THEMATIC REVIEW

65 YEARS OF THE DOUBLE HELIX

One gene, many endocrine and metabolic syndromes: PTEN-opathies and precision medicine

Lamis Yehia1 and Charis Eng1,2,3,4

1Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA
2Taussig Cancer Institute, Cleveland Clinic, Cleveland, Ohio, USA
3Department of Genetics and Genome Sciences, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA
4Germline High Risk Cancer Focus Group, CASE Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio, USA

Correspondence should be addressed to C Eng: enge@ccf.org

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Abstract

An average of 10% of all cancers (range 1–40%) are caused by heritable mutations and over the years have become powerful models for precision medicine practice. Furthermore, such cancer predisposition genes for seemingly rare syndromes have turned out to help explain mechanisms of sporadic carcinogenesis and often inform normal development. The tumor suppressor PTEN encodes a ubiquitously expressed phosphatase that counteracts the PI3K/AKT/mTOR cascade – one of the most critical growth-promoting signaling pathways. Clinically, individuals with germline PTEN mutations have diverse phenotypes and fall under the umbrella term PTEN hamartoma tumor syndrome (PHTS). PHTS encompasses four clinically distinct allelic overgrowth syndromes, namely Cowden, Bannayan-Riley-Ruvalcaba, Proteus and Proteus-like syndromes. Relatedly, mutations in other genes encoding components of the PI3K/AKT/mTOR pathway downstream of PTEN also predispose patients to partially overlapping clinical manifestations, with similar effects as PTEN malfunction. We refer to these syndromes as ‘PTEN-opathies.’ As a tumor suppressor and key regulator of normal development, PTEN dysfunction can cause a spectrum of phenotypes including benign overgrowths, malignancies, metabolic and neurodevelopmental disorders. Relevant to clinical practice, the identification of PTEN mutations in patients not only establishes a PHTS molecular diagnosis, but also informs on more accurate cancer risk assessment and medical management of those patients and affected family members. Importantly, timely diagnosis is key, as early recognition allows for preventative measures such as high-risk screening and surveillance even prior to cancer onset. This review highlights the translational impact that the discovery of PTEN has had on the diagnosis, management and treatment of PHTS.

Key Words

- PTEN
- PTEN hamartoma tumor syndrome
- PTEN-opathies
- molecular diagnosis
- cancer risk assessment
- medical management

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Introduction

One predisposition gene to one inherited cancer syndrome was the rule when RB1 was associated with inherited retinoblastoma (Knudson 1971, Comings 1973, Cavenee et al. 1983, Friend et al. 1986, Lee et al. 1987). This held true for many years until germline RET proto-oncogene mutations were shown to predispose to multiple endocrine neoplasia type 2 and to Hirschsprung disease (Mulligan et al. 1994, Eng & Mulligan 1997) (see review in this issue, and the MEN 2 anniversary issue). In 1997, germline PTEN mutations were shown to predispose to Cowden syndrome (Nelen et al. 1996, Liaw et al. 1997) and over the last two decades, have been shown to predispose to many disparate endocrine neoplasia syndromes and non-neoplastic disorders serving as a model for precision medicine (Eng 2000, Tan et al. 2012, Mester & Eng 2013, Tilot et al. 2015, Ngeow et al. 2017).

Cowden syndrome (CS; MIM 158350) is an autosomal dominant multi-system disorder characterized by multiple hamartomas and increased lifetime risks of breast, thyroid and other cancers (Lloyd & Dennis 1963, Starink et al. 1986). Other clinical phenotypes involve multiple organ systems such as the brain, uterus, colon and mucocutaneous tissues (Marsh et al. 1998, Orloff & Eng 2008). Because of this high degree of phenotypic variability and the presence of cancers that can also occur sporadically in the general population, CS remains underdiagnosed and estimated to affect about 1 in 200,000 individuals (Nelen et al. 1999). The syndrome was first described in 1963 by Dr Kenneth Lloyd and Dr Macey Dennis in a 20-year-old female named Rachel Cowden, after whom the disease was named (Lloyd & Dennis 1963). Rachel Cowden presented with multiple developmental overgrowths and defects, including but not limited to scrotal tongue, papillomatous papules, thyroid adenomas, extensive fibrocystic breast disease and malignancy and a family history of the forme fruste of this complex multi-systemic disorder. Indeed, her physicians suspected the involvement of an aberrant gene giving rise to her symptoms. However, it was not until 1996 that the first susceptibility gene for CS was mapped to 10q22-23 (Nelen et al. 1996) and identified 1 year later as the tumor suppressor gene phosphatase and tensin homolog, PTEN (OMIM 601728) on 10q23.3 (Liaw et al. 1997).

Heritable cancers are important to healthcare because they develop much earlier than their sporadic counterparts (younger age at onset) and confer a much higher risk of developing bilateral (in paired organs), multifocal and often multiple primary cancers in other organs, than is expected in the general population. The majority of these syndromes are inherited autosomal dominantly, implying a 50% probability of passing on a causative germline mutation to offspring. Therefore, once a germline mutation is identified in a proband, predictive gene-specific testing can be instituted in other family members, thus allowing for early detection with high-risk clinical surveillance or prophylactic measures. Notably, of 468 genes frequently somatically mutated in tumors, 49 (10%) are also cancer-predisposing genes when existing in the germline. Relatedly, of 114 established distinct cancer predisposing genes, 65 (40%) also play significant roles in oncogenesis when somatically mutated in tumors (Rahman 2014). Therefore, studying the dual role of such gene discoveries will not only provide fundamental knowledge about gene function in the inherited cancers, but also offer invaluable insights toward the biology and cellular and molecular pathways in the more common sporadic cancers.

As a tumor suppressor gene, PTEN has been shown to be somatically mutated in multiple cancer types such as prostate, kidney, endometrial, breast and brain cancers (Li & Sun 1997, Li et al. 1997, Risinger et al. 1997, Steck et al. 1997) and considered as one of the most frequently somatically mutated genes in human cancers (Cantley & Neel 1999, Simpson & Parsons 2001, Hollander et al. 2011). PTEN has also been shown to play an important role in normal development and physiology, besides its classical role as a tumor suppressor (Di Cristofano et al. 1998, Knobbe et al. 2008). Relatedly, the clinical spectrum of disorders that are associated with germline PTEN mutations has historically expanded beyond CS (Marsh et al. 1998, Zhou et al. 2003, Tan et al. 2011), to include, seemingly disparate syndromes (Fig. 1). These include Bannayan–Riley–Ruvalcaba syndrome (BRRS; OMIM 153480) (Marsh et al. 1997, 1998, 1999, Longy et al. 1998, Zhou et al. 2003), Proteus syndrome (PS; OMIM 176920) and Proteus-like syndrome (Zhou et al. 2001, Smith et al. 2002, Eng 2003, Loffeld et al. 2006, Orloff & Eng 2008). BRRS is a congenital disorder classically characterized by macrocephaly in combination with intestinal hamartomatous polyposis, vascular malformations, lipomas, hemangiomas and genital
PTEN-opathies and precision medicine

L Yehia and C Eng

Endocrine-Related Cancer

Figure 1
PTEN hamartoma tumor syndrome clinical spectrum. Subsets of patients with Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome, and Proteus and Proteus-like syndrome represent a spectrum of heritable conditions associated with germline mutations in the PTEN tumor suppressor gene. Regardless of clinical phenotype, such individuals with germline PTEN mutations have PTEN hamartoma tumor syndrome.

Discovery of PTEN and relevance to neoplasia

In 1997, four landmark papers uncovered the identity of PTEN as the frequently lost tumor suppressor on chromosome 10 and the cause of CS when mutated in the germline (Li & Sun 1997, Li et al. 1997, Liaw et al. 1997, Steck et al. 1997). By the end of 1997, at least 25 more PTEN-related studies were published. More than 20 years later, and with >14,000 PTEN-related reports through mid-2018, PTEN is not only appreciated as a bona fide tumor suppressor gene but also as a critical gene regulating normal development and physiology (Di Cristofano et al. 1998, Knobbe et al. 2008).

PTEN encodes a 403 amino acid protein (Fig. 2) that contains a catalytic signature motif, HCXXGXXR (X is any amino acid), which is also typical of active sites of other protein tyrosine phosphatases or PTPs (Denu et al. 1996, Ren et al. 2009). However, unlike PTPs, the amino-terminal region of PTEN shares homology with the cytoskeletal actin-binding protein tensin (TNS1) and

accurate PTEN-informed cancer risk assessment and medical management. When the gene is known, it is also possible to perform genetic testing in the patient’s family members. Ultimately, the goal is to minimize morbidity and mortality through cancer prevention or at least early detection, when the disease is still manageable.

Interestingly, mutations in other genes that encode components of the PI3K/AKT/mTOR pathway downstream of PTEN also predispose patients to partially overlapping sets of clinical manifestations, with similar effects as PTEN malfunction. We refer to these syndromes as ‘PTEN-opathies’, with clinical phenotypes ranging from overgrowth to cancer (Mester & Eng 2013). Accordingly, the PTEN-opathies are also characterized by variable multi-systemic manifestations that occur throughout the life span.

Figure 2
Functional domains of PTEN. PTEN is a 403 amino-acid protein that comprises a phosphatidylinositol-4,5-bisphosphate (PIP2)-binding domain (PBD), a phosphatase domain, a C2 domain and a PDZ-binding domain. The active site is included within amino acid residues 123 and 130. PEST (Pro, Glu, Ser, Thr) sequences within the C-tail may contribute to PTEN stability and activity. The PDZ domain is important for protein–protein interactions that play a vital role in cellular signaling transduction.
auxilin, the ATPase heat shock cognate 70 (HSC70) cofactor (Li et al. 1997, Steck et al. 1997). The latter two features, namely, being a phosphatase and sharing homology with tensin, endowed PTEN its name as Phosphatase and TENsin homolog. Functionally, PTEN is an ubiquitously expressed dual-specificity phosphatase that can dephosphorylate both lipid (Myers et al. 1998) and protein (Li & Sun 1997) substrates. The N-terminus contains the phosphatase domain, responsible for the enzymatic activity of PTEN (Song et al. 2012). The catalytic core within the phosphatase domain spans amino acids 123–130. The N-terminal tail also includes a phosphatidylinositol 4,5-bisphosphate (PIP2)-binding motif and nuclear and cytoplasmic localization sequences (Chung et al. 2005, Denning et al. 2007, Gil et al. 2015). The C-terminus contains multiple domains important for modulating molecular interactions and cellular signaling transduction (Fanning & Anderson 1999, Goffin et al. 2002, Zbuk & Eng 2007, Mester & Eng 2013). As such, the C2 domain can bind phospholipid membranes and plays a role in inhibiting cell migration (Lee et al. 1999, Raftopoulou et al. 2004). In addition, the PDZ-binding motif within the C-terminal tail allows protein–protein interactions (Adey et al. 2000, Wu et al. 2000a,b). The C-terminus also contains PEST (Pro, Glu, Ser, Thr) sequences and several phosphorylation sites located in the last 50 amino acids, both critical for modulating PTEN protein stability (Georgescu et al. 1999, 2000, Vazquez et al. 2000).

At the molecular level, PTEN classically functions as a key negative regulator of phosphatidylinositol 3’ kinase (PI3K) signaling (Maehama & Dixon 1998, Stambolic et al. 1998, Carnero et al. 2008). Under growth factor stimulation, PI3K is activated and catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-triphosphate (PIP3). PIP3 recruits PDK1 to the plasma membrane, which then contributes to the activation of AKT. Activated AKT phosphorylates TSC2, thus removing the TSC1/2 complex’s inhibition of mTOR signaling (Inoki et al. 2002). The lipid phosphatase activity of PTEN counteracts PI3K by dephosphorylating PIP3 to PIP2, thereby dampening AKT activation (Maehama & Dixon 1998, Stambolic et al. 1998). PTEN function has also been shown to be dependent on subcellular localization (Trotman et al. 2007, Planchon et al. 2008). Although originally believed to be an exclusively cytoplasmic phosphatase, multiple studies have reported canonical nuclear roles of PTEN in cell cycle regulation (Wu et al. 1998, Weng et al. 2001a,b), double-strand break repairs and maintenance of genomic stability (Baker 2007, Shen et al. 2007). More recently, PTEN has been found to exist in cell nucleoli, regulating ribosomal DNA (rDNA) transcription and cellular proliferation (Liang et al. 2017). Hence, it then became evident that PTEN can have multiple protein isoforms characterized by distinct subcellular localizations and functions (Hopkins et al. 2013, Liang et al. 2014, 2017). All such molecular events are tightly orchestrated, with PTEN being expressed in all three germ cell layers (endoderm, mesoderm, ectoderm) throughout development (Di Cristofano et al. 1998, Podospanina et al. 1999, Gimm et al. 2000, Knobbe et al. 2008). Accordingly, PTEN and downstream pathway perturbation often lead to aberrant cellular phenotypes manifesting as variable multi-systemic phenotypes.

**PTEN hamartoma tumor syndrome**

CS, BRRS and Proteus and Proteus-like syndrome represent a spectrum of heritable conditions associated with germline mutations in the PTEN tumor suppressor gene (Eng 2016). Clinically, the differential diagnosis includes other genetic disorders with overlapping phenotypes of macrocephaly, gastrointestinal polyposis, benign tumors and cancer predisposition, such as juvenile polyposis syndrome (JPS), Peutz-Jeghers syndrome (PJS), neurofibromatosis type 1 (NF 1) and others (Table 1). A diagnosis of PHTS is given to patients with identifiable pathogenic germline PTEN mutation irrespective of clinical syndrome (Eng 2016). PTEN was the first susceptibility gene identified for CS (Nelen et al. 1996, Liaw et al. 1997). Consensus diagnostic criteria for CS (Table 2) were first developed in 1996 by the International Cowden Consortium (ICC) and form the basis for the National Comprehensive Cancer Network Guidelines (Eng 2000). Early studies on families with CS meeting strict ICC diagnostic criteria identified that germline PTEN mutations accounted for up to 85% of CS (Marsh et al. 1998, Zhou et al. 2003). Subsequent analysis of 3042 prospective community-accrued CS and CS-like probands (Tan et al. 2011) estimated that ~25% of patients who met more relaxed diagnostic criteria harbored pathogenic PTEN mutations. Germline PTEN mutations have been reported in up to 60% of BRRS patients (Marsh et al. 1997, 1998, 1999, Longy et al. 1998, Zhou et al. 2003). Among those BRRS patients who remain mutation negative, approximately 10% were found to harbor large deletions of PTEN (Zhou et al. 2003). Data accrued over the last 20 years also suggest that 7–20% of individuals who meet the clinical diagnostic criteria of PS and 50–67% with Proteus-like syndrome have germline mutations in PTEN.
Table 1  Differential diagnosis for PTEN hamartoma tumor syndrome (PHTS).

<table>
<thead>
<tr>
<th>Disorder</th>
<th>OMIM</th>
<th>Gene(s)</th>
<th>Mode of inheritance</th>
<th>General</th>
<th>Overlapping with PHTS</th>
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<tr>
<td>Juvenile polyposis syndrome (JPS)</td>
<td>174900</td>
<td>BMPR1A SMAD4</td>
<td>AD</td>
<td>Predisposition to hamartomatous polyps in the gastrointestinal (GI) tract</td>
<td>Hamartomatous GI polyps</td>
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<td>Polyps usually present by age 20 years</td>
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<td>Polyps range from 4 to &gt;100 over a lifetime</td>
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<td>Untreated polyps may cause bleeding and anemia</td>
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<td>Polyps typically benign although malignant transformation may occur</td>
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<td>Peutz-Jeghers syndrome (PJS)</td>
<td>175200</td>
<td>STK11</td>
<td>AD</td>
<td>GI polyposis</td>
<td>Hamartomatous GI polyps</td>
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<td>Mucocutaneous pigmentation (pathognomonic within the perioral region, particularly if crosses vermilion border)</td>
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<td>Cancer predisposition (intestinal and extraintestinal)</td>
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<td>Neurofibromatosis type 1 (NF 1)</td>
<td>162200</td>
<td>NF1</td>
<td>AD</td>
<td>Café-au-lait spots on the skin</td>
<td>GI ganglieneuromas</td>
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<td></td>
<td>Neurofibromas (fibromatous tumors of the skin)</td>
<td>Café-au-lait macules</td>
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<td>Lisch nodules in the eyes, optic gliomas</td>
<td>Fibromatous skin tumors</td>
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<td>Bone deformities, short stature</td>
<td>Macrocephaly</td>
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<td>Macrocephaly</td>
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<td>Learning disabilities</td>
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<td>Benign skin tumors</td>
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<td>Pulmonary cysts, high risk for pneumothorax</td>
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<td>Renal tumors</td>
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<td>Birt-Hogg-Dube syndrome (BHD)</td>
<td>135150</td>
<td>FLCN</td>
<td>AD</td>
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<td>Cutaneous lesions (e.g., trichiepitheliomas can be mistaken for trichilemmomas)</td>
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<td>Renal cell cancer</td>
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<td>Hamartomatous gastric polyps</td>
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<tr>
<td>Nevoid basal cell carcinoma syndrome (Gorlin syndrome)</td>
<td>109400</td>
<td>PTCH1 SUFU</td>
<td>AD</td>
<td>Multiple jaw keratocysts and/or basal cell carcinomas</td>
<td>Macrocephaly</td>
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<td>Lamellar (sheet-like) calcification of the falx</td>
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<td>Tumors and cancers such as fibromas and medulloblastomas</td>
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<td>Palmar/plantar pits (≥2)</td>
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<td>Macrocephaly</td>
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<td>Progressive segmental or patchy overgrowth and disfigurement</td>
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<td>Rare tumors (e.g., cystadenoma of the ovary, parotid monomorphic adenomas)</td>
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<td>Pulmonary complications, increased susceptibility to deep vein thrombosis and pulmonary embolism</td>
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<td></td>
<td>Connective tissue nevi, epidermal nevi, and vascular malformations</td>
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<td>AKT1-related Proteus syndrome</td>
<td>176920</td>
<td>AKT1</td>
<td>Somatic (mosaic)</td>
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<td>Macrocephaly</td>
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<td>Overgrowth</td>
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<td>Vascular malformations</td>
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</tbody>
</table>
Table 2  International Cowden Consortium (ICC) operational diagnostic criteria.

<table>
<thead>
<tr>
<th>Pathognomonic</th>
<th>Major</th>
<th>Minor</th>
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<tbody>
<tr>
<td>Adult Lhermitte-Duclos disease (LDD)</td>
<td>Breast cancer</td>
<td>Other thyroid lesions (e.g., adenoma,</td>
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<tr>
<td>Mucocutaneous lesions</td>
<td>Thyroid cancer (nonmedullary)</td>
<td>multinodular goiter)</td>
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<tr>
<td>Trichilemmomas, facial</td>
<td>Macrocephaly (i.e., ≥97th percentile)</td>
<td>Mental retardation (i.e., IQ ≤ 75)</td>
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<tr>
<td>Acral keratoses</td>
<td>Endometrial cancer</td>
<td>GI hamartomas</td>
</tr>
<tr>
<td>Papillomatous papules</td>
<td></td>
<td>Fibrocystic breast disease</td>
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<tr>
<td>Mucosal lesions</td>
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<td>Lipomas</td>
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<td></td>
<td></td>
<td>Fibromas</td>
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<td>Genitourinary tumors (especially renal cell</td>
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<td></td>
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<td>carcinoma)</td>
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<tr>
<td></td>
<td></td>
<td>Genitourinary malformations</td>
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<td></td>
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<td>Uterine fibroids</td>
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</table>

Operational diagnosis in an individual
Any of 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 CS
Any one pathognomonic criterion
Any one major criteria ± minor criteria
Two minor criteria
History of Bannayan–Riley–Ruvalcaba syndrome

Reproduced, with permission, from Eng C, 2000, Will the real Cowden syndrome please stand up: revised diagnostic criteria, *Journal of Medical Genetics* 36 828–830.

Pathogenic PTEN mutations (Zhou et al. 2001, Smith et al. 2002, Eng 2003, Loffeld et al. 2006, Orloff & Eng 2008). Although these conditions are inherited autosomally, a recent study showed that de novo PTEN mutations occur in 10–44% of PHTS probands (Mester & Eng 2012).

Clinical utility of identifying a germline PTEN mutation

PTEN-informed cancer risk assessment and medical management

Even before the discovery of PTEN as the first CS susceptibility gene, it was recognized that CS patients have an elevated lifetime risk of developing breast and thyroid cancers (Brownstein et al. 1978, Starink et al. 1986). The identification of germline PTEN mutations in individuals with CS (Liaw et al. 1997) subsequently led to the expansion of the spectra of cancers associated with CS and the derivation of more accurate cancer risk estimates. An age-adjusted cancer incidence and age-related penetrance study conducted on 368 individuals having deleterious germline PTEN mutations (out of 3399 prospectively recruited patients meeting relaxed ICC diagnostic criteria) revealed similarly elevated lifetime risks of specific component cancers (Tan et al. 2012) (Fig. 3 and Table 3). Breast cancer lifetime risk was the most pronounced, beginning at around 30 years of age and reaching an estimated lifetime risk of 85%. Relatedly noted were lifetime risks of 35% for thyroid cancer (epithelial and never medullary with risk beginning at birth), 28% for endometrial cancer, 34% for renal cell carcinoma (RCC), 9% for colorectal carcinoma and 6% for melanoma. Such elevated cancer risks have been independently replicated by other groups (Bubien et al. 2013, Nieuwenhuis et al. 2014). As with other hereditary cancer syndromes, the risk of bilateral (in paired organs such as the breasts and kidneys) and multifocal cancer is elevated. Another more recent study showed that individuals with germline PTEN mutations have a 7-fold increased risk of developing a second primary malignant neoplasm as compared to the US general population (Ngeow et al. 2014). These clinical risk assessment outcomes resulted in improving existing surveillance recommendations and clinical management of PTEN mutation-positive CS patients. The ultimate goal behind increased cancer surveillance is to detect any tumors at the earliest, most manageable stages.
Endocrine neoplasia risks in PHTS

The two most well-documented component neoplasias in PHTS are carcinomas of the breast and epithelial thyroid gland (Starink et al. 1986, Zbuk & Eng 2007, Hobert & Eng 2009, Ngeow et al. 2011, 2012). As relevant to endocrine neoplasia, benign thyroid disease such as multinodular goiter, adenomatous nodules and follicular adenomas commonly occur in up to 75% of individuals with CS (Harach et al. 1999). Before the discovery of PTEN, the lifetime risk for differentiated thyroid cancer was estimated to be 10% in individuals with CS (Starink et al. 1986). However, prospective studies revealed that individuals with germline pathogenic PTEN mutations have a 35% lifetime risk of epithelial thyroid carcinoma (compared to ~1% in the US general population), with an age at diagnosis as early as 7 years. The median age of onset was 37 years (Ngeow et al. 2011, Tan et al. 2012). Follicular thyroid carcinoma (FTC) and follicular variant of papillary thyroid carcinoma (FVPTC) tend to be overrepresented in PHTS patients (Harach et al. 1999, Ngeow et al. 2011). The ratio of FTC to the more common papillary thyroid cancer (PTC) was 1 in 2 among PHTS patients as compared with approximately 1 in 14 in the general population. Therefore, FTC is considered as a major diagnostic criterion and important feature in PHTS. Germline frameshift PTEN mutations were found in 31% of patients with thyroid cancer compared to 17% in those without thyroid cancer (Ngeow et al. 2011). Yet, the prevalence of germline PTEN mutations in unselected differentiated thyroid cancer is low (<1%) (Nagy et al. 2011). Pediatric onset of thyroid cancer, male gender, history of thyroid nodules and/or thyroiditis and FTC histology were factors that were found to be predictive of PTEN mutation status in a series of CS/CS-like patients with thyroid cancer (Ngeow et al. 2011). Relatedly, PHTS individuals do not seem to be at significantly elevated lifetime risks of developing cancers in other endocrine glands/organisms. Based on isolated case reports and our clinical observations, a minority of patients could present with ovarian and testicular cancers (particularly germ cell tumors) and pituitary adenomas (Devi et al. 2007, Cho et al. 2008, Rasalkar & Paunipagar 2010, Efstathiadou et al. 2014). However, these tumors are rather incidental and not component features of PHTS (Tan et al. 2012).

Endocrine-related neoplasia risks in PHTS

As relevant to endocrine-related neoplasia, individuals with CS are at an increased risk for the development of benign and malignant tumors of the breast. Women with CS have up to 67% risk for benign breast disease (Eng 2016). Earlier reports estimated that females with CS have a 25–50% lifetime risk of developing breast cancer, with a mean age of diagnosis between 38 and 46 years (Starink et al. 1986). More recent studies reevaluating the lifetime risks for cancer in CS patients with germline PTEN mutations indicated higher risks than was previously estimated. In a large prospective study of PTEN mutation-positive CS and CS-like patients, Tan et al. reported an 85% lifetime risk (in contrast to ~12% in the US general population) for female breast cancer, starting around age 30 years, with 50% penetrance by age 50 years (Tan et al. 2012). Bubien et al. similarly found that females with...
PTEN mutations had a cumulative risk of 77% for female breast cancer at age 70 years (Bubien et al. 2013). Additionally, Nieuwenhuis et al. reported a 67% breast cancer risk by age 60 years in females with germline PTEN mutations (Nieuwenhuis et al. 2014). Relatedly, although male breast cancer had been associated with CS (Fackenthal et al. 2001), an increased lifetime risk in PTEN mutation-positive males was not noted in a recent study with >3000 patients (Tan et al. 2011). Importantly, PTEN germline mutations have also been associated with the occurrence of second primary cancer diagnoses (Ngeow et al. 2014). This study identified that women with germline PTEN mutations who have had a diagnosis of breast cancer have a 29% risk of developing a second breast cancer within 10 years (Ngeow et al. 2014). The histopathology of CS-associated breast cancer is adenocarcinoma of the breast, both ductal and lobular (Schrager et al. 1998).

Individuals with germline PTEN mutations also have a 28% lifetime risk of developing endometrial cancer. PTEN-related endometrial cancer risk begins at age 25, rising to 30% by age 60 (Tan et al. 2012). A recent prospective study showed that the mean age of endometrial cancer diagnosis in patients with PTEN mutations was 44 years, with three-quarters diagnosed under age 50 years. In this study, age ≤50 years, macrocephaly, high phenotypic burden and/or coexisting RCC were strong clinical predictors for the existence of germline PTEN mutations in endometrial cancer CS/CS-like patients (Mahdi et al. 2015). These observations may guide PTEN genetic testing and age range for consideration of surveillance or prophylactic strategies. Individuals with germline PTEN mutations are also at increased risk of developing benign endometrial diseases, such as uterine fibroids (Tan et al. 2012). Relatedly, although somatic PTEN alterations are common in prostate cancer (Li et al. 1997), this type of cancer is rarely observed in males with CS (Barbosa et al. 2011).

### Table 3  Cancer risks and PTEN-enabled clinical management of PHTS patients.

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Population risk (SEER (%))</th>
<th>Lifetime risk in PHTS (%)</th>
<th>Screening recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (female)</td>
<td>12</td>
<td>67–85</td>
<td>Starting at age 30 years: annual mammogram; breast MRI</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1</td>
<td>6–38</td>
<td>Annual ultrasound</td>
</tr>
<tr>
<td>Endometrial</td>
<td>2.6</td>
<td>21–28</td>
<td>Starting at age 30 years: annual endometrial biopsy or transvaginal ultrasound</td>
</tr>
<tr>
<td>Renal cell</td>
<td>2.6</td>
<td>2–34</td>
<td>Starting at age 40 years: renal imaging every 2 years</td>
</tr>
<tr>
<td>Colon</td>
<td>5</td>
<td>9–17</td>
<td>Starting at age 40 years: colonoscopy every 2 years</td>
</tr>
<tr>
<td>Melanoma</td>
<td>2</td>
<td>2–6</td>
<td>Annual dermatologic examination</td>
</tr>
</tbody>
</table>

Data from Tan et al. (2012), Bubien et al. (2013) and Nieuwenhuis et al. (2014). Lifetime risks calculated to age 70 by Tan et al. (2012) and Bubien et al. (2013), and to age 60 by Nieuwenhuis et al. (2014). Cancer risk percent ranges reflect lowest and highest frequencies reported in all three studies.

PTEN -opathies and precision medicine

**Spectrum of non-endocrine PHTS-related neoplasia**

In addition to elevated lifetime risks of endocrine-related neoplasias, PHTS individuals have increased risks of other cancers including renal and colon cancers and melanoma (Tan et al. 2012). RCC has a starting age at risk of approximately 40 years (Tan et al. 2012). The predominant histology is papillary (Mester et al. 2012). Because renal ultrasound screening is not sensitive for detecting papillary RCC, particularly if the tumor is small, CT or MRI examinations are preferred for PHTS patients with suspected RCC (Mester et al. 2012). As relevant to the gastrointestinal tract, the majority (>90%) of PTEN mutation carriers who had a colonoscopy performed as part of clinical care, had colorectal polyps typically with a mix of histologic subtypes. Polyp histologies varied and included ganglieneuromas, hamartomatous polyps, juvenile polyps and adenomatous polyps (Heald et al. 2010). Patients who developed colorectal carcinomas also tended to have pre-/coexisting colonic polyposis. A subset of PTEN mutation positive patients also show upper gastrointestinal polyps and approximately 20% had glycogenic acanthosis (McGarrity et al. 2003, Nishizawa et al. 2009, Heald et al. 2010). Finally, several individual case reports have previously noted the existence of melanoma in CS patients (Siegel & Reed 1976, Greene et al. 1984). A subsequent large prospective study resulted in the addition of melanoma in the clinical cancer spectrum of PHTS (Tan et al. 2012). The earliest age of onset of melanoma was 3 years.

**Non-neoplastic manifestations**

Besides its classical role as a *bona fide* tumor suppressor, PTEN has also been shown to have key functions in normal development and physiology (Di Cristofano et al. 1998, Knobbe et al. 2008). Accordingly, individuals with germline PTEN mutations also manifest with various...
non-cancer phenotypes. Individuals with germline PTEN or pathway-associated gene mutations could also manifest with several neurodevelopmental phenotypes such as megalencephaly, autism spectrum disorder (ASD) and developmental delay (Goffin et al. 2001, Butler et al. 2005, Hansen-Kiss et al. 2017). Isolated studies identified germline PTEN pathogenic mutations in patients with macrocephaly and VATER association or with macrocephaly and autism (Reardon et al. 2001, Eng 2016). The first estimate of mutation frequency in a prospective series of patients with macrocephaly and autism was derived by Butler et al. (2005). This study found that approximately 20% of individuals with ASD and macrocephaly have germline pathogenic PTEN mutations (Butler et al. 2005). A frequency of 10–20% of germline PTEN mutations in ASD with macrocephaly has been independently reported by other groups (Herman et al. 2007a, b, Orrico et al. 2009, Varga et al. 2009). The extent of macrocephaly (defined by an occipital-frontal circumference or OFC measurement ≥2 standard deviations or S.D. over the mean) observed in ASD patients with germline PTEN mutations is often more severe than is observed in ASD patients with WT PTEN (Mester et al. 2011). Combined with ease of measurement of head circumference, these observations make macrocephaly an important endophenotype within ASD.

An increasing number of benign manifestations such as mucocutaneous features (trichilemmomas, papillomatous papules, acral and plantar keratoses), GI polyposis, Lhermitte-Duclos disease (LDD), lipomas and arteriovenous malformations have also been found to be associated with PHTS (Starink et al. 1986, Eng 2000, Heald et al. 2010). Although the majority of such features are rarely life threatening compared to malignancies, some of these rarer features such as adult-onset LDD are considered as pathognomonic and hence important for clinicians to recognize as ‘red flags’ for establishing a CS diagnosis and excluding other differential diagnoses (Eng 2016).

A subset of patients also manifest with features associated with increased insulin sensitivity and obesity (Pal et al. 2012). Compared to controls, PTEN mutation carriers showed significantly lower fasting plasma insulin levels, higher glucose infusion rate and increased measures of obesity. The increased BMI was attributed to augmented adiposity without corresponding changes in fat distribution. 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. At the molecular level, the enhanced insulin sensitivity could be explained through amplified insulin signaling upstream of the PI3K-AKT pathway, the latter known to be modulated by PTEN (Ozes et al. 2001, Simpson et al. 2001). Interestingly, studies in animal models showed opposite effects with increased PTEN levels (Ortega-Molina & Serrano 2013), with one study showing ‘Super-PTEN’-mutant mice having reduced body size, increased energy expenditure, reduced body fat accumulation and tumor resistance (Garcia-Cao et al. 2012).

**Identification of patients for genetic risk assessment**

Because individuals with PHTS have individual features that are also seen in the general population, recognizing such patients for referral to genetics evaluation is a challenge (Eng 2000, Mester & Eng 2015). Moreover, due to the high degree of phenotypic heterogeneity and high frequency of de novo (10-47%) germline PTEN mutations (Mester & Eng 2012), a family history of component cancers may not be as evident in CS. To aid in clinical diagnosis, a nomogram-based clinical predictor named Cleveland Clinic PTEN Risk Calculator was developed to evaluate the pretest probability of harboring a germline PTEN mutation (Tan et al. 2011). This nomogram was generated through multiple logistic regression analysis of comprehensive PTEN mutation data from prospective accrual of >3000 probands meeting at least the relaxed ICC operational criteria from multiple centers, including the community. Individual weights are given to each phenotypic feature based on comparison of prevalence relative to the general population and age of onset of cancer, when present (Fig. 4). The sum of weights of all clinical features results in a semi-quantitative score referred to as the PTEN Cleveland Clinic score or CC score. Because the key phenotypic features present in children and adults are different, two sets of criteria are utilized depending on age groups. In adults, a CC score of 10, corresponding to a pretest probability of 3% and a sensitivity of 90%, is a recommended threshold for referral to genetic specialists for PTEN genetic testing (Tan et al. 2011). The latter study also showed a strong inverse association between PTEN protein expression and CC score (P<0.001), further highlighting the utility of this tool in identifying individuals with potential mutations in PTEN. More recently, it was also demonstrated that a CC score of 15, corresponding to a 10% a priori risk of positive PTEN mutation status, is the most cost-effective cut-off to refer CS-like patients for PTEN germline testing (Ngeow et al. 2015). In the research setting,
the CC score has also been utilized to identify CS patients for gene discovery efforts (Orloff et al. 2013, Yehia et al. 2015). This is particularly pertinent for CS individuals who have a high CC score (hence, strongly predictive of an underlying germline PTEN mutation) but who test negative for germline PTEN mutations. For pediatric patients (<18 years of age), distinct criteria were developed to guide selection for germline PTEN mutation testing (Table 4) (Tan et al. 2011). Macrocephaly (occipitofrontal head circumference \( >2 \) s.d. over the population mean or 97.5th percentile) was identified as a key criterion for diagnosis, based on 100% prevalence at diagnosis. Neurodevelopmental (e.g., autism, developmental delay) and dermatologic (e.g., lipomas, oral papillomas) features were also present in 100% of pediatric patients with germline PTEN mutations. However, since dermatologic features are often overlooked and difficult to recognize, less prevalent features such as vascular malformations, thyroid goiter, gastrointestinal polyps and early-onset cancers (e.g., thyroid, germ cell) also warrant referral for PTEN testing. The CC score questionnaire-based clinical decision tool is freely available online to assist clinicians at the point of patient care and to guide for referral to genetics professionals (http://www.lerner.ccf.org/gmi/ccscore/).

**PTEN mutation spectrum and complexity of genotype-phenotype associations**

The PTEN gene consists of nine exons encoding a 403 amino acid protein. Pathogenic germline PTEN mutations have been reported in all nine exons of the protein. These include various types of mutations such as missense, nonsense, splice site, intragenic deletions or insertions and large deletions (Tan et al. 2011, 2012, Mester & Eng 2013, Ngeow et al. 2014). Virtually all germline missense PTEN mutations within the coding region are considered pathogenic (Zbuk & Eng 2007, Tan et al. 2011). The most commonly observed PTEN mutations include nonsense and frameshift mutations in exons 5, 6, 7 and 8 (Tan et al. 2011). Three common nonsense truncating mutations, namely R130X, R233X and R335X, have been well characterized in exons 5, 7 and 8, respectively. The latter three exons are overrepresented in the PTEN germline mutation spectrum, likely reflecting domain function (Tan et al. 2011). Exon 5, which encodes the catalytic core motif of PTEN, is a hotspot for mutations likely due to its biological significance (Waite & Eng 2002, Eng 2003, Tan et al. 2011). Large deletions and duplications affecting PTEN are less common and can be found over the entire coding sequence (Tan et al. 2011, 2012, Mester & Eng 2013).

<table>
<thead>
<tr>
<th><strong>Table 4</strong> Pediatric criteria for consideration of PTEN hamartoma tumor syndrome.</th>
</tr>
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<tbody>
<tr>
<td><strong>Required criterion</strong></td>
</tr>
<tr>
<td>Macrocephaly (( \geq 2 ) s.d.)</td>
</tr>
<tr>
<td><strong>Secondary criteria</strong></td>
</tr>
<tr>
<td>At least 1 of the following should be present:</td>
</tr>
<tr>
<td>- Autism spectrum disorder or developmental delay</td>
</tr>
<tr>
<td>- Dermatologic features (lipomas, oral papillomas, trichilemmomas, penile freckling)</td>
</tr>
<tr>
<td>- Vascular features (arteriovenous malformations or hemangiomas)</td>
</tr>
<tr>
<td>- Gastrointestinal polyps</td>
</tr>
<tr>
<td>- Pediatric-onset thyroid cancer or germ cell tumors</td>
</tr>
</tbody>
</table>

Mester & Eng 2013). Moreover, although limited data are currently available for variants identified in the PTEN promoter region, some promoter variants have been shown to affect gene expression and are hence considered as pathogenic (Zhou et al. 2003, Mester & Eng 2015). More recently, it was shown that certain PTEN intronic variants result in exon skipping, alternative splicing or the use of cryptic splice sites (Chen et al. 2017).

With the existence of various PTEN mutation types and varied clinical phenotypic spectra, it became prudent to evaluate the validity of genotype–phenotype associations in PHTS. Historically, genotype–phenotype analyses revealed an association between PTEN germline mutations and breast cancer (Marsh et al. 1998, 1999). Another study showed that missense mutations and mutations within and 5′ of the phosphatase core motif appeared to be associated with clinical phenotypic features in five or more organs, serving as a surrogate of disease severity (Marsh et al. 1998). Other groups could not find clear genotype–phenotype associations, although the sample size of identified PHTS patients (n=13) was small and hence lacked statistical power (Nelen et al. 1999). Unfortunately, even the largest subsequent cohorts of PHTS patients were insufficient to identify clear and consistent PTEN genotype–phenotype associations.

The PTEN-opathies: intact PTEN, disrupted PTEN pathway

The existence of multiple hamartoma tumor syndrome patients who are WT for PTEN allowed the exploration of whether mutations in other genes within the PTEN signaling pathway are germane in these disorders. Indeed, it was hypothesized that non-PHTS CS/CS-like individuals who have a high CC score (strongly predictive of an underlying germline PTEN mutation) but who are PTEN mutation negative, have a high probability of harboring mutations in genes that are downstream of PTEN signaling. To address this hypothesis, 91 PTEN WT CS/CS-like patients were tested for germline mutations in AKT1, PIK3CA, PIK3R1 and PIK3R2 (Orloff et al. 2013). These genes have been shown to harbor somatic mutations in thyroid, breast and endometrial cancers, component neoplasia of CS (Engelman et al. 2006, Cheung et al. 2011, Du et al. 2012). Indeed, this study confirmed the existence of germline AKT1 (2.2%) and PIK3CA (8.8%) gain-of-function mutations that resulted in significant phosphorylation of AKT1 at Threonine 308 and an increase of intracellular PI3P levels, both mimicking loss-of-function effects of PTEN.

More recently, a gain-of-function germline EGFR mutation (c.977G>T, p.Cys326Phe) has been identified in a unique family presenting with LDD, a cerebellar hamartoma and pathognomonic CS feature. Functionally, the mutation resulted in increased activation of the ERK and AKT signaling pathways, also mimicking loss-of-function PTEN mutations (Colby et al. 2016). As relevant to PS, a somatic mosaic-activating mutation in AKT1 (c.49G>A, p.Glu17Lys) has been identified in >90% of individuals meeting clinical diagnostic criteria (Lindhurst et al. 2011). As such, it is hypothesized that the latter AKT1 mutation would be lethal if inherited in the germline. Since gain-of-function AKT1 mutations lead to similar downstream effects as upstream PTEN loss of function, this finding confirms that a subset of PS and PS-like are indeed PTEN-opathies.

Mutations within the PTEN pathway genes could also cause disorders other than CS/CS-like, BRRS and PS/PS-like. PIK3CA-related overgrowth spectrum encompasses distinct clinical entities with phenotypic overlap between the different syndromes (Kepper-Noreuil et al. 2015). These overgrowth disorders are typically associated with postzygotic somatic mosaic PIK3CA mutations in affected tissues and are characterized by segmental overgrowth affecting the body (e.g. CLOVES syndrome, fibroadipose hyperplasia) or the brain (e.g. megalencephaly-capillary malformation syndrome (MCAP), hemimegalencephaly). PIK3CA activation results in phosphorylation and activation of AKT, ultimately resulting in overgrowth-promoting downstream effects within the PI3K/AKT/mTOR signaling pathway downstream of PTEN.

Non-PTEN pathway genes associated with CS and BRRS

Germline PTEN mutations are found in ~25% of CS/CS-like individuals meeting ICC criteria (Tan et al. 2011), and AKT1/PIK3CA in up to 11% of PTEN-WT patients (Orloff et al. 2013). Germline PTEN mutations are also found in up to 60% of BRRS patients (Marsh et al. 1997, 1998, 1999, Longy et al. 1998, Zhou et al. 2003). Therefore, non-PTEN gene and canonical pathway-related etiologies could explain the remaining patients. The identification of other relevant CS/CS-like and BRRS susceptibility genes is important because while PTEN mutation-negative patients can be diagnosed clinically, they do not have the benefit of specific gene- or genotype-informed genetic counseling, precise risk assessment and subsequent management; without a known gene, it is...
impossible to offer predictive testing to the probands' family members. Moreover, knowledge about gene function in the inherited cancers offers insights toward the biology and cellular and molecular pathways in the more common sporadic cancers.

Recent research efforts resulted in the identification of other germline susceptibility genes in CS/CS-like and BRRS patients. Approximately 10% of individuals with CS or CS-like phenotypes harbor germline heterozygous variants in the genes encoding three of the four subunits of succinate dehydrogenase or mitochondrial complex II (SDHB, SDHC and SDHD, collectively referred to as SDHx) (Ni et al. 2008). SDHx mutations and variants caused an increase in reactive oxygen species (ROS) and activation of AKT and/or MAPK signaling pathways downstream of PTEN (Ni et al. 2008). Individuals carrying the SDHx variants showed an increased risk of breast, papillary thyroid and renal cell cancers that surpassed the risk mediated by mutant PTEN alone (Ni et al. 2012). Functional studies demonstrated that the SDHx variants resulted in ROS-mediated stabilization of HIF-1α, destabilization and decreased protein expression of p53 due to defective interaction with NQO1 and resistance to apoptosis. This study also mechanistically revealed how mitochondrial dysfunction could lead to tumorigenesis subsequent to elevated flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD⁺), the cofactor and product of NQO1 enzymatic catalysis, respectively (Ni et al. 2012). KLLN (OMIM 612105), a gene that resides upstream of PTEN, was identified as a CS predisposition gene. Similar to PTEN, KLLN is regulated by p53 and induces apoptosis (Cho & Liang 2008) and the two genes share a bidirectional promoter. Up to 30% of CS/CS-like patients without germline PTEN and SDHx mutations were found to have germline KLLN promoter hypermethylation (Bennett et al. 2010). These patients showed a 2- to 3-fold increase in invasive breast and RCCs as compared to those with germline PTEN mutations. More recently, germline heterogeneous change-of-function variants in SEC23B have been identified in approximately 4% of CS patients and enriched in apparently sporadic thyroid cancer patients (Yehia et al. 2015). Relatedly, an in-frame compound heterozygous germline deletion in USF3 was found in a multi-generation CS-like family with PTC, in up to 29% of unrelated CS/CS-like individuals, and 27% of individuals with apparently sporadic thyroid cancer (Ni et al. 2016). Finally, germline TTN variants were found to be enriched in a subset of PTEN-WT patients with classic BRRS clinical features (Yehia et al. 2017). Functionally, focal adhesion kinase (FAK)-related contact inhibition of proliferation was found to account for the observed overgrowth phenotypes. FAK is known to be regulated by PTEN (Tamura et al. 1999) and to regulate contact inhibition itself (McLean et al. 2005, Takai et al. 2008). Most of the identified CS/CS-like and BRRS susceptibility genes resulted in cellular and molecular phenotypes similar to PTEN loss of function, suggesting possible non-canonical pathway cross-talks as relevant to the observed clinical phenotypes.

Modifiers of malignancy risks in PHTS

As with other heritable cancer syndromes, while it is possible to predict gene- and organ-specific lifetime cancer risks, it remains challenging to accurately predict which subset of patients with germline PTEN mutations will develop which component malignancy. It is also difficult to accurately predict the severity of disease presentation based on the underlying germline PTEN mutation. Indeed, identical pathogenic PTEN mutations have been observed in PHTS patients with seemingly disparate clinical presentations (e.g., cancer vs ASD). These data suggest that additional genetic, epigenetic and even environmental factors could act as phenotypic modifiers in PHTS.

A proof-of-principle study showed that 6% (26/444) of PTEN mutation-positive CS/CS-like individuals also harbor germline variants in SDHx (Ni et al. 2012). While individuals with SDHx variants alone showed the highest prevalence of thyroid cancer (91% of papillary histology), the coexistence of a PTEN mutation was associated with a 77% rate of breast cancer, as compared to 32% with PTEN mutations alone and 57% with SDHx variants alone. Although the prevalence of thyroid cancer was not significantly elevated in individuals with both PTEN mutations and SDHx variants, it is noteworthy to state that the histology was papillary for all tumors (vs follicular in individuals with PTEN mutations). This hence indicated a potential role of SDHx variants in modifying the histology of these thyroid carcinomas from the dominantly follicular variant seen in PTEN mutation-positive individuals. Mechanistically, it was demonstrated that SDHD G12S and H50R variants lead to impaired PTEN function by altering its subcellular localization through SRC-induced oxidation, accompanied by apoptosis resistance and induction of migration in both thyroid and CS patient-derived lymphoblastoid cell lines (Yu et al. 2015). Importantly, Bosutinib, a specific SRC inhibitor, could rescue SDHD dysfunction-induced cellular phenotype and tumorigenesis in thyroid cell lines,
only when WT PTEN was expressed. More recently, it was shown that SDHD G12S and H50R variants resulted in decreased autophagy, and this cellular phenotype was similarly dependent on the presence of WT PTEN (Yu et al. 2017). These findings could provide an explanation to the clinically observed increased prevalence of thyroid cancer in CS patients with SDHx variants compared to those with PTEN mutations alone and the decreased prevalence of thyroid cancer in the setting of coexisting PTEN mutations and SDHx variants. Therefore, these studies uncovered evident cross-talk between PTEN and SDHx, and an important functional role for SDHx germline variants as modifiers of CS-related cancer risks and component cancer histology (Fig. 5).

Irrespective of PTEN mutation status, it is also possible that distinct PTEN regulation changes and consequently variable PTEN expression could result in particular clinical presentations. It is widely accepted that PTEN is haploinsufficient as a tumor suppressor. Studies in murine models provided conclusive evidence suggesting subtle reductions in Pten dose predispose to tumorigenic phenotypes in a tissue-specific manner, also serving as a key determinant in cancer progression (Trotman et al. 2003, Alimonti et al. 2010). In humans, it is also possible that such subtle variations in PTEN levels could represent one of the mechanisms underlying the remarkable heterogeneity of PHTS clinical manifestations. One example is the identification of miRNAs that serve as genetic modifiers in CS by modulating PTEN levels (Pezzolesi et al. 2008). More specifically, miR-19a and miR-21 were found to be differentially expressed in PHTS patients and in PTEN mutation-negative CS/CS-like individuals. Therefore, irrespective of mutation status, the differentially increased expression of these PTEN-targeting miRNAs resulted in decreased PTEN protein levels and modulation of CS-related phenotypes. Another example is the identification of approximately 10% of germline PTEN mutations in the promoter region of CS patients (Teresi et al. 2007, Tan et al. 2011). These mutations also resulted in altered translation and reduced PTEN protein levels. Patients with such promoter mutations had a high prevalence of breast, thyroid and endometrial cancers. Ultimately, the identification of robust modifiers could help further tailor surveillance guidelines based on more accurate organ-specific cancer risks and estimates of severity that are unique for individual PHTS patients.

**Molecularly targeted therapies in PHTS**

Perturbation of PTEN and upregulation of the downstream PI3K/AKT/mTOR pathway provides a rational basis for therapeutically targeting this pathway in patients with PHTS and associated PTEN-opathies. Altered PI3K/AKT/mTOR signaling suggests that PI3K, AKT and mTOR inhibitors are germane for the effective treatment or prevention of associated overgrowth phenotypes and other syndromic features. Although there are many inhibitors of the PI3K/AKT/mTOR pathway in development as anti-cancer drugs, the most promising and most clinically developed pathway inhibitors are those that target mTOR. Indeed, a proof-of-concept report by Marsh et al. demonstrated the clinical utility of the mTOR inhibitor rapamycin in the treatment of a severe form of PHTS (Marsh et al. 2008). In this case study, treatment of a child suffering from PTEN-related PS with rapamycin for 14 months resulted in marked reduction in soft-tissue masses and normalization of respiratory and gastrointestinal functioning. A more recent report also demonstrated successful treatment with rapamycin of a PHTS infant suffering from LDD (Zak et al. 2017). While these and other reports discussed promising results from single patients, it is important to note that mTOR inhibitors have also been successfully utilized in

**Figure 5**

SDHx as modifier of PTEN-associated cancer risk and tumor histology. Increased cancer frequencies in SDHx variant-only carriers and in PTEN mutation and SDHx variant double-carriers compared with PTEN mutation-only carriers. While individuals with SDHx variants alone show the highest prevalence of thyroid cancer, the coexistence of a PTEN mutation with SDHx variants was associated with substantially increased prevalence of breast cancer, as compared to either gene mutation alone. FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; FvPTC, follicular variant papillary thyroid cancer; mut+, mutation positive; var+, variant positive.
patients with other hereditary cancer syndromes affecting the mTOR pathway, such as tuberous sclerosis complex (TSC; OMIM 191100) (Crino et al. 2006, Yalon et al. 2011, Bisler et al. 2013, Krueger et al. 2013) and PJS (OMIM 175200) (Wei et al. 2008). Indeed, TSC1/2 and STK11/LKB1, the susceptibility genes for TSC and PJS respectively, are not only upstream of mTOR (Gao et al. 2002, Inoki et al. 2002), but are also downstream of PTEN signaling (Huang & Manning 2008). This provides a rational basis for also targeting mTOR signaling in PHTS patients. In fact, the mTOR inhibitor sirolimus has been used in a Phase II open-label clinical trial (NCT00971789) in individuals with PHTS. Collection of follow-up data and analysis are underway. Relatedly, an mTOR inhibitor trial is currently accruing pediatric, adolescent and young adult patients with germline pathogenic PTEN mutations and ASD (Eng 2016). In addition to mTOR inhibition, other upstream components of the PTEN signaling pathway can also serve as candidates for pharmacologic inhibition. These include PI3K and AKT inhibitors, although the clinical utility for the treatment of PHTS had not been explored yet.

From a biological point of view, it is important to keep in mind that while PTEN is the central negative regulator of the PI3K/AKT/mTOR signaling pathway, this tumor suppressor has other versatile functions beyond its classical lipid phosphatase activity. Hence, one caveat is the possibility that targeting the PI3K/AKT/mTOR signaling pathway would be ineffective in situations where disease-associated PTEN alterations impact lipid phosphatase-independent functions (e.g., protein phosphatase activity or subcellular localization). Relatedly, another caveat is that inhibiting mTOR results in feedback activation of upstream cascade components such as AKT through insulin receptor substrate 1 (IRS1) or through direct phosphorylation at Ser473 by mTORC2 (Mahalingam et al. 2009). Such loss of negative feedback control (and hence activation of AKT) have been observed in a Phase I neoadjuvant trial of rapamycin in patients with recurrent PTEN-deficient glioblastoma (Cloughesy et al. 2008). Interestingly, rebound upregulation of AKT during mTOR inhibition can be abrogated by pre-treatment or co-treatment of breast cancer cells with resveratrol, at least in vitro (He et al. 2011).

We speculate that the next generation of therapies would represent combinations of multiple agents that would, in theory, effectively target growth-promoting signals without loss of feedback controls. Importantly, because PHTS patients have a diverse spectrum of germline PTEN mutations, we also predict that the most effective treatments would target unique vulnerabilities imparted by specific mutations. For example, mutations occurring within the C-terminal region would be good candidates for proteasome inhibition to mitigate PTEN instability and degradation (Georgescu et al. 1999). Similarly, targeted therapies for mutations that impact the subcellular localization of PTEN also warrant further investigation. For example, mutant PTEN K289E retains catalytic activity but is characterized by a nuclear import defect due to loss of mono-ubiquitination at this particular amino acid (Trotman et al. 2007). This certainly makes it more plausible to target nuclear-specific functions of PTEN (Planchon et al. 2008). Indeed, given the essential role of PTEN in maintaining genomic integrity (Planchon et al. 2008, Yin & Shen 2008), several studies have shown the possibility of therapeutically harnessing such a role of nuclear PTEN through the use of polyadenosine diphosphate ribose polymerase inhibitors (Mendes-Pereira et al. 2009, Dedes et al. 2010, Dillon & Miller 2014). Overall, pathway-targeted and mutation-specific agents will likely underscore therapeutic management of PHTS and other overgrowth syndromes in the era of precision medicine.

**Perspectives and future considerations**

Patients with PHTS may present with a variety of benign and malignant clinical features, as well as neurodevelopmental disorders such as ASD. Over the last two decades, significant progress has been made in understanding the molecular and genetic mechanisms related to PTEN and associated pathway dysfunction. The existence of a known gene allows for the establishment of a molecular diagnosis. Diagnosing PHTS in patients in turn allows for more accurate PTEN-informed cancer risk assessment, medical management and counseling of the patients’ family members. The ultimate goal is to reduce morbidity and mortality through cancer prevention or at least early detection, when the disease is still manageable. Relatedly, understanding the cellular and molecular pathways and mechanisms behind PTEN and associated pathway disruption had also paved the way for targeted cancer therapeutics. Importantly, the crux of the matter now is to be able to accurately predict which subsets of PHTS patients will eventually develop cancer (i.e. beyond probabilities), and more specifically which type of cancer. This allows even more accurate cancer risk assessment and medical management tailored to the patient’s molecular bio-profile. Modifier studies are burgeoning and have indeed indicated promising results in more accurately predicting organ-specific cancers. At the 65th anniversary...
of the Double Helix, one cannot but ponder what would have happened to Rachel Cowden had we known she had a germline PTEN mutation. Would she have died of metastatic breast cancer at age 31?

‘Ipse scientia potestas est’ (‘Knowledge itself is power’)
– Sir Francis Bacon, Meditationes Sacrae (1597).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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PTEN-opathies and precision medicine


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