THEMATIC REVIEW

65 YEARS OF THE DOUBLE HELIX

One gene, many endocrine and metabolic syndromes: *PTEN*-opathies and precision medicineLamis Yehia¹ and Charis Eng^{1,2,3,4}¹Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA²Taussig Cancer Institute, Cleveland Clinic, Cleveland, Ohio, USA³Department of Genetics and Genome Sciences, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA⁴Germline High Risk Cancer Focus Group, CASE Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio, USACorrespondence should be addressed to C Eng: engc@ccf.org

This paper is part of a thematic review section celebrating 65 Years of the Double Helix. The guest editors for this section were Charis Eng, William Foulkes and Jérôme Bertherat. C Eng was not involved in the review or editorial process for this paper, on which she is listed as an author.

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

Endocrine-Related Cancer
(2018) 25, T121–T140

*'Superior doctors prevent the disease,
Mediocre doctors treat the disease before evident,
Inferior doctors treat the full-blown disease'*

– Huangdi Neijing, First Chinese Medical Text (2600
BCE)

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

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.

freckling (Ruvalcaba *et al.* 1980, Gorlin *et al.* 1992). The true prevalence of BRRS is unknown, although it is generally considered rare. PS is a rare, complex and highly variable hamartomatous overgrowth disorder, clinically characterized by congenital malformations and segmental overgrowths of multiple tissues of all germ layers, most commonly affecting the skin, skeleton, adipose and central nervous system (Cohen & Hayden 1979). PS-like refers to individuals who exhibit clinical manifestations for PS but who do not fulfill diagnostic criteria. Hence, individuals with germline *PTEN* mutations can have diverse clinical phenotypes and are defined under the umbrella term of *PTEN* hamartoma tumor syndrome (PHTS). The diagnosis of PHTS is established upon the identification of a pathogenic heterozygous germline *PTEN* mutation on molecular genetic testing, regardless of the phenotype. Diagnosing PHTS allows for more

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.

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

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.* 2001, 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, Podsypanina *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

Table 1 Differential diagnosis for *PTEN* hamartoma tumor syndrome (PHTS).

Disorder	OMIM	Gene(s)	Mode of inheritance	Clinical features of the disorder	
				General	Overlapping with PHTS
Juvenile polyposis syndrome (JPS)	174900	<i>BMPR1A</i> <i>SMAD4</i>	AD	<ul style="list-style-type: none"> Predisposition to hamartomatous polyps in the gastrointestinal (GI) tract Polyps usually present by age 20 years Polyps range from 4 to >100 over a lifetime Untreated polyps may cause bleeding and anemia Polyps typically benign although malignant transformation may occur 	<ul style="list-style-type: none"> Hamartomatous GI polyps
Peutz-Jeghers syndrome (PJS)	175200	<i>STK11</i>	AD	<ul style="list-style-type: none"> GI polyposis Mucocutaneous pigmentation (pathognomonic within the perioral region, particularly if crosses vermilion border) Cancer predisposition (intestinal and extraintestinal) 	<ul style="list-style-type: none"> Hamartomatous GI polyps GI and breast cancers
Neurofibromatosis type 1 (NF 1)	162200	<i>NF1</i>	AD	<ul style="list-style-type: none"> Café-au-lait spots on the skin Neurofibromas (fibromatous tumors of the skin) Lisch nodules in the eyes, optic gliomas Bone deformities, short stature Macrocephaly Learning disabilities 	<ul style="list-style-type: none"> GI ganglioneuromas Café-au-lait macules Fibromatous skin tumors Macrocephaly
Birt-Hogg-Dube syndrome (BHD)	135150	<i>FLCN</i>	AD	<ul style="list-style-type: none"> Benign skin tumors Pulmonary cysts, high risk for pneumothorax Renal tumors 	<ul style="list-style-type: none"> Cutaneous lesions (e.g., trichiepitheliomas can be mistaken for trichilemmomas) Renal cell cancer
Nevoid basal cell carcinoma syndrome (Gorlin syndrome)	109400	<i>PTCH1</i> <i>SUFU</i>	AD	<ul style="list-style-type: none"> Multiple jaw keratocysts and/or basal cell carcinomas Lamellar (sheet-like) calcification of the falx Tumors and cancers such as fibromas and medulloblastomas Palmar/plantar pits (≥ 2) Macrocephaly 	<ul style="list-style-type: none"> Hamartomatous gastric polyps Macrocephaly
<i>AKT1</i> -related Proteus syndrome	176920	<i>AKT1</i>	Somatic (mosaic)	<ul style="list-style-type: none"> Progressive segmental or patchy overgrowth and disfigurement Rare tumors (e.g., cystadenoma of the ovary, parotid monomorphic adenomas) Pulmonary complications, increased susceptibility to deep vein thrombosis and pulmonary embolism Connective tissue nevi, epidermal nevi, and vascular malformations 	<ul style="list-style-type: none"> Macrocephaly Overgrowth Vascular malformations

AD, autosomal dominant; OMIM, Online Mendelian Inheritance in Man.

Table 2 International Cowden Consortium (ICC) operational diagnostic criteria.

Pathognomonic	Major	Minor
Adult Lhermitte-Duclos disease (LDD)	Breast cancer	Other thyroid lesions (e.g., adenoma, multinodular goiter)
Mucocutaneous lesions	Thyroid cancer (nonmedullary)	Mental retardation (i.e., IQ $\leq$ 75)
Trichilemmomas, facial	Macrocephaly (i.e., $\geq$ 97th percentile)	GI hamartomas
Acral keratoses	Endometrial cancer	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 following:

- Mucocutaneous lesions alone, if $\geq$ six facial papules (three of which must be trichilemmomas)
- Cutaneous facial papules and oral mucosal papillomatosis
- Oral mucosal papillomatosis and acral keratoses
- $\geq$ Six palmo-plantar keratoses
- $\geq$ Two major criteria (one of which must be macrocephaly or LDD)
- One major and $\geq$ three minor criteria
- $\geq$ Four minor criteria

Operational diagnosis in a family where one individual is diagnostic for CS

- Any one pathognomonic criterion
- Any one major criteria $\pm$ minor criteria
- Two minor criteria
- History of Bannayan–Riley–Ruvalcaba syndrome

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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 autosomal dominantly, 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.

Figure 3

Lifetime risks of component cancers for patients with PHTS. Lifetime cancer risks of organ-specific cancers are depicted in black for the general population and in magenta for individuals with *PTEN* hamartoma tumor syndrome (PHTS). Reprinted from *Endocrinology & Metabolism Clinics of North America*, vol 46, Ngeow J, Sesock K & Eng C, Clinical Implications for Germline *PTEN* Spectrum Disorders, pages 503–517, Copyright (2017), with permission from Elsevier.

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/organs. 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

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

Cancer	Population risk (SEER) (%)	Lifetime risk in PHTS (%)	Screening recommendations
Breast (female)	12	67–85	Starting at age 30 years: annual mammogram; breast MRI
Thyroid	1	6–38	Annual ultrasound
Endometrial	2.6	21–28	Starting at age 30 years: annual endometrial biopsy or transvaginal ultrasound
Renal cell	1.6	2–34	Starting at age 40 years: renal imaging every 2 years
Colon	5	9–17	Starting at age 40 years: colonoscopy every 2 years
Melanoma	2	2–6	Annual dermatologic examination

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. PHTS, *PTEN* hamartoma tumor syndrome; SEER, Surveillance, Epidemiology, and End Results.

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).

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 ganglioneuromas, 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,

Figure 4

The Cleveland Clinic (CC) score risk calculator nomogram. The CC score represents sum of weights of all clinical phenotypes reported in an individual and corresponds to the *a priori* risk of finding a germline pathogenic *PTEN* mutation. Figure illustrates three hypothetical cases corresponding to CC scores of 5, 10 and 15 and associated point probabilities of <1, 3 and 10%, respectively. Adapted minimally from *American Journal of Human Genetics*, vol 88, Tan MH, Mester J, Peterson C *et al.*, A clinical scoring system for selection of patients for *PTEN* mutation testing is proposed on the basis of a prospective study of 3042 probands, pages 42–56, Copyright (2011), with permission from Elsevier.

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,

Table 4 Pediatric criteria for consideration of *PTEN* hamartoma tumor syndrome.

Required criterion	Secondary criteria
Macrocephaly (≥ 2 s.d.)	At least 1 of the following should be present: <ul style="list-style-type: none"> 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

Reproduced, with permission, from Tan MH, Mester J, Peterson C *et al.* (2011) A clinical scoring system for selection of patients for *PTEN* mutation testing is proposed on the basis of a prospective study of 3042 probands, *American Journal of Human Genetics* 88 42–56.

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 PIP3 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 (Keppler-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-1 α , 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 heterozygous 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, Bissler *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?

'Ipsa 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.

Funding

This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Acknowledgements

The authors are grateful to their patients and families who have participated in their research studies throughout the years. Charis Eng is the Sondra J and Stephen R Hardis Chair of Cancer Genomic Medicine at the Cleveland Clinic and an American Cancer Society Clinical Research Professor.

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Received in final form 17 May 2018

Accepted 23 May 2018

Accepted Preprint published online 23 May 2018