Y16X)	1	Nonsense	3
	p.Gln17X (Q17X)	1	Nonsense	2
	p.Phe21X (F21X)	1	Nonsense	1
	p.Ser59X (S59X)	3	Nonsense	2
	p.Glu106X (E106X)	5	Nonsense	1
	p.Gln110X (Q110X)	5	Nonsense	1
	p.Arg130X (R130X)	5	Nonsense	14
	p.Tyr180X (Y180X)	6	Nonsense	3
	p.Leu182X (L182X)	6	Nonsense	2
	p.Cys211X (C211X)	6	Nonsense	1
	p.Gln214X (Q214X)	7	Nonsense	1
	p.Gln219X (Q219X)	7	Nonsense	2
	p.Ser229X (S229X)	7	Nonsense	1
	p.Arg233X (R233X)	7	Nonsense	7
	p.Gln245X (Q245X)	7	Nonsense	1
	p.Trp274X (W274X)	8	Nonsense	1
	p.Glu299X (E299X)	8	Nonsense	1
	p.Glu307X (E307X)	8	Nonsense	1
	p.Leu320X (L320X)	8	Nonsense	1
	p.Arg335X (R335X)	8	Nonsense	13
c.164+1delGTAAG		2	Splice	1
c.165-1G>A		3	Splice	1
c.209+1G>A		3	Splice	1
c.209+1insT		3	Splice	1
c.209+1G>T		3	Splice	1
c.209+1_2delGT		3	Splice	1
c. 210-3_-7del CTTTT		4	Splice	1
c.492+2delT		5	Splice	2
c.634+2T>C		6	Splice	3
c.635-1G>A		7	Splice	1
c.1027-2A>G		9	Splice	1
c.1027-2A>C		9	Splice	1

Because of the relatively small number of cancers, analysis was only possible by grouping thyroid, breast, and endometrial cancers together. Given the ages for mutation carriers in this cohort at the time of *PTEN* testing, the expected combined number of invasive thyroid, breast, and endometrial cancer cases in the general population would be 2.2. We observed 54 cases, giving a ratio of observed to expected cases of 24.5. This difference was statistically significant ($p<0.001$).

Logistic regression analyses

Table 4 shows the results of the logistic regression model for males with macrocephaly. There were a total of 267 males, of which 149 were ≤18 years of age with macrocephaly. Of these, 44 (29.5%) were found to have a mutation. Lipomas, papillomatous papules, and penile freckling were found to be significant predictors of the presence of a mutation for this gender/age group. The probability of having a mutation for a macrocephalic male ≤18 years of age with all of these features would be 99.1%. There were only 59 males >18 years of age with macrocephaly in our cohort, of which 31 were found to have a mutation (52.5%). The presence of benign thyroid disease and benign GI

Table 3 Percentage of *PTEN* related clinical features reported in the entire cohort of 172 mutation positive patients, and broken down by gender and age

Clinical features in *PTEN* positive patients	All patients (172)	Female all (90)	Female ≤18 (21)	Female >18 (69)	Males all (82)	Males <18 (44)	Male >18 (38)
Macrocephaly	84	77	90	72	91	100	82
Lhermitte–Duclos	3	03	0	4	4	0	8
CNS tumour, benign	3	1	0	1	5	2	8
Brain cancer	0	0	0	0	0	0	0
MR/DD*	17	10	18	7	26	46	4
Benign thyroid	38	52	14	62	23	2	47
Thyroid cancer	8	10	0	13	5	0	11
Benign breast	20	38	10	46	0	0	0
Breast cancer	17	32	0	41	0	0	0
Lipoma	47	43	29	46	50	43	58
Trichelemmomas	19	26	5	32	12	5	21
Papillomatous papules	41	48	19	55	34	20	50
Penile freckling	23	0	0	0	48	61	32
Other benign skin	37	38	43	36	35	25	47
Any benign skin (combined)	77	81	81	81	73	64	84
Skin cancer	4	6	0	7	2	0	5
Benign GI	40	37	19	41	43	14	76
Glycogenic acanthosis	4	4	5	4	4	2	5
GI cancer	3	3	0	4	4	0	8
Uterine fibroids	10	20	0	26	0	0	0
Endometrial cancer	7	13	0	17	0	0	0
Kidney/renal cell cancer	3	1	0	1	5	0	11
GU development anomalies	1	1	0	1	0	0	0
*Data on mental retardation/ developmental disabilities were collected only on a subset of patients	N=110	N=60	N=17	N=44	N=50	N=26	N=24

CNS, central nervous system; GI, gastrointestinal; GU, genitourinary; MR/DD, mental retardation/developmental delay.

lesions were found to be significant in the predictive model. If a male with macrocephaly >18 years of age presented with these two characteristics his probability of having a mutation was 93.3%.

Table 5 shows the logistic regression results for females, which were not restricted to subjects with macrocephaly. There were 105 females ≤18 years of age in this group, of which 21 (20.0%) had a mutation. The analysis showed that benign skin disease and macrocephaly were significant predictors of the presence of a mutation for females ≤18 years of age. However, the probability of having a mutation for a female ≤18 years old with these features would only be 44.3%. For females >18 years, macrocephaly, endometrial cancer, trichilemommas, papillomatous papules, benign thyroid disease, and benign GI lesions were all found to be significant predictors of having a mutation. If a female >18 years presented with all of these features, the probability of a mutation was 99.7%. The data obtained from these analyses were used to develop a model which predicts the likelihood of finding a mutation in a patient with various combinations of these clinical features. The model is available online at http://internalmedicine.osu.edu/genetics/research/tools-for-providers/.

DISCUSSION

CS and BRRS are complex disorders with multiple component features, many of which are common by themselves in the general population. Because of the rarity of these syndromes, the existing literature on the component clinical features of CS/BRRS has been based on compilations of case reports (with their inherent selection biases) and studies of relatively small numbers of patients and families. More importantly, the majority of these reports predate the adoption of the CS Consortium diagnostic criteria in 1996. The current diagnostic criteria used for CS are based on these reported features, and are thus sensitive to their accuracy. As the primary laboratory in the USA offering clinical testing for *PTEN* gene mutations during the study period, we were in a unique position to assemble the world's largest reported series of clinically tested, mutation positive patients on which to assess the clinical features and performance of the Consortium diagnostic criteria.

Benign clinical features

The frequencies of a number of benign clinical features differed significantly for our cohort, compared to the literature. Consistent with more recent reports,[9 29 30] the frequency of macrocephaly

Table 4 Logistic regression models results for probability of mutation for males with macrocephaly overall and by age group

Variable	Coefficient estimate 95% CI	p Value
Males all ages		
Benign gastrointestinal lesions	1.041 (0.15 to 1.94)	0.022
Lipomas	1.476 (0.71 to 2.25)	<0.001
Papillomatous papules	1.700 (0.67 to 2.73)	0.001
Penile freckling	1.576 (0.84 to 2.31)	<0.001
Intercept	−1.995 (−2.54 to −1.45)	
Males ≤18 years		
Penile freckling	2.185 (1.23 to 3.14)	<0.001
Lipomas	2.465 (1.63 to 3.57)	<0.001
Papillomatous papules	2.396 (0.54 to 4.24)	0.011
Intercept	−2.369 (−3.07 to −1.67)	
Males >18 years		
Benign thyroid disease	1.759 (0.21 to 3.31)	0.026
Benign gastrointestinal lesions	2.307 (0.98 to 3.63)	0.001
Intercept	−1.417 (−2.34 to −0.49)	

Table 5 Logistic regression models results for probability of mutation for females overall and by age group

Variable	Coefficient estimate 95% CI	p Value
Females all ages		
Benign thyroid disease	1.188 (0.55 to 1.83)	<0.001
Trichilemmomas	1.599 (0.68 to 2.51)	0.001
Papillomatous papules	1.300 (0.66 to 1.94)	<0.001
Benign gastrointestinal lesions	0.945 (0.27 to 1.62)	0.006
Macrocephaly	2.270 (1.61 to 2.93)	<0.001
Lhermitte duclos	3.000 (0.32 to 5.67)	0.028
Cancer	−0.659 (−1.3 to −0.02)	0.043
Endometrial cancer	2.000 (1.01 to 2.99)	<0.001
Intercept	−4.024 (−4.75 to −3.30)	
Females ≤18 years		
Benign skin disease	1.869 (0.73 to 3.01)	0.001
Macrocephaly	1.962 (0.38 to 3.55)	0.015
Intercept	−4.058 (−5.84 to −2.28)	
Females >18 years		
Benign thyroid disease	1.358 (0.62 to 2.09)	<0.001
Benign gastrointestinal lesions	1.076 (0.30 to 0.85)	0.007
Macrocephaly	2.365 (1.61 to 3.11)	<0.001
Trichilemmomas	1.815 (0.83 to 2.80)	<0.001
Papillomatous papules	1.680 (0.94 to 2.42)	<0.001
Endometrial cancer	2.171 (1.11 to 3.23)	<0.001
Intercept	−4.744 (−5.63 to −3.86)	

among mutation positive patients (84%) was much higher than prior estimates.[15] This most likely reflects the fact that head circumferences have been more routinely obtained on CS patients since the adoption of the Consortium criteria.

Adult females had frequencies of benign breast disease (46%) and uterine fibroids (26%) that were significantly lower than the reported rates for CS patients, and not above the background rates of 60% and 70–80%, respectively, reported for the general US population.[31–33] This raises into question the usefulness of these features as part of the diagnostic criteria, and indeed neither was found to be a significant predictor of a mutation in our modelling.

The rate of benign skin lesions among all patients (77%) was also significantly lower than commonly reported. Based on early reports in the dermatology literature, papillomatous papules, trichilemmomas, acral keratoses, and mucosal lesions were thought to affect nearly all patients with CS.[34 35] While it is possible that CS related skin lesions may have been under-reported in our cohort, it is also likely that prior reports over-estimated the frequency of skin lesions with CS. Regardless, our findings suggest that readily apparent skin manifestations may be less common among patients with *PTEN* mutations than previously reported, and that a formal dermatological examination may be helpful in the diagnostic workup of suspected patients.

Malignant features

The high frequencies of breast, endometrial, and thyroid cancers in our cohort were expected. Given the overall young age of our cohort, and that additional cancers might be diagnosed at later ages, our study may well underestimate the lifetime cancer risks. Among adult women with mutations in our cohort, 28/69 (41%) had a diagnosis of invasive breast cancer at the time of testing, with a mean age at molecular testing of 47.6 years (range 33–64 years). No males with breast cancer were identified, consistent with the literature in which only two cases have been reported.[7 36] An association between *PTEN* mutations and risk for male breast cancer is therefore not supported by our data, although it cannot be ruled out.

Endometrial cancer was seen in 12/69 (17%) adult women, which is significantly higher than previous estimates. Six of these women had also had breast cancer, but it is unknown whether their breast cancers predated their endometrial cancer diagnoses, nor whether they had been treated with tamoxifen, which would have increased their endometrial cancer risk. Thyroid cancer was seen in 13 patients (7.6% overall). Interestingly, it was seen at similar rates among adult women (13%) and men (11%), in contrast to the approximately 3 to 4:1 female to male ratio seen in the general population. Given the young mean age of our cohort there was also a suggestion in our data of increased rates of GI cancers, and renal cell carcinoma. A recent report summarising cancers identified in CS cases reported in the medical literature found cumulative risks of cancer to age 70 of 81% for breast cancer, 21% for thyroid cancer, 19% for renal cancer, and 16% for colon cancer.[37] However, the diagnostic criteria used were slightly different from Consortium criteria and only 46% of their cases had a documented *PTEN* mutation. Another recent report of patients with *PTEN* mutations found colorectal cancer in 7.1% of patients overall, and in 13% of those who had had endoscopies.[23]

A number of other clinical features have been suggested to be increased among patients with *PTEN* mutations, but were not supported by our data. Specifically, none of the 172 patients had reported brain cancers and only one had a reported genitourinary malformation. While melanoma has been suggested to be increased in CS, it was reported in only 2/172 mutation positive cases in our study. These were in women ages 45 and 49 years at testing, but we do not know their ages of diagnosis. SEER data estimate the probability of a woman developing melanoma by age 50 as approximately 0.4%.[18]

CS Consortium diagnostic criteria

Our data also suggests that the specificity of the Consortium criteria are lower than previously estimated. Only 79 of 230 subjects (34%) meeting CS diagnostic criteria in our cohort had a detectable mutation, which is significantly lower than the 80% previously reported.[7] This suggests that the Consortium diagnostic criteria are not as robust at identifying patients with germline *PTEN* mutations as previously thought. In comparison, 23 of 42 (55%) BRRS patients had a detectable mutation, which is consistent with the literature.[13]

Specific components of the Consortium criteria are also called into question. For example, looking only at the simplest pairings of two major features, the mutation detection rates in our study were generally low: macrocephaly/breast cancer—23/64 (36%); macrocephaly/thyroid cancer—10/25 (40%); macrocephaly/endometrial cancer—8/10 (80%); breast/thyroid cancers—1/56 (1.8%); breast/endometrial cancers 6/17 (35%); and thyroid/endometrial cancers—3/7 (43%). Yet these are almost certainly overestimates for individuals presenting with only two major features since the majority of patients in our cohort had several other features in addition to the pairs indicated. This was most apparent for the macrocephaly/endometrial cancer category, where eight of 10 women were found to have a mutation: of the eight, five also had a primary breast cancer and a sixth patient had a primary thyroid cancer. These patients also had numerous minor clinical features as well. A recent publication on a small cohort of women with breast cancer also found that macrocephaly was not a significant differentiator between women with or without mutations, and that only 17% of women meeting CS Consortium diagnostic criteria were found to have a mutation.[38] This raises questions as to the validity of the 'two major features' diagnostic criterion.

The low rate of mutation detection in this study may be partially attributed to inaccurate diagnoses or incomplete ascertainment of clinical features by the heterogeneous group of clinicians who referred patients for testing. This is in contrast to some prior research studies in which the diagnoses were made by a single research team.[7 13] However, we believe that the conditions leading to referral for clinical testing in this study represent more 'real world' conditions under which CS/BRRS clinical features are ascertained and testing is ordered. In addition, the vast majority of tests (approximately 90%) were ordered by genetics or cancer genetic programmes, regardless of whether or not a mutation was found in the patient. These centres would be expected to be the most experienced at identifying patients meeting CS criteria. This would argue for modification of the CS diagnostic criteria to focus on clinical features more strongly indicative of the presence of a *PTEN* mutation, and less on features such as benign breast disease and uterine fibroids, which are common in the general population and were not seen at increased rates in our cohort.

Limitations

This study has a number of limitations. Most significantly, there is an inherent referral bias present because those patients with more clinical features of CS/BRRS are obviously more likely to get tested. In addition, the vast majority of subjects (142) were the first identified affected member in their family and thus were more likely to have significant clinical features. However, since only 30 subjects were tested because of having an affected relative, we were not able to exclude the probands from our analyses. Because we found no significant differences in the frequencies of the clinical features between probands and tested relatives (data not shown), these groups were combined in our analyses. Our data thus may overestimate the frequency of *PTEN* related clinical features. A less biased assessment would come from studying family members only and excluding the proband. However, given the rarity of this disease, this will be very difficult to do. As the only clinical laboratory in the USA testing for *PTEN* mutations during much of our 8 year accrual period, we were still able to collect only 30 relatives with mutations. Statistically significant conclusions cannot be made on such a small cohort. We thus felt it was important and clinically informative to provide the data we had available on the large cohort of mutation positive probands.

As noted above, the patients in our study were not directly evaluated by our team and the accuracy of the clinical histories reported by the referring clinicians may vary widely. While this was guided by the required use of a clinical features checklist, it is possible that incomplete and/or inaccurate histories may have been reported. However, incomplete histories would lead to an underestimate of the frequencies of clinical features.

A final limitation was that only sequencing was used for analysis of the *PTEN* gene, and thus some mutations may have been missed. However, the existing literature suggests that these are likely to be few. One group found variations in the *PTEN* promoter in approximately 2% of patients meeting CS diagnostic criteria.[11] The same group found large rearrangements or deletions in 0 of 95 CS patients and three of 27 BRRS patients who had no detectable mutation on DGGE analysis. More recently, deletions were found in three of 15 CS patients who were negative for *PTEN* mutations on DGGE analysis.[10] Assuming that DGGE detects mutations in 40–80% of patients meeting CS diagnostic criteria (based on the literature and the current study), this extrapolates to deletions being detected in 4–12% of patients with CS.

Conclusions

In conclusion, our findings support the previously reported increased frequencies of breast, endometrial, and thyroid cancers and many but not all of the component non-malignant features in patients with *PTEN* mutations. Our data also suggest that endometrial cancer may be more frequent than previously reported, and that kidney and colon cancer may also be increased in frequency. Further work is needed to confirm these possible associations.

We also found evidence that the Consortium diagnostic criteria for CS are significantly less specific than previously estimated. In particular, most of the pairings of macrocephaly and one other major criteria, which meet current CS diagnostic criteria, led to low mutation detection rates. An association between *PTEN* mutations and certain minor diagnostic criteria, such as benign breast disease and uterine fibroids, was not found in our cohort. This work suggests that the Consortium diagnostic criteria should be revised to put greater emphasis on the most predictive clinical features. Based on our data, we have developed a model (http://internalmedicine.osu.edu/genetics/research/tools-for-providers/) which can be used to predict the likelihood of a *PTEN* mutation for a patient based on their constellation of clinical features. We hope that it can provide guidance in clinical practice in identifying patients appropriate for *PTEN* testing.

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REFERENCES

1. **Nelen MR,** Padberg GW, Peeters EAJ, Lin AY, van den Helm B, Frants RR, Coulon V, Goldstein AM, van Reen MMM, Easton DF, Eeles RA, Hodgson S, Mulvihill JJ, Murday VA, Tucker MA, Mariman ECM, Starink TM, Ponder BAJ, Ropers HH, Kremer H, Longy M, Eng C. Localization of the gene for Cowden disease to 10q22-23. *Nat Genet* 1996;**13**:114–16.
2. **Eng C.** Will the real Cowden syndrome please stand up: revised diagnostic criteria. *J Med Genet* 2000;**37**:828–30.
3. **Pilarski R,** Eng C. Will the real Cowden syndrome please stand up (again)? Expanding mutational and clinical spectra of the PTEN hamartoma tumour syndrome. *J Med Genet* 2004;**41**:323–6.
4. **Zbuk KM,** Stein JL, Eng C. PTEN Hamartoma Tumor Syndrome (PHTS). *GeneReviews at GeneTests: Medical Genetics Information Resource [database online]*. http://www.genetests.org 2006.
5. **Nelen MR,** van Staveren CG, Peeters EAJ, Ben Hassel M, Gorlin RJ, Hamm H, Lindboe CF, Fryns J-P, Sijmons RH, Woods DG, Mariman ECM, Padberg GW, Kremer H. Germline mutations in the *PTEN/ MMAC1* gene in patients with Cowden disease. *Hum Mol Genet* 1997;**6**:1383–7.
6. **Liaw D,** Marsh DJ, Li J, Dahia PLM, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R. Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* 1997;**16**:64–7.
7. **Marsh DJ,** Coulon V, Lunetta KL, Rocca-Serra P, Dahia PLM, Zheng Z, Liaw D, Caron S, Duboué B, Lin AY, Richardson A-L, Bonnetblanc J-M, Bressieux J-M, Cabarrot-Moreau A, Chompret A, Demange L, Eeles R, Yahanda AM, Fearon ER, Fricker J-P, Gorlin RJ, Hodgson SV, Huson S, Lacombe D, LePrat F, Odent S, Toulouse C, Olopade OI, Sobol H, Tishler S, Woods CG, Robinson BG, Weber HC, Parsons R, Peacocke M, Longy M, Eng C. Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline PTEN mutation. *Hum Mol Genet* 1998;**7**:507–15.
8. **Tsou HC,** Teng DH, Ping XL, Brancolini V, Davis T, Hu R, Xie XX, Gruener AC, Schrager CA, Christiano AM, Eng C, Steck P, Ott J, Tavtigian SV, Peacocke M. The role of MMAC1 mutations in early-onset breast cancer: causative in association with

Cowden syndrome and excluded in BRCA1-negative cases. *Am J Hum Genet* 1997;**61**:1036—43.
9. **Nelen MR,** Kremer H, Konings IBM, Schoute F, van Essen AJ, Koch R, Woods CG, Fryns J-P, Hamel B, Hoefsloot LH, Peeters EAJ, Padberg GW. Novel *PTEN* mutations in patients with Cowden disease: absence of clear genotype-phenotype correlations. *Eur J Hum Genet* 1999;**7**:267—73.
10. **Chibon F,** Primois C, Bressieux JM, Lacombe D, Lok C, Mauriac L, Taieb A, Longy M. Contribution of PTEN large rearrangements in Cowden disease: a MAPH screening approach. *J Med Genet* 2008;**45**:657—65.
11. **Zhou XP,** Waite KA, Pilarski R, Hampel H, Fernandez MJ, Bos C, Dasouki M, Feldman GL, Greenberg LA, Ivanovich J, Matloff E, Patterson A, Pierpont ME, Russo D, Nassif NT, Eng C. Germline PTEN Promoter Mutations and Deletions in Cowden/Bannayan-Riley-Ruvalcaba Syndrome Result in Aberrant PTEN Protein and Dysregulation of the Phosphoinositol-3-Kinase/Akt Pathway. *Am J Hum Genet* 2003;**73**:404—11.
12. **Gorlin RJ,** Cohen MM, Condon LM, Burke BA. Bannayan-Riley-Ruvalcaba syndrome. *Am J Med Genet* 1992;**44**:307—14.
13. **Marsh DJ,** Kum JB, Lunetta KL, Bennett MJ, Gorlin RJ, Ahmed SF, Bodurtha J, Crowe C, Curtis MA, Dazouki M, Dunn T, Feit H, Geraghty MT, Graham JM, Hodgson SV, Hunter A, Korf BR, Manchester D, Miesfeldt S, Murday VA, Nathanson KA, Parisi M, Pober B, Romano C, Tolmie JL, Trembath R, Winter RM, Zackai EH, Zori RT, Weng LP, Dahia PLM, Eng C. *PTEN* mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. *Hum Mol Genet* 1999;**8**:1461—72.
14. **Pilarski R.** Cowden syndrome: a critical review of the clinical literature. *J Genet Couns* 2009;**18**:13—27.
15. **Eng C.** *PTEN*: One Gene, Many Syndromes. *Hum Mutat* 2003;**22**:183—98.
16. **Marsh DJ,** Roth S, Lunetta KL, Hemminki A, Dahia PL, Sistonen P, Zheng Z, Caron S, van Orsouw NJ, Bodmer WF, Cottrell SE, Dunlop MG, Eccles D, Hodgson SV, Jarvinen H, Kellokumpu I, Markie D, Neale K, Phillips R, Rozen P, Syngal S, Vijg J, Tomlinson IP, Aaltonen LA, Eng C. Exclusion of PTEN and 10q22-24 as the susceptibility locus for juvenile polyposis syndrome. *Cancer Res* 1997;**57**:5017—21.
17. **Marsh DJ,** Caron S, Dahia PLM, Kum JB, Frayling IM, Tomlinson IPM, Hughes KS, Hodgson SV, Murday VA, Houlston R, Eng C. Germline *PTEN* mutations in Cowden syndrome-like families. *J Med Genet* 1998;**35**:881—5.
18. **National Cancer Institute SRaAB.** *DevCan: Probability of Developing or Dying of Cancer Software, Version 6.5.0*. Bethesda, MD, USA: US National Cancer Institute, 2005.
19. **Fay MP,** Pfeiffer R, Cronin KA, Le C, Feuer EJ. Age-conditional probabilities of developing cancer. *Stat Med* 2003;**22**:1837—48.
20. **Fay MP.** Estimating age conditional probability of developing disease from surveillance data. *Popul Health Metr* 2004;**2**:6.
21. **Vega A,** Torres J, Torres M, Cameselle-Teijeiro J, Macia M, Carracedo A, Pulido R. A novel loss-of-function mutation (N48K) in the PTEN gene in a Spanish patient with Cowden disease. *J Invest Dermatol* 2003;**121**:1356—9.
22. **Bonneau D,** Longy M. Mutations of the human PTEN gene. *Hum Mutat* 2000;**16**:109—22.
23. **Heald B,** Mester J, Rybicki L, Orloff MS, Burke CA, Eng C. Frequent Gastrointestinal Polyps and Colorectal Adenocarcinomas in a Prospective Series of PTEN Mutation Carriers. *Gastroenterology* 2010;**139**:1927—33.
24. **Kato H,** Kato S, Kumabe T, Sonoda Y, Yoshimoto T, Kato S, Han SY, Suzuki T, Shibata H, Kanamaru R, Ishioka C. Functional evaluation of p53 and PTEN gene mutations in gliomas. *Clin Cancer Res* 2000;**6**:3937—43.
25. **Han SY,** Kato H, Kato S, Suzuki T, Shibata H, Ishii S, Shiiba K, Matsuno S, Kanamaru R, Ishioka C. Functional evaluation of PTEN missense mutations using in vitro phosphoinositide phosphatase assay. *Cancer Res* 2000;**60**:3147—51.
26. **Sharma MR,** Petty EM, Lesperance MM. Airway obstruction caused by PTEN hamartoma (Bannayan-Riley-Ruvalcaba) syndrome. *Arch Otolaryngol Head Neck Surg* 2007;**133**:1157—60.
27. **Orrico A,** Galli L, Buoni S, Orsi A, Vonella G, Sorrentino V. Novel PTEN mutations in neurodevelopmental disorders and macrocephaly. *Clin Genet* 2009;**75**:195—8.
28. **Zhou XP,** Marsh DJ, Morrison CD, Chaudhury AR, Maxwell M, Reifenberger G, Eng C. Germline inactivation of PTEN and dysregulation of the phosphoinositol-3-kinase/Akt pathway cause human Lhermitte-Duclos disease in adults. *Am J Hum Genet* 2003;**73**:1191—8.
29. **Tan WH,** Baris HN, Burrows PE, Robson CD, Alomari AI, Mulliken JB, Fishman SJ, Irons MB. The spectrum of vascular anomalies in patients with PTEN mutations: implications for diagnosis and management. *J Med Genet* 2007;**44**:594—602.
30. **Lachlan KL,** Lucassen AM, Bunyan D, Temple IK. Cowden syndrome and Bannayan Riley Ruvalcaba syndrome represent one condition with variable expression and age-related penetrance: results of a clinical study of PTEN mutation carriers. *J Med Genet* 2007;**44**:579—85.
31. **Love SM,** Gelman RS, Silen W. Sounding board. Fibrocystic "disease" of the breast—a nondisease? *N Engl J Med* 1982;**307**:1010—14.
32. **Santen RJ,** Mansel R. Benign breast disorders. *N Engl J Med* 2005;**353**:275—85.
33. **Day Baird D,** Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. *Am J Obstet Gynecol* 2003;**188**:100—7.
34. **Salem OS,** Steck WD. Cowden's disease (multiple hamartoma and neoplasia syndrome). A case report and review of the English literature. *J Am Acad Dermatol* 1983;**8**:686—96.
35. **Starink TM,** Hausman R. The cutaneous pathology of facial lesions in Cowden's disease. *J Cutan Pathol* 1984;**11**:331—7.
36. **Fackenthal J,** Marsh DJ, Richardson AL, Cummings SC, Eng C, Robinson BG, Olopade OI. Male breast cancer in Cowden syndrome patients with germline *PTEN* mutations. *J Med Genet* 2001;**38**:159—64.
37. **Riegert-Johnson DL,** Gleeson FC, Roberts M, Tholen K, Youngborg L, Bullock M, Boardman LA. Cancer and Lhermitte-Duclos disease are common in Cowden syndrome patients. *Hered Cancer Clin Pract* 2010;**8**:6.
38. **Shiovitz S,** Everett J, Huang SC, Orloff MS, Eng C, Gruber SB. Head circumference in the clinical detection of PTEN hamartoma tumor syndrome in a clinic population at high-risk of breast cancer. *Breast Cancer Res Treat* 2010;**124**:459—65.