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The Clinical Spectrum of *PTEN* Mutations

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Abstract

PTEN is a tumor suppressor gene that classically dampens the PI3K/AKT/mTOR growth-promoting signaling cascade. *PTEN* dysfunction causes dysregulation of this and other pathways, resulting in overgrowth. Cowden syndrome, a hereditary cancer predisposition and overgrowth disorder, was the first Mendelian condition associated with germline *PTEN* mutations. Since then, significant advances by the research and medical communities have elucidated how clinical phenotypic manifestations result from the underlying germline *PTEN* mutations. With time, it became evident that *PTEN* mutations can result in a broad phenotypic spectrum, causing seemingly disparate disorders from cancer to autism. Hence, the umbrella term of *PTEN* hamartoma tumor syndrome (PHTS) was coined. Timely diagnosis and understanding the natural history of PHTS are vital because early recognition enables gene-informed management, particularly as related to high-risk cancer surveillance and addressing the neurodevelopmental symptoms.

INTRODUCTION

Germline mutations in *PTEN* (OMIM 601728), a tumor suppressor gene on 10q23 (1), have been found in subsets of seemingly disparate syndromes (2). Thus, the umbrella genetic diagnosis *PTEN* hamartoma tumor syndrome (PHTS) was coined for the subsets of individuals with any clinical diagnosis characterized by germline *PTEN* mutations (3, 4). The first recognized clinical diagnosis was Cowden syndrome (CS; OMIM 158350), an autosomal dominant multisystem disorder characterized by multiple hamartomas and elevated lifetime risks of breast, thyroid, and other cancers (5, 6). Hamartomas are developmentally disorganized benign growths affecting at least two (strictly three) components normally found in the affected tissue. The syndrome was first described in 1963 by Lloyd & Dennis (5) in a 20-year-old patient, Rachel Cowden.

It is estimated that CS has an incidence of 1:200,000 individuals, although this is likely an underestimate (4). Indeed, CS is difficult to recognize and remains underdiagnosed, particularly because of the protean manifestations that may occur in isolation in the general population. The lack of uniform diagnostic criteria for CS prior to 1995 motivated the founding of the International Cowden Consortium (ICC; C. Eng, Coordinator and Chair), with the original intent of creating operational diagnostic consensus criteria to select affected CS individuals or families for the purposes of mapping and characterizing the causative gene (7).

The first susceptibility gene for CS was mapped to 10q22–23 in 1996 (8) and identified a year later as the tumor suppressor gene *PTEN* (phosphatase and tensin homolog). Early studies on CS families meeting strict ICC diagnostic criteria identified germline *PTEN* mutations in ∼85% of CS cases (9, 10). Subsequent analysis of >3,000 prospective community-accrued CS and CS-like (full diagnostic criteria minus one) probands (11) estimated that ∼25% of patients who met more relaxed diagnostic criteria harbored germline *PTEN* mutations.

The clinical spectrum of *PTEN* mutations has expanded since the identification of *PTEN* as the CS susceptibility gene. Bannayan-Riley-Ruvalcaba syndrome (BRRS; OMIM 153480) was first described as a congenital or pediatric-onset disorder classically characterized by macrocephaly in combination with intestinal hamartomatous polyposis, vascular malformations, lipomas, hemangiomas, and genital freckling (12). BRRS is allelic to CS, as subsets of both disorders have been associated with germline *PTEN* mutations, including identical *PTEN* mutations in families segregating both clinical phenotypes (3). It is this observation that initially led to the coining of the term PHTS. Germline intragenic *PTEN* mutations have been reported in ∼60% of BRRS individuals (9, 13, 14). Among those without identified intragenic mutations, another 10% harbor large deletions encompassing or including *PTEN* (10).

Besides CS and BRRS, germline *PTEN* mutations have been identified in up to 20% of individuals with Proteus syndrome (PS; OMIM 176920) and in Proteus-like syndrome (15). PS is a rare, complex, and highly variable hamartomatous overgrowth disorder, characterized by congenital malformations and segmental overgrowths of multiple tissues of all germ layers, most commonly affecting the skin, skeleton, adipose tissue, and central nervous system (16). The term PS-like refers to individuals who exhibit clinical manifestations of PS but do not fulfill diagnostic criteria. Additionally, ∼10–20% of individuals with autism spectrum disorder (ASD) and macrocephaly harbor germline *PTEN* mutations (17–21). Single cases of VATER (vertebral defects, anal atresia, tracheoesophageal fistula with esophageal atresia, and radial or renal dysplasia) syndrome, megalencephaly, and hemimegencephaly have been reported to carry germline *PTEN* mutations as well (22, 23).

Based on the clinical spectrum and unified genetic etiology, a diagnosis of PHTS has profound clinical implications for the proband and at-risk family members. Here, we review the clinical spectrum of germline *PTEN* mutations and the broad medical implications and challenges of a PHTS diagnosis.

RECOGNITION OF THE CLINICAL PHENOTYPIC SPECTRUM OF *PTEN* HAMARTOMA TUMOR SYNDROME

Clinical features of PHTS are extensively variable and affect multiple organ systems (**Figure 1**). Accordingly, there are several scenarios in which PHTS patients may come to the attention of healthcare providers. CS usually presents by the late twenties, with variable expression and age-related penetrance. The most commonly reported manifestations are macrocephaly, mucocutaneous lesions, thyroid abnormalities, fibrocystic disease and carcinoma of the breast, gastrointestinal hamartomas, multiple early-onset uterine leiomyomas, and developmental delay (4, 24). Pathognomonic mucocutaneous lesions are believed to exist in 100% of CS patients by age 30. The operational diagnostic consensus criteria for CS form the basis for the US-based National Comprehensive Cancer Network (NCCN) guidelines (**Table 1**) (7). Relatedly, pediatric patients typically come to medical attention due to macrocephaly, neurodevelopmental abnormalities, dermatologic features, and rarely, early-onset cancers (**Table 2**).

It is crucial for clinicians to be mindful of the genetic differential diagnosis of PHTS. A careful history and physical examination, as well as a meticulous family history to look for other component symptoms and signs, are warranted. For example, gastrointestinal hamartomatous polyposis can be observed in other hamartomatous syndromes such as juvenile polyposis syndrome (OMIM 174900) and Peutz-Jeghers syndrome (OMIM 175200). Additionally, undiagnosed patients may

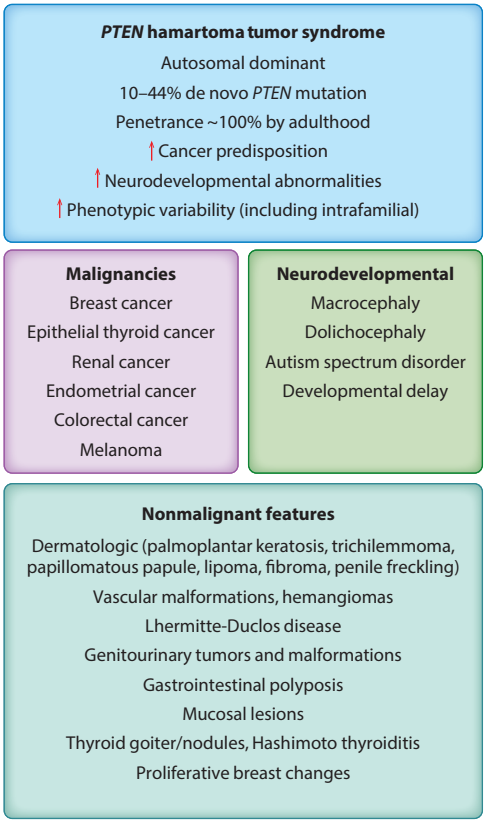

Figure 1

Clinical characteristics of *PTEN* hamartoma tumor syndrome.

Table 1 International Cowden Consortium operational diagnostic criteria (7)

Pathognomonic	Major	Minor
Adult Lhermitte-Duclos disease (LDD)	Breast carcinoma	Other thyroid lesions (e.g., adenoma, multinodular goiter)
Mucocutaneous lesions	Thyroid carcinoma (non-medullary), especially follicular thyroid carcinoma	Mental retardation (i.e., IQ ≤ 75)
Trichilemmomas, facial	Macrocephaly (occipital frontal circumference ≥97th percentile)	Gastrointestinal hamartomas
Acral keratoses	Endometrial carcinoma	Fibrocystic breast disease
Papillomatous papules		Lipomas
Mucosal lesions		Fibromas
		Genitourinary tumors (especially renal cell carcinoma)
		Genitourinary malformations
		Uterine fibroids
Operational diagnosis in an individual (any of the following)		
Mucocutaneous lesions alone, if ≥ six facial papules (three of which must be trichilemmomas)		
Cutaneous facial papules and oral mucosal papillomatosis		
Oral mucosal papillomatosis and acral keratoses		
≥ Six palmoplantar keratoses		
≥ Two major criteria (one of which must be macrocephaly or LDD)		
One major and ≥ three minor criteria		
≥ Four minor criteria		
Operational diagnosis in a family where one individual is diagnostic for Cowden syndrome		
Any one pathognomonic criterion		
Any one major criterion ± minor criteria		
Two minor criteria		
History of Bannayan-Riley-Ruvalcaba syndrome		

seek medical attention because of abnormal thyroid function or a thyroid mass. Over two-thirds of CS patients have thyroid problems, which may occur at any age (4). Finding multifocal lesions, especially in young patients, should raise suspicion. Hence, recognizing PHTS is facilitated by examining patients for clinical "red flags" that guide clinicians toward distinguishing pathognomonic features, given their rarity in the general population (25).

To aid clinicians at the point of care, a nomogram-based clinical predictor named the Cleveland Clinic *PTEN* Risk Calculator (**http://www.lerner.ccf.org/gmi/ccscore/**) was developed to evaluate the pretest probability of harboring a germline *PTEN* mutation (11). This clinical decision support tool provides an age-adjusted weighted sum of phenotypic features referred to as the *PTEN* Cleveland Clinic score (CC score). A CC score of 10 is a recommended threshold for referral of adult patients to genetic specialists for *PTEN* genetic testing (11). A CC score of 15, corresponding to a 10% a priori risk of positive *PTEN* mutation status, is the most cost-effective cut-off at which to refer CS-like patients for *PTEN* germline testing (26). For pediatric patients, distinct criteria should be followed to guide selection for germline *PTEN* mutation testing (**Table 2**) (11).

Table 2 Pediatric criteria for diagnosis of *PTEN* hamartoma tumor syndrome (11)

Required criterion	Secondary criteria
Macrocephaly (≥2 standard deviations)	At least one of the following criteria should be present: ■ Autism spectrum disorder or developmental delay ■ Dermatologic features (lipomas, oral papillomas, trichilemmomas, penile freckling) ■ Vascular features (arteriovenous malformations or hemangiomas) ■ Gastrointestinal polyps ■ Pediatric-onset thyroid cancer or germ cell tumors

PTEN GERMLINE MUTATION SPECTRUM

Pathogenic germline mutations have been reported in all nine exons of *PTEN* (11, 27, 28). These mutations include missense, nonsense, and splice site mutations; intragenic deletions or insertions; and large deletions (11, 27–29). Exons 5, 7, and 8 are overrepresented in the *PTEN* germline mutation spectrum (11). Three common nonsense mutations (R130X, R233X, R335X) have been well-characterized in exons 5, 7, and 8, respectively. Exon 5 is a hotspot for germline mutations as it harbors the catalytic core motif (30, 31). Additionally, although limited data are available for *PTEN* promoter variants, some pathogenic promoter mutations have been shown to affect *PTEN* transcription, as well as translation due to altered RNA secondary structure (10). Particular pathogenic *PTEN* intronic variants can cause exon skipping, alternative splicing, or the use of cryptic splice sites (32). Large deletions and duplications affecting *PTEN* are less common and can be found over the entire coding sequence (11, 29). To facilitate evidence-based and clinically relevant classification of germline *PTEN* mutations, the Clinical Genome Resource (ClinGen) established the *PTEN* Variant Curation Expert Panel to guide and complete these efforts (33).

CLINICAL IMPLICATIONS

PTEN-Related Lifetime Neoplasia Risks

An age-adjusted cancer incidence and age-related penetrance study (27) revealed elevated lifetime risks in PHTS for breast (85%), thyroid (35%), kidney (34%), endometrial (28%), and colon (9%) cancers, as well as melanoma (6%) (**Table 3**). Elevated cancer risks have been independently validated by other groups (34, 35). As is typical of hereditary cancer syndromes, the risk of early-onset, bilateral, and multifocal cancer is elevated.

Among all component cancers, female breast cancer lifetime risk is the most pronounced, beginning around age 30. Although male breast cancer has been reported in CS (36), an increased lifetime risk in *PTEN* mutation–positive males was not noted in the expanded series of prospective patients (11). Women with CS also have up to 67% risk for benign breast disease (4).

Benign thyroid diseases such as multinodular goiter, adenomatous nodules, and follicular adenomas commonly occur in up to 75% of individuals with CS (37). Epithelial thyroid cancer is observed in PHTS, contrary to medullary thyroid carcinoma characteristic of multiple endocrine neoplasia type 2 (OMIM 155240). More specifically, follicular thyroid carcinoma and follicular variant of papillary thyroid carcinoma tend to be overrepresented in PHTS patients (37, 38). Pediatric onset of thyroid cancer, male gender, history of thyroid nodules and/or thyroiditis, and follicular thyroid carcinoma histology were found to be predictive of *PTEN* mutation status in a series of CS/CS-like patients with thyroid cancer (38).

The risk for renal cell carcinoma in PHTS patients begins at ~40 years (27). The predominant histology is papillary (39). Because renal ultrasound screening is not sensitive for detecting papillary renal cell carcinoma, particularly if the tumor is small, computed tomography or magnetic resonance imaging examinations are preferred for PHTS patients with suspected renal cell carcinoma (39). However, a subset of urologists screen with ultrasound, reasoning that resection would not occur until the carcinoma reaches a certain size, which would be detectable by ultrasound.

PTEN-related endometrial cancer risk begins at age 25, rising to 30% by age 60 (27). Age ≤50 years, macrocephaly, high phenotypic burden, and/or coexisting renal cell carcinoma are strong clinical predictors for the existence of germline *PTEN* mutations in endometrial cancer in CS/CS-like patients (40). Individuals with germline *PTEN* mutations are also at increased risk of developing benign endometrial diseases, such as uterine fibroids (27).

Additionally, >90% of *PTEN* mutation carriers who had a colonoscopy had colorectal polyps, typically with mixed histologies. Polyp histologies may include ganglioneuromas, hamartomatous

Table 3 Component cancer risks, clinical surveillance, and management guidelines for PHTS[a]

Organ or organ system	Population cancer risk (SEER)	Lifetime cancer risk in PHTS[b]	Screening/surgical guidelines[c]
Breast (female)	12%	67–85%	Starting at age 18: Consistent breast awareness and self-exam; report changes to healthcare provider Starting at age 25[d]: Clinical breast exam every 6–12 months Starting at age 30–35[d]: Annual mammogram with consideration of tomosynthesis and breast MRI with contrast Starting at age of diagnosis in adults: Discuss mastectomy, as needed
Thyroid	1%	6–38%	Starting at age of diagnosis (including childhood): Annual thyroid ultrasound
Kidney	1.6%	2–34%	Starting at age 40: Consider renal ultrasound every 1–2 years
Endometrium	2.6%	21–28%	Personalized management: ■ Encourage patient education and prompt response to symptoms (e.g., abnormal bleeding) ■ Consider endometrial biopsy screening every 1–2 years ■ Transvaginal ultrasound in postmenopausal women at the clinician's discretion and as needed ■ Discuss hysterectomy upon completion of childbearing and as needed
Colon	5%	9–17%	Starting at age 35[d] (unless symptomatic): Colonoscopy every 5 years; more frequently if patient is symptomatic or polyps are found
Dermatologic[e]	2%	2–6%	Personalized management: Dermatologic exam at the clinician's recommendation
Developmental	NA	NA	Starting at age of diagnosis: At the clinician's recommendation, consider psychomotor assessment in children; brain MRI if symptomatic

[a]Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment: Breast and Ovarian V.3.2019. © National Comprehensive Cancer Network, Inc. 2019. All rights reserved. Accessed January 18, 2019. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

[b]Cancer lifetime risks calculated to age 70 by Tan et al. (27) and Bubien et al. (34), and to age 60 by Nieuwenhuis et al. (35). Cancer risk percent ranges reflect lowest and highest frequencies reported in all three studies.

[c]Annual comprehensive physical exam starting at age 18 years, or 5 years before the youngest age of diagnosis of a component cancer in the family (whichever comes first), with particular attention to thyroid exam. Encourage patient education regarding the signs and symptoms of cancer.

[d]Cancer screening should begin 5–10 years before the earliest known component cancer in the family or according to the ages listed in this table, whichever comes first.

[e]Lifetime cancer risk estimates of skin cutaneous melanoma.

Abbreviations: MRI, magnetic resonance imaging; NA, not applicable; PHTS, *PTEN* hamartoma tumor syndrome; SEER, Surveillance, Epidemiology, and End Results.

polyps, juvenile polyps, and adenomatous polyps (41). Patients who developed colorectal carcinomas also tended to have pre- or coexisting colonic polyposis. A subset of PHTS patients also show upper gastrointestinal polyps, and ~20% have glycogenic acanthosis (41–43).

Melanoma was observed in several individual case reports and formally added as a CS component cancer, with the earliest age of onset reported at three years (27, 44, 45).

Individuals with PHTS also have a sevenfold increased risk of developing a second primary malignant neoplasm as compared to the US general population (28). Notably, women with germline *PTEN* mutations who have had a diagnosis of breast cancer have a 29% risk of developing a second

primary breast cancer within ten years. Importantly, these risk assessment estimates guide existing surveillance and medical management recommendations for PHTS patients (**Table 3**), with the ultimate goal of preventing or at least detecting any tumors at the earliest, most manageable stages (31).

Brain Abnormalities and Neurodevelopmental Disorders

Individuals with germline *PTEN* mutations can also manifest with neurodevelopmental phenotypes such as megalencephaly, ASD, and developmental delay (17, 21, 46). Isolated studies identified germline *PTEN* pathogenic mutations in patients with macrocephaly (occipital-frontal circumference measurement ≥2 standard deviations over the mean) and VATER association or with macrocephaly and ASD (4, 22). A frequency of 10–20% of germline *PTEN* mutations in ASD with macrocephaly has been independently reported by multiple groups (17–19, 47, 48). The extent of macrocephaly observed in ASD patients with germline *PTEN* mutations is often more severe than in ASD patients with wild-type *PTEN* (49). These observations, combined with ease of measurement of head circumference, make macrocephaly an important endophenotype within ASD. ASD-related macrocephaly has been linked to increased brain mass and white matter volumes (50–52). Neurological studies of PHTS patients with ASD show increases in cortical white matter and a distinctive cognitive profile, including delayed language development with poor working memory and processing speed (53). Neurocognitive assessment of individuals with PHTS revealed a wide range of intellectual abilities, with compromised motor function, executive function, and memory recall in the majority of participants (54). Interestingly, a broad spectrum of neuropsychiatric phenotypes has been observed in individuals with PHTS, macrocephaly, and brain leukoencephalopathies (55). These phenotypes range from normal development and intelligence to severely debilitating neurocognitive and motor aberrations, including generalized anxiety, adult-onset movement disorders, obsessive-compulsive disorder, and psychosis. In addition to white matter abnormalities, features such as meningiomas, arteriovenous malformations, prominent perivascular spaces, cortical dysplasia, and gray matter heterotopias have also been reported (55–57).

As pertinent to overgrowth, Lhermitte-Duclos disease, a hamartomatous overgrowth of the cerebellum, is a pathognomonic feature observed in (adult) CS (7). Histologically, the lesion is characterized by large neuronal cells expanding into the granular and molecular layers of the cerebellar cortex (58). Imaging findings typically encompass unilateral cerebellar hypertrophy and widened cerebellar folia with a "tiger-striped" appearance (59). Such distinct features are important for clinicians to recognize as red flags for establishing a PHTS diagnosis and excluding differential diagnoses (4, 31). Collectively, these studies provide strong evidence for the association of germline *PTEN* mutations with brain-related and neurodevelopmental disorders, forming the basis for the recommendation of *PTEN* genetic testing in this subset of patients.

Immunologic and Metabolic Aberrations

Autoimmunity and lymphoid hyperplasia are observed in ∼40% of PHTS patients. These patients show dysregulated immune function as manifested by lymphopenia, CD4+ T cell reduction, and changes in T and B lymphocyte subsets (60). Indeed, clinicians have observed various autoimmune-related phenotypes such as Hashimoto thyroiditis, eosinophilic esophagitis, colitis, and vasculitis in PHTS patients (38, 61–63). Relatedly, an ex vivo model utilizing innate immune cells from PHTS patients with germline *PTEN* loss-of-function mutations revealed elevated lactate production and a proinflammatory phenotype of monocytes, particularly in the context of *PTEN*-deficient thyroid cancer (64). These data suggest that *PTEN* may affect multiple aspects

of immune function, including modulation of the microenvironment as relevant to the efficacy of cancer therapeutics (65, 66). Finally, a subset of PHTS patients show features associated with increased insulin sensitivity and obesity (67). Compared to controls, *PTEN* mutation carriers show lower fasting plasma insulin levels, higher glucose infusion rate, and increased measures of obesity. This insulin hypersensitivity phenotype reflected an apparently divergent effect of *PTEN* mutations—increased risks of obesity and cancer but a decreased risk of type 2 diabetes.

MEDICAL MANAGEMENT, GENETIC COUNSELING, AND PSYCHOSOCIAL IMPLICATIONS

Because of the broad spectrum of phenotypic manifestations and variable expressivity, many PHTS patients may go undiagnosed for years. Hence, medical practitioners should be familiar with clinical red flags associated with PHTS (**Figure 1** and **Table 2**). Moreover, clinicians should be cognizant of the role of family history and genetic counseling referral guidelines, while paying close attention to age-specific penetrance of PHTS manifestations in family members (4, 68).

A relationship connecting the patient, primary care provider, and genetics team can be crucial for patient care. The key to successful management of PHTS patients and their families is a multidisciplinary team (an example may be viewed at **https://my.clevelandclinic.org/departments/genomics/specialties/pten-clinic**). Because PHTS is an autosomal dominant disorder, offspring of an affected person have a 50% probability to inherit the gene mutation, and relatives are at an increased risk as well. Up to 44% of patients have a de novo *PTEN* mutation, which means the mutation is not carried by either of their parents (69) and there will be no obvious family history. If a family-specific mutation is known, then screening for that particular mutation in at-risk family members would yield results that are 100% accurate. *PTEN* is included on multigene testing panels curated for multiple hereditary cancer syndromes, ASD, global development delay, epilepsy, and overgrowth syndromes. Although multigene panels are useful to test for multiple overlapping syndromes, data interpretation may be challenging in terms of maximizing actionable findings while limiting the identification of variants of uncertain significance and PHTS-unrelated secondary pathogenic mutations. Relatedly, patients not meeting clinical diagnostic criteria for syndromes under the PHTS umbrella or without a contributory family history may be identified as harboring a germline *PTEN* mutation.

As PHTS is a hereditary syndrome, the identification of a germline *PTEN* mutation in an individual will often lead to the identification of the same *PTEN* mutation in other family members, in whom a PHTS diagnosis may not have been suspected (70). Hence, clinicians responsible for delivering genetic testing results should be aware of the psychosocial implications of a genetic diagnosis, including apprehensiveness about sharing genetic information with family members (68). It is important that a PHTS diagnosis is delivered as an opportunity for prevention ("knowledge is power"). Genetic counseling can also be useful in family planning. Preimplantation testing can prevent having children with PHTS; other options, including alternative reproduction, may also be considered (4).

Because *PTEN* alterations classically result in enhanced PI3K/AKT/mTOR signaling, this pathway represents a rational therapeutic target in PHTS. A phase II open-label clinical trial (NCT00971789) utilized the mTOR inhibitor sirolimus in adult PHTS patients (81). Another mTOR inhibitor trial is currently accruing pediatric, adolescent, and young adult patients with germline pathogenic *PTEN* mutations and ASD (NCT02461446). Exploring the clinical utility of pharmacologically inhibiting upstream components of the PTEN pathway such as PI3K and AKT is warranted. From a biological perspective, since PTEN regulates a vast array of cellular processes

independent of PI3K/AKT/mTOR signaling, it is also critical to explore targeting such vulnerabilities. For example, since nuclear PTEN participates in maintaining genomic integrity, PARP inhibitors would make sense in individuals with mutations that disrupt nuclear PTEN function (71).

KEY CHALLENGES

Missing Heritability

It is not uncommon to encounter individuals who meet the diagnostic criteria and yet test negative for germline *PTEN* mutations and large deletions. Indeed, ~15% of classic CS and ~95% of CS-like individuals lack detectable *PTEN* mutations (27). Germline *PTEN* mutations exist in ~60% of BRRS patients (3, 9, 10, 13). One possibility is that non-*PTEN* etiologies exist in patients with wild-type *PTEN*; this has been an area of active research. These studies have identified multiple susceptibility genes for *PTEN*-wild-type CS/CS-like and BRRS, including *SDHB-D*, *KLLN*, *AKT1*, *PIK3CA*, *SEC23B*, *USF3*, and *TTN* (2). More recently, known cancer predisposition genes such as *BRCA1*, *BRCA2*, *RET*, and others were associated with a rare subset of *PTEN*-wild-type CS/CS-like and BRRS patients (72). While some genes (e.g., *AKT1*, *PIK3CA*) make sense biologically as being key effectors within the PTEN signaling cascade, other genes have required meticulous functional interrogation to explain their contribution to PHTS-related phenotypes. For example, *SEC23B* has been found to play noncanonical roles in the ribosome biogenesis pathway, a pro-growth cellular activity known to be regulated by *PTEN* (73, 74). The identification of other CS/CS-like and BRRS susceptibility genes reflects the phenotypic heterogeneity observed in these disorders.

These observations do not preclude the possibility that nongenetic (e.g., epigenetic) factors may also be etiologic in the subset of CS/CS-like and BRRS patients without identified germline *PTEN* alterations. However, *PTEN* is currently the only clinically actionable gene for PHTS. All clinically diagnosed individuals are counseled and medically managed as though they have a germline *PTEN* mutation, regardless of underlying genotype (4). Hence, validating and characterizing non-*PTEN* etiologies are important because while *PTEN* mutation–negative patients can be diagnosed clinically, they do not have the benefit of specific gene-informed genetic counseling, predictive testing of family members, and precision in risk assessment and subsequent management.

Variable Genotype–Phenotype Associations

Once the broad phenotypic and *PTEN* genotypic spectra in individuals with PHTS were known, it became prudent to explore whether predictive genotype–phenotype correlations exist. This is critical because, although all germline *PTEN* mutation carriers are counseled as high-risk cancer-predisposed patients, it is still impossible to predict at an individual level who will go on to develop any particular component cancer. Relatedly, some PHTS individuals do not develop cancer but instead have neurodevelopmental and cognitive disorders such as ASD and developmental delays. A minority of patients manifest with both malignancies and neurodevelopmental features. Hence, in these contexts, genotype–phenotype associations will further personalize medical management, with respect to surveillance, of individual PHTS patients.

Earlier analyses identified an association between germline *PTEN* mutations and malignant breast disease, as well as an association between missense mutation and/or position of mutation within the phosphatase core motif and the development of multiorgan disease (3, 9). Another group did not detect such genotype–phenotype correlations (75), likely because of small sample

size ($n = 13$), compared to the 44 families and 43 probands of the preceding studies (3, 9). Analyses on expanded series of PHTS individuals revealed that germline *PTEN* frameshift mutations are overrepresented, but not absolute, in thyroid cancer (38), nonsense mutations in colorectal cancer (27), promoter mutations in breast cancer (27), and missense mutations in ASD (53). In addition to cancer risk, research efforts have also identified differences in *PTEN* missense mutations impacting the three-dimensional dynamics and stability of PTEN protein structure to influence cancer versus ASD phenotypes (76, 77). Such effects cannot be extrapolated from the secondary structure of the PTEN protein and indeed provide an important dimension to consider for assessing *PTEN* genotype–PHTS phenotype associations. Studies have also shown that ASD-associated mutations tend to retain higher PTEN activity relative to non-ASD-associated mutations (78–80). Relatedly, *PTEN* mutations that result in accumulation of stable inactive PTEN protein are predicted to lead to more severe PHTS-related developmental phenotypes and malignancies (80). Collectively, these data corroborate the complex genotype–phenotype associations and the likely existence of multiple modifiers of disease risk and manifestations in individuals with PHTS. More precise predictions of disease manifestations in relation to particular *PTEN* genotypes necessitate longitudinal studies to characterize the natural history of a PHTS diagnosis. The latter is an ongoing effort in which pediatric PHTS patients presenting to clinic with or without an ASD diagnosis are followed throughout their lifespan for other manifestations with age-related penetrance, such as cancer (Natural History Study of Individuals with Autism and Germline Heterozygous *PTEN* Mutations, NCT02461446).

SUMMARY AND PERSPECTIVE

As the availability and regularity of genetic testing rise, there is an increasing need for both clinician and patient education surrounding genetic etiology of disease and attention to family history and clinical red flags. PHTS encompasses a wide spectrum of variable clinical phenotypes affecting multiple organ systems, including benign and malignant neoplasias, as well as neurodevelopmental disorders such as ASD. The diagnosis of PHTS is established upon the identification of a pathogenic germline *PTEN* mutation on molecular genetic testing. PHTS patients benefit from specific *PTEN*-informed genetic counseling, predictive testing of family members, and precise risk assessment and subsequent management. Precisely managing PHTS engages both the patient and caregiver. Efforts must be made to disseminate knowledge so that those living on the broad phenotypic spectrum can be identified and screened appropriately so as to receive the care they need.

Importantly, the *PTEN* mutation landscape cannot independently account for the variability in observed phenotypes. This poses a clinical challenge, as individuals carrying identical *PTEN* mutations may have completely different disease manifestations and natural histories. Intriguingly, burgeoning data indicate that genetic and nongenetic modifiers may be key contributors dictating specific clinical phenotypes in PHTS. Ongoing research and clinical trials preface an era of predictive medicine, whereby treatment is not only preventive but also specific to the individual. One major question that remains is how to translate the predictive value of such *PTEN*-modifier combinations into the clinic. Systematic and longitudinal studies will be fundamental for unraveling the complexity of *PTEN*-modifier–phenotype interactions.

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