The Clinical Spectrum of PTEN Hamartoma Tumor Syndrome: Exploring the Value of Thyroid Surveillance

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Abstract
Introduction: Phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome (PHTS) comprises a collection of clinical features characterized by constitutional variants in PTEN. Several guidelines recommend thyroid screening, beginning at the pediatric age at the time of PHTS diagnosis; however, the benefits of early surveillance has not been well defined. Methods: We conducted a retrospective investigation of patients followed up at the Children’s Hospital of Philadelphia with a diagnosis of PHTS between January 2003 and June 2019. In total, 81 patients younger than 19 years were identified. Results: The most common clinical feature at presentation was macrocephaly (85.1%), followed by impaired development (42.0%), skin/oral lesions (30.9%), and autism spectrum disorder (27.2%). A total of 58 of 81 patients underwent thyroid surveillance, with 30 patients (51.7%) found to have a nodule(s). Ultimately, 16 patients underwent thyroidectomy, with 7.4% (6/81) diagnosed with thyroid cancer. All thyroid cancer patients were older than 10 years at diagnosis, and all displayed low-invasive behavior. Of the patients younger than 10 years at the time of thyroid ultrasound (US) surveillance, 71.4% (15/21) had a normal US. The remaining 6 patients had thyroid nodules, including 4 undergoing thyroid surgery with benign histology. Discussion/Conclusion: Patients with macrocephaly, impaired cognitive development and thyroid nodules, and/or early-onset gastrointestinal polyps should undergo constitutional testing for PHTS. There does not appear to be a clinical advantage to initiating thyroid US surveillance before 10 years of age. In PHTS patients with a normal physical examination, thyroid US surveillance can be delayed until 10 years of age.
Introduction

Phosphatase and tensin homolog (PTEN), located on chromosome ten at position q21.31, is a tumor suppressor gene in the P13K/AKT/mTOR signaling pathway essential for the regulation of cellular growth, migration, and apoptosis [1, 2]. Heterozygous constitutional pathogenic/likely pathogenic (P/LP) variants in the PTEN gene are associated with what was previously thought to be distinct clinical entities, including but not limited to Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, PTEN-related Proteus syndrome, and Proteus-like syndrome. Such autosomal dominant syndromes are now more aptly described as PTEN hamartoma tumor syndrome (PHTS) with the understanding that the phenotypic features of these syndromes comprise the clinical spectrum related Proteus syndrome, and Proteus-like syndrome. PTEN syndrome, Bannayan-Riley-Ruvalcaba syndrome, -related Proteus syndrome, and Proteus-like syndrome. Such autosomal dominant syndromes are now more aptly described as PTEN hamartoma tumor syndrome (PHTS) with the understanding that the phenotypic features of these syndromes comprise the clinical spectrum associated with variants in PTEN [3–5]. Childhood manifestations of PHTS include macrocephaly; autism spectrum disorder (ASD); impaired development, including intellectual disability (ID) and/or developmental delay (DD); lipomas; vascular malformations; pigmented macules on the glans penis; gastrointestinal (GI) polyps; and thyroid nodules [6]. Over an individual’s lifetime, P/LP variants in the PTEN gene predispose affected individuals to an increased risk for developing malignant tumors of the thyroid, breast, endometrium, GI tract, kidneys, and skin (melanoma), with differentiated thyroid cancer (DTC) representing the prevailing risk in pediatrics [7]. As PHTS displays variable expression and age-dependent clinical manifestations, there is likely underdiagnosis of this condition in pediatrics secondary to the rarity and difficulty in recognizing the clinical phenotype [6]. The missed opportunity of diagnosis may result in a lack in proper medical surveillance, as well as anticipatory guidance with regard to risk to family members and future offspring.

The lifetime risk of developing DTC for individuals with PHTS is between 35 and 38%, with previous recommendations to initiate thyroid ultrasound (US) surveillance after 18 years of age [7–11]. After several reports documenting the diagnosis of thyroid nodules and DTC in patients younger than 18 years, with the youngest reported case of PHTS-related DTC in a 4-year-old patient, multiple groups suggested that US surveillance should be initiated earlier – at the time of diagnosis, by 10 years of age, by 7 years of age, or even as early as 2–3 years depending on the age of diagnosis and the patient’s ability to cooperate with the examination [8, 12–16]. Despite these recommendations, the benefit of early thyroid surveillance in pediatrics with PHTS has not been well defined. Initiation of US surveillance may result in early detection of malignancy, but it may also lead to an increased risk of false-positive results, resulting in unnecessary interventions, including fine-needle aspiration (FNA) and thyroidectomy [15].

We investigated patients with PHTS at the Children’s Hospital of Philadelphia (CHOP) to better understand the clinical presentation and spectrum of disease and ultimately, to optimize testing, surveillance, and management. We focused on the screening, incidence, and spectrum of thyroid disease in children with PHTS in an effort to determine whether patients are undergoing recommended medical surveillance and whether recent recommended changes for pediatric thyroid cancer surveillance are improving clinical outcomes. In order to identify the complete spectrum of PHTS, genetic indicators and clinical manifestations were reviewed to (1) understand the rationale of diagnosis, (2) identify opportunities to improve selection for genetic testing and screening, and (3) assess whether there was a genotype-phenotype correlation with a spectrum of disease.

Materials and Methods

A retrospective, single-site cohort investigation was conducted among pediatric patients (<19 years at the time of diagnosis) followed up at CHOP. An initial list of 57 patients with a confirmed P/LP PTEN variant was retrieved from CHOP’s Cancer Predisposition Program. Six additional individuals seen at CHOP’s Thyroid Center diagnosed with PHTS were identified and included in the study, yielding a cohort of 63 patients. By querying the “Problem List” in the hospital electronic medical record (Epic) for the terms PHTS, PTEN variant, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and/or genetic disorder, a new list was generated comprising 96 patients with PHTS. The list of patients from CHOP’s Cancer Predisposition Program and the list generated from Epic were cross-checked, ascertaining 36 additional potential patients for inclusion in the study. Of the 36 patients, 18 were excluded because the presence of a P/LP PTEN variant could not be confirmed (n = 11), or there was limited medical information available because they were primarily followed up by another institution (n = 6), or the patient had deceased (n = 1). Thus, a final cohort of 81 patients younger than 19 years at the time of diagnosis with confirmation of a constitutional pathogenic PTEN P/LP variant between January 2003 and June 2019 was included in the data analysis (shown in Fig. 1).

The electronic medical records between January 2003 and June 2019 of the 81 patients were then searched to collect and analyze information pertaining to presenting clinical features and surveillance, as well as the PTEN variant pattern of inheritance. Surveillance by genetics, oncology (Cancer Predisposition Program), endocrinology (Thyroid Center), developmental pediatrics or neurology, dermatology, and/or gastroenterology was reviewed. Clinical features, including the presence of macrocephaly, DD

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and/or ID, ASD, skin and oral lesions, freckling on the glans penis for males, hypotonia, breast lesions for females, GI polyps, thyroid nodules, and/or DTC were reviewed at the time of diagnosis of PHTS as well as post-diagnosis. Endocrinology records, thyroid US reports, FNA biopsy reports, and thyroidectomy pathology reports, as well as gastroenterology records relating to presenting signs and symptoms that preceded discovery of polyps, endoscopy and colonoscopy reports, and polypectomy pathology reports were recorded and reviewed. P/LP PTEN variant sequencing reports were retrieved and compared to PHTS-associated diagnosis to assess possible genotype-phenotype associations.

**Results**

**Demographic and Clinical Characteristics of Children with PHTS**

A total of 81 pediatric patients (42 males and 39 females) at CHOP with a documented diagnosis of PHTS were evaluated. The majority of patients in this cohort were white (59/81, 72.8%). By mutational analysis, PHTS resulted from an apparent de novo variant in 19 patients.
(23.5%) and familial inheritance in 16 patients (19.8%), including 10 patients (12.3%) and 6 patients (7.4%) with confirmation of PHTS from maternal and paternal lineages, respectively. Parental mutational status to confirm the pattern of inheritance of PHTS was unknown or not available for 46 patients (56.8%) secondary to the family declining PTEN testing based on the absence of PTEN-associated clinical features and/or financial reasons. Age at the time of PHTS diagnosis varied between 13 months and 19 years, with the average and median age of diagnosis of PHTS of 7.8 and 7.0 years, respectively (shown in Table 1).

The majority of the patients, 79 of 81 (97.5%), were evaluated by genetics for PTEN molecular testing. Subsequent to the diagnosis, 61 patients (75.3%) were seen by the Cancer Predisposition Program in oncology, 58 patients (71.6%) were seen by the Thyroid Center in endocrinology for initial US surveillance, 56 patients (69.1%) were seen by neurology or developmental pediatrics for a neurodevelopmental evaluation, 44 patients (54.3%) were seen by dermatology for a skin examination, and 30 patients (37.0%) were seen by gastroenterology to be screened for GI polyps (shown in Table 2).

Presenting clinical features associated with PHTS at initial diagnosis and postdiagnosis, along with the youngest age of documented diagnosis, are reported in Table 3. The most common clinical feature at presentation was macrocephaly (85.1%) followed by ID (42.0%), skin/oral lesions (30.9%), and ASD (27.2%). Of the 30 patients with dermatological features, 22 (73.3%) were diagnosed with lipomas, 15 (50.0%) with freckling of the glans penis, 9 (30.0%) with vascular malformations, 9 (30.0%) with trichilemmomas, 6 (20.0%) with hamartomas, 4 (13.0%) with papillomas, 3 (10.0%) with fibromas, 3 (10.0%) with acral keratoses, 3 (10.0%) with mucocutaneous lesions, and 1 (3.3%) with an arteriovenous malformation. Thirty patients were also evaluated by GI with the most common reason for referral secondary to symptoms, including a history of constipation (20/29, 69.0%), abdominal pain (14/29, 48.3%), and/or blood per rectum (9/29, 31.0%). One patient was referred for GI polyps secondary to the diagnosis of PHTS. Ultimately, 14/30 (46.7%) patients were found to have GI polyps between the ages of 18 months and 18 years, with the average age of presentation at 8.7 years. In 5 of the 14 patients, the diagnosis of PHTS was made after referral for polyp-related GI symptoms. In 9 of the 14 patients, the polyps were diagnosed during PHTS-associated surveillance. All of the GI polyps that were resected were found to be histologically benign.

**Thyroid Features in Patients with Constitutional P/LP PTEN Variants**

A total of 58 of 81 patients underwent thyroid surveillance, including 41 patients (70.7%) secondary to diagnosis of PHTS, 16 patients (27.6%) secondary to a pre-existing thyroid condition (thyroid nodule(s), Hashimoto’s thyroiditis, multinodular goiter, and/or asymmetric thyroid), and 1 patient (1.7%) secondary to a complaint of throat pain. Thyroid US surveillance was initiated between 19 months and 20 years of age, with the average and median ages of surveillance of 10.9 and 11.0 years, respectively. Thirty patients were discovered to have thyroid
Thyroid nodules by presymptomatic US imaging (n = 23) or after identification of a palpable mass on physical examination (n = 7). The average ages of discovery of the nodules were 12.0 and 13.6 years, respectively.

Thyroid nodules were identified on initial US screening in 27 patients and subsequent thyroid US monitoring in 3 patients. Of the 30 patients with thyroid nodules, 66.7% (20/30) had multinodular disease (2 or more thyroid nodules) and 33.3% (10/30) had a solitary thyroid nodule. There was no difference in age between the patients with multinodular disease and patients with a solitary nodule, presenting a mean of 10.9 and a median of 11.0 years compared to a mean of 10.6 and a median of 10.5 years, respectively. Nineteen patients (63.3%, 19/30) underwent FNA; 1 of 19 (5.3%) had nondiagnostic cytology based on The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC, category I), 6 of 19 (31.6%) had benign cytology (TBSRTC, category II), 12 of 19 (63.2%) had indeterminate cytology (TBSRTC categories III, IV, or V), and none had malignant cytology (TBSRTC category VI) (Fig. 2). Ultimately, 16 of the 19 patients underwent thyroidectomy, with 6 of 16 (37.5%) diagnosed with DTC, including follicular thyroid carcinoma (FTC, n = 2) and papillary thyroid carcinoma (PTC, n = 4).

**Fig. 2.** Thyroid features among PTEN variant-positive patients. PTEN, phosphatase and tensin homolog; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology; FNA, fine-needle aspiration; US, ultrasound.

**Fig. 3.** Thyroid features among PTEN variant-positive patients who underwent thyroidectomy. PTEN, phosphatase and tensin homolog; FTC, follicular thyroid carcinoma; PTC, papillary thyroid carcinoma.
There were no permanent complications from FNA or thyroidectomy. Four individuals with nondiagnostic ($n = 1$) or benign ($n = 3$) cytology elected total thyroidectomy due to a pre-existing thyroid condition ($n = 1$), a family history of thyroid malignancy ($n = 1$), or to minimize exposure of general anesthesia during surveillance procedures in patients with severe autism ($n = 2$). On surgical pathology, 3 of these 4 specimens were benign and one had a micro-focus of PTC (the tumor that was nondiagnostic by FNA). Of the 20 patients with multinodular disease, 14 underwent surgery with 35.7% (5/14) found to have malignant histology, including a classic papillary thyroid microcarcinoma (PTMC, $n = 1$), an encapsulated follicular variant PTC ($n = 1$), a minimally invasive FTC with one focus of PTMC ($n = 1$), a classic variant of PTC with 2 foci of PTMC ($n = 1$), and a minimally invasive FTC ($n = 1$). Of the 10 patients with a solitary nodule, 2 underwent surgery, with 1 patient found to have an oncocyctic PTC and the other found to have a benign, follicular adenoma.

All thyroid cancer patients were older than 10 years at diagnosis, with the youngest being 12 years old, and all thyroid cancers displayed low-invasive features classified as low risk for persistent postsurgical disease by the American Thyroid Association pediatric guidelines (shown in Fig. 3) [11]. One patient with PTC, a 17-year-old male, had 2 foci of uptake on postoperative radioiodine whole body scan consistent with central neck lymph node metastasis. The remaining 5 patients had no radiological or surgical evidence of extrathyroidal disease. All patients with DTC have achieved remission, defined as an undetectable, non-TSH-stimulated thyroglobulin level, an undetectable antithyroglobulin level, and no evidence of persistent disease on postsurgical radiological imaging.

Of the patients younger than 10 years at the time of initiation of thyroid US surveillance, 15/21 (71.4%) had a normal US and the remaining 6 patients younger than 10 years had thyroid nodule(s). Four of the 6 patients underwent FNA biopsy, and all of the nodules either had benign ($n = 1$) or indeterminate ($n = 3$) cytopathology including follicular lesion of unknown significance ($n = 2$; TBSRTC category III) and follicular neoplasm ($n = 1$; TBSRTC category IV). These 4 patients underwent thyroid surgery, and all had benign histology. Of the 23 patients who did not undergo thyroid US monitoring, 10 (43.5%) had been referred for US, but the study was never completed, and 13 (56.5%) were not referred based on the patient’s age and the contemporary thyroid US surveillance recommendations at the time of clinical evaluation (Fig. 2).

### Constitutional PTEN Variant Spectrum for Genotype-Phenotype Analysis

Of the 77 patients who had genetic testing performed, PTEN sequencing results from 12 patients were excluded from analysis because the precise DNA variant, predicted protein change, and/or location of the variant could not be confirmed. Individuals with identical PTEN variants presented with similar clinical features (shown in Fig. 4). Of the 65 patients in our analysis, 11 patients (11/65, 16.9%) were found to have variants located in previously reported hot spots, including R233X in exon 7 ($n = 5$), R130X in exon 5 ($n = 3$), and R335X in exon 8 ($n = 3$) [17, 18]. Individuals with variants R130X in exon 5 ($n = 3$), R335X in exon 8 ($n = 3$), and R173C in exon 6 ($n = 2$) presented with thyroid nodules, demonstrating potential genotype-phenotype associations of PHTS; however, there was no observed association between PHTS and thyroid carcinoma identified in this cohort.

### Discussion/Conclusion

We analyzed 81 patients with PHTS diagnosed prior to 19 years of age, making this the largest comprehensive review of clinical features in pediatric patients with PHTS from a single institution. Previous studies have comprised smaller pediatric cohorts, segmented review of the clinical spectrum of PHTS, and/or combined analysis of both pediatric and adult patients [7, 10, 12–14, 19–21]. Overall, our results support that pediatric patients with macrocephaly in combination with DD/ID/ASD, thyroid nodules, GI polyps, and/or skin lesions (lipomas, penile freckling, vascular malformations, trichilemmomas, hamartomas, papillomas, fibromas, acral keratoses, mucocutaneous lesions, and arteriovenous malformations) should undergo PTEN testing via referral to genetic consultation (shown in Table 3). Patients diagnosed with PHTS presenting with persistent abdominal pain, rectal bleeding, and/or severe constipation are recommended to undergo endoscopy and/or colonoscopy to assess for early-onset GI polyps. This corroborates previous studies suggesting PTEN testing for pediatric patients with macrocephaly and DD as well as the pediatric-specific guidelines in GeneReviews® and Cleveland Clinic PTEN Risk Calculator [12, 14, 20, 22, 23].

Based on the current guidelines, the majority of patients (71.6%) in our cohort had a thyroid US completed at the time of data analysis (shown in Table 2) [6, 24, 25]. Approximately 50% of patients (30/58) that had a US were found to have a nodule; however, only 6 noninvasive
DTCs were ultimately diagnosed (6/81, 7.4%), including 2 FTCs and 4 PTCs with no distant metastasis. These results are consistent with previously published data reporting that 5% of individuals with PHTS younger than 20 years develop DTC [7, 16]. There were no patients in our cohort diagnosed with DTC prior to 10 years of age, with the youngest diagnosed at 12 years old (shown in Table 3). Within the cohort of patients younger than 10 years, 6 patients were found to have thyroid nodules (6/19, 31.6%), with 4 having benign or indeterminate (TBSRTC categories III or IV) cytology after FNA, and all diagnosed with benign disease after thyroidectomy (shown in Fig. 2, 3). None had recurrent laryngeal nerve damage or hypoparathyroidism, although all thyroidectomy patients (16/16) are on life-long thyroid hormone replacement therapy.

Previous studies exploring the clinical spectrum of PHTS in pediatrics have confirmed a diagnosis of thyroid cancer in 9 patients younger than 10 years, with the youngest presenting DTC at 4 years old [13, 16]. Smith and colleague’s report of one patient diagnosed with DTC at 7 years of age [13]. This report, along with data from the Cleveland Clinical PTEN registry reporting a 9-fold risk of thyroid cancer in patients younger than 19 years, prompted a change in recommendation to begin thyroid monitoring before 18 years of age [26]. Most recently, the National Comprehensive Cancer Network recommended US monitoring begin at 7 years old [25].

Although early thyroid monitoring provided an opportunity for early detection of thyroid nodules, our results do not demonstrate a clinical advantage of initiating US surveillance prior to 10 years of age. With the high prevalence of benign thyroid nodules in pediatric patients with PHTS, the high rate of indeterminate cytology (63%, 12/19; Fig. 2), and the observation that DTC in patients with PHTS most commonly displays low-invasive disease...
behavior (i.e., a low risk for regional and distant metastasis; Fig. 3), detection of thyroid nodules in prepubertal children does not appear to provide a distinct benefit [10, 13, 14, 27]. While it is well accepted that the development of PHTS-associated DTC is progressive with increasing risk over advancing age, the lack of a difference in the mean or median age between patients in our cohort with solitary versus multinodular disease (a mean of 10.6 and a median of 10.5 years vs. a mean of 10.9 and a median of 11.0 years, respectively) suggests that the rate of progression is slow and that the risk may not increase until the 2nd or 3rd decade of life. A recent, retrospective, single-site review of adult patients with PHTS reported a mean age of 28.5 years at the time of diagnosis of DTC, with a range between 16 and 41 years [28]. Similar to patients with sporadic nodules not associated with PHTS, the rate of malignancy was higher in patients with solitary nodules (50%, 1/2) than in patients with multinodular goiter (35.7%, 5/14) [29]. Unfortunately, the small number of patients with a solitary nodule who underwent surgical resection in our cohort was too small to draw a meaningful conclusion.

Based on these observations, we agree with the proposed recommendation of Jonker and colleagues that children with a pathogenic PTEN variant begin thyroid US monitoring no earlier than 10 years of age [16] and at the latest by 18 years of age, in keeping with the recent recommendation by the European PHTS Guideline Development Group [30]. This approach would provide the potential benefit of early detection of PHTS-associated DTC while reducing the risk for invasive procedures (FNA and/or thyroidectomy) that do not appear to have a clinical advantage. Our data also suggest that there is a high likelihood for indeterminate cytology. Within this population, however, it is unlikely that somatic oncogene analysis of indeterminate cytology would provide additional information to stratify patients for observation versus surgery as driver mutations are mutually exclusive events in the majority of noninvasive DTC [31]. In the setting of low-risk invasive DTC, one could offer observation to patients with TBSRTC category III cytology; however, the burden of surveillance must be considered within the context of the benefit of a confirmed diagnosis and the high likelihood of achieving surgical remission. An improved understanding of PHTS-associated DTC is critical to optimize anticipatory guidance and education so that patients and families can participate in an informed decision-making process.

As with any retrospective chart review, this case series is limited by what is accessible in the hospital electronic medical record and variation of patient information documented by medical professionals. The relatively short amount of time included in this review, between 2003 and 2019, decreases but does not eliminate the variance in the approach to care based on individual providers and changes in consensus guidelines. Since 2010, the variance in the approach to surveillance and care has improved with the formation of the CHOP Thyroid Center in 2009 and the recent formation of a multidisciplinary PHTS clinic in 2020, which includes evaluation by specialists from the Cancer Predisposition Program, the Comprehensive Vascular Anomalies program, genetics, the Thyroid Center, gastroenterology, dermatology, neurology, immunology, developmental pediatrics, and psychology.

**Conclusion**

There does not appear to be a clinical advantage for initiating thyroid US surveillance prior to 10 years of age secondary to the low risk for developing PHTS-associated DTC prior to adolescence. In addition, early thyroid monitoring may not significantly improve outcomes of thyroid cancer as the majority of PHTS-related DTC exhibit low-invasive behavior with no reports of distant metastasis found in a PubMed literature search at the time of submission of this article. We support the proposal that children with a pathogenic PTEN variant begin thyroid US examination after 10 years of age, with an annual thyroid US thereafter to ensure early detection of DTC while reducing the possibility of unnecessary interventions, such as FNA and thyroid surgery. Pediatric patients with macrocephaly and associated impaired cognitive development, thyroid nodules, and/or early-onset GI polyps should undergo constitutional testing for pathogenic variants of PTEN.

**Statement of Ethics**

This retrospective study involving human subjects was reviewed and approved by the Children’s Hospital of Philadelphia Institutional Review Board (CHOP IRB #17-014224). Written informed consent from the participant and/or participants’ legal guardian was not required per the CHOP IRB; a waiver of consent/parental permission has been approved per 45 CFR 46.116(d).

**Conflict of Interest Statement**

There are no conflicts of interest.
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Author Contributions

A.J.B. devised the project. J.A.B. collected the data, performed the analysis, and created the tables and figures. A.J.B. and J.A.B. wrote the manuscript with support and critical review by S.D.T., I.A., G.M.B., S.P.M., K.Z., D.A., and A.F.T. All authors discussed the results and provided editorial review of the manuscript.

References