ANTICANCER RESEARCH *42*: 1481-1485 (2022)
doi:10.21873/anticanres.15619

Thyroid Follicular Cell-derived Carcinomas in a Background of Multiple Adenomatous Nodules Leading to a Diagnosis of *PTEN* Hamartoma Tumor Syndrome in an Adult Patient With a Novel *RECQL4* Mutation

ALEX LIU[1], PAULA M. BORGES[2], YOU SHER TAY[3], LESTER D.R. THOMPSON[4], MAX X. KONG[5] and JINPING LAI[5]

[1]*Amador Valley High School, Oakland, CA, U.S.A.;*
[2]*Department of Otolaryngology-Head and Neck surgery, Kaiser Permanente Sacramento Medical Center, Sacramento, CA, U.S.A.;*
[3]*Department of Endocrinology, Kaiser Permanente Sacramento Medical Center, Sacramento, CA, U.S.A.;*
[4]*Head and Neck Pathology Consultations, Woodland Hills, CA, U.S.A.;*
[5]*Department of Pathology and Laboratory Medicine, Kaiser Permanente Sacramento Medical Center, Sacramento, CA, U.S.A.*

Abstract. *Background: Phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome (PHTS) is a complex disorder. Carriers develop hamartomatous tumors, with an increased risk for developing malignant tumors in multiple organs. Surveillance to facilitate the early detection and treatment of malignancies is extremely important. Case Report: A 31-year-old male presented with a 10 cm left lobe thyroid gland mass. After fine needle aspiration a left hemithyroidectomy was performed, which demonstrated a minimally invasive follicular thyroid carcinoma (FTC, stage pT3a) and microscopic classical papillary thyroid carcinoma (PTC) in the background of about 50 separate adenomatous nodules (0.2-5 mm). Immunostaining showed loss of PTEN protein in the minimally invasive FTC and in all of the nodules tested, with uninvolved parenchyma serving as an internal control. Kaiser Permanente Northern California (KPNC) Hereditary Cancer Panel, testing for 62 genes, was performed and showed germline mutations in PTEN and RecQ like helicase 4 (RECQL4) genes. Completion thyroidectomy subsequently performed demonstrated about 60 follicular cell-derived adenomatous nodules (0.3-10 mm). Genetic counseling and evaluation documented Cowden syndrome (CS) in the family. Thus, PHTS was confirmed. Conclusion: This report documents synchronous FTC and PTC in a background of multiple follicular adenomatous nodules with a novel RECQL4 mutation in an adult patient with PHTS. As such, documented the loss of PTEN protein in a thyroid gland affected by multiple adenomatous nodules aided in diagnosing PHTS.*

Correspondence to: Jinping Lai, MD, Ph.D., Department of Pathology and Laboratory Medicine, Kaiser Permanente Sacramento Medical Center, Sacramento, CA 95825, U.S.A. Tel: +1 9169737260, Fax: +1 9169737283, e-mail: Jinping.x.lai@kp.org

Key Words: Minimally invasive follicular thyroid carcinoma, *PTEN* gene mutation, Cowden syndrome, PTEN hamartoma tumor syndrome, *RECQL4* gene, germline mutation, surveillance.

Papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC) are the two most common thyroid malignancies arising from thyroid follicular epithelial cells (1). There are a heterogenous group of familial predisposition cancer syndromes that have other clinical findings that generally predominate, with thyroid gland manifestations also seen. One of these familial syndromes is the *PTEN* hamartoma tumor syndrome (PHTS), which is a highly variable condition characterized by hamartomatous lesions developing in multiple organs along with showing an increased risk for the development of malignancies. Most affected individuals show macrocephaly, Lhermitte-Duclos disease, mucocutaneous lesions (trichilemmomas; papules), gastrointestinal hamartomas, lipomas, follicular cell-derived thyroid lesions, pigmented macules of the glans penis, benign and malignant breast tumors, cerebrovascular malformations, and several types of cancer in patients with germline *PTEN* mutations. Associated cancers include breast (up to 85% risk), thyroid (typically FTC, up to 35% risk), kidney (up to 34%), endometrial (up to 28% risk), colorectal

(9-16% risk) and melanoma (6% risk) (2). The protein produced from *PTEN* is a tumor suppressor, functioning to normally prevent cells from proliferating in an uncontrolled fashion. Thus, a mutation prevents the protein from regulating cell proliferation effectively, and uncontrolled growth leads to hamartomas and malignancies. PHTS is also associated with developmental delay, intellectual disability, and autism spectrum disorder. Biological relatives are at risk for *PTEN*-related conditions and should consider testing as clinically appropriate; first-degree relatives (siblings, children, parents) have a 50% chance of also carrying this mutation (2), as it is an autosomal dominant disorder.

With respect to the thyroid gland, PHTS presents most frequently with clinical multinodular goiter, reflected as multiple hamartomatous nodules histologically, while carcinoma is detected at a lifetime risk of 3-10% in most series (3). Benign thyroid gland disease is common in PHTS, with thyroid adenomatous nodules (hamartomas) and thyroid follicular adenoma reported in 30-68% of adults and 2-14% of children with *PTEN* mutations (3).

We report a case of the initial presentation of PHTS/Cowden syndrome (CS) with synchronous FTC, PTC, and multiple adenomatous (hamartomatous) nodules. We emphasize the importance of PTEN immunostaining screening when innumerable, tightly formed adenomatous nodules are identified in a thyroid gland removed in a young patient. Further, in addition to *PTEN*, a novel mutation of uncertain significance in *RECQL4* was also identified. With additional studies, this mutation may be included in surveillance testing.

Case Report

The patient was a 31-year-old male with a long-standing history of thyroid enlargement. At 14 years old, he had thyroid gland enlargement and was sent for a thyroid gland fine needle aspiration (FNA). It was diagnosed as benign. He presented for evaluation of difficulty breathing at rest in certain positions and an inability to rotate his neck in certain positions. Physical exam documented an enlarged left thyroid gland lobe and macrocephaly. No penile freckling, generalized skin pigmentation or papillomas were identified. Ultrasound examination and computed tomography scans of the neck showed an enlarged left thyroid gland lobe containing a 10 cm hypervascular, heterogeneous appearing mass (Figure 1A and B). Serologies showed normal T4 free (1.2 ng/dl) and TSH (1.6 mIU/ml) levels. FNA of the left thyroid gland mass was interpreted as Bethesda II (benign thyroid nodule). For symptomatic relief, a left hemithyroidectomy was performed.

The macroscopic hemithyroidectomy showed a 10 cm encapsulated mass with a heterogeneous and hemorrhagic cut surface, along with a calcified center (Figure 1C). The tumor-to-capsule-to-adjacent parenchymal interface was entirely submitted for microscopic evaluation in 93 blocks. Microscopically, a well-formed tumor capsule was identified, with smooth muscle-walled vessels within the fibrous connective tissue, helping to support the presence of a true capsule. No areas of lymphatic or vascular invasion were identified, with only capsular invasion (Figure 1D) identified in four areas. The tumor was arranged in a follicular architecture with easily identified colloid throughout. There were no nuclear features of PTC. There was no tumor necrosis and no increased mitoses. These changes supported a diagnosis of minimally invasive FTC. In addition, there was a microscopic focus (0.2 mm) of classical PTC (on slide 78), showing all of the characteristic nuclear and architectural features of PTC. The margins were free from tumor. It was the background non-neoplastic thyroid gland parenchyma that was of note for about 50 adenomatous (hamartomatous) nodules that ranged from 0.2 to 5 mm. These nodules were cellular, showing a monotonous follicular architecture with nuclei that were round and regular with nuclear hyperchromasia. These nodules were quite different from the surrounding parenchyma. With such tight, well-formed nodules lacking any fibrous connective tissue capsules and having a general monotony to them, raised the possibility of a *PTEN* or *DICER1* abnormality. Immunohistochemistry for PTEN was performed, and showed nuclear loss of PTEN in the FTC (Figure 2A) and in the hamartomatous nodules randomly tested from the submitted parenchyma (Figure 2B-D). The PTC was not identified in the immunohistochemistry slide as it was so small. The pathology diagnoses included a minimally invasive FTC, which based on a tumor size of 10×8×4.7 cm, was placed in the pT3a AJCC stage, with "M" used to designated multifocal tumors (the PTC), along with loss of PTEN suggested a germline alteration in this gene and possible association with PHTS. Further genetic consultation and counselling resulted in molecular testing and completion thyroidectomy. The completion thyroidectomy specimen showed no additional malignancy, but demonstrated about 60 adenomatous/hamartomatous nodules, similar to the contralateral side. The patient recovered well after surgery and was without evidence of disease 6 months after surgery.

Molecular characteristics and genetic consultation. A PCR test for Invitae KPNC Hereditary Cancer Panel, which included 62 genes, was performed. In addition to the pathogenic *PTEN* mutation [*PTEN* c.388C>T (p.Arg130)], two mutations in *RECQL4* [*RECQL4* c.2755G>A (p.Ala919Thr); *RECQL4* c.940C>T (p.Pro314Ser)] of uncertain significance were also identified.

A genetics consultation developed a family pedigree. The patient's family was of Hispanic ancestry. The patient's mother was diagnosed with breast cancer at 40 years old and died of disease 2 years later. The patient's father is currently cancer free. The patient's son has macrocephaly with speech

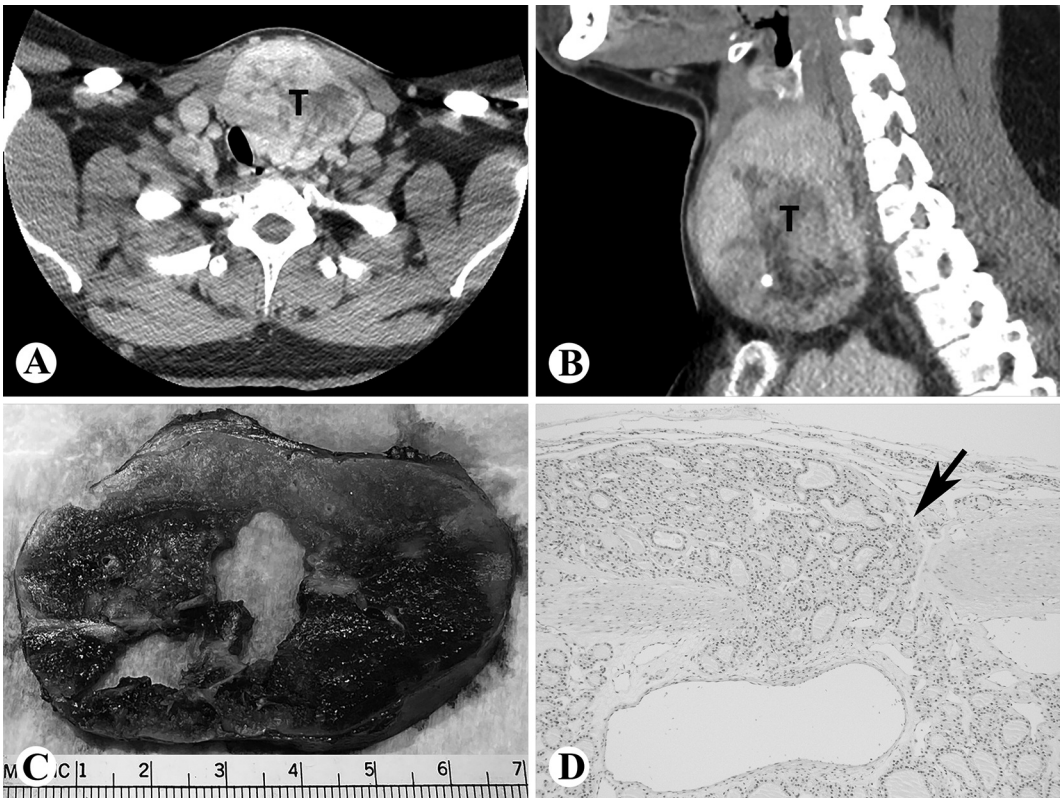

Figure 1. *Minimally invasive follicular thyroid carcinoma. A and B) Computer tomography scans show a 10 cm left thyroid gland mass (T) (A, Axial; B, Sagittal). C) Grossly, the left hemithyroidectomy specimen shows an encapsulated mass with heterogeneous cut surface. D) Microscopically, H&E stained section shows tumor capsular invasion (arrow) (100×).*

delay. The histological findings, molecular testing and family pedigree supported Cowden syndrome (CS), one of the subtypes within PHTS.

Discussion

PTEN immunohistochemical loss is highly suggestive of PHTS, including CS, but it is not diagnostic without additional clinical correlation to confirm the diagnosis. Barletta, *et al*. (4) showed PTEN loss by IHC has 100% sensitivity and 92.3% specificity. As CS confers a significant risk for cancer development (4), PTEN loss by IHC is useful to suggest PHTS, triggering genetic counseling and further molecular testing, which can then be used for gene–informed management, particularly regarding high-risk cancer surveillance and addressing neurodevelopment symptoms (4).

The histologic features in thyroidectomy specimens from patients with CS have been well described (5). The salient thyroid features in this disorder are multicentric follicular adenomas and multiple, bilateral adenomatous (hamartomatous) nodules. Multiple tiny cellular foci, sometimes called microadenomas, can be seen. It is the multifocal adenomatous nodules in this case that suggested the diagnosis, which was confirmed by PTEN immunohistochemistry. This case also included unilateral synchronous PTC (6) and FTC, with PTEN loss in both the carcinoma and background hamartomas.

PHTS results from germline inactivation mutations in the *PTEN* gene. The primary findings in PHTS include increased risk for certain types of cancer. Individuals with *PTEN* mutations should undergo cancer screening at the time of diagnosis to enable healthcare providers to detect any tumors at the earliest, most treatable stages, similar to this reported case. Due to the patient's young age and family history of early presentation of breast carcinoma, genetic testing for hereditary cancer predisposition (Invitae KPNC Hereditary Cancer 62 gene panel) was performed, which showed a pathogenic variant, c.388C>T (p.Arg130*) of *PTEN*. This molecular finding supports a diagnosis of PTEN-related condition. It seems the patient reported inherited the pathogenic gene from his mother, with presumed transmission to his son, although not confirmed as genetic testing for the son was declined.

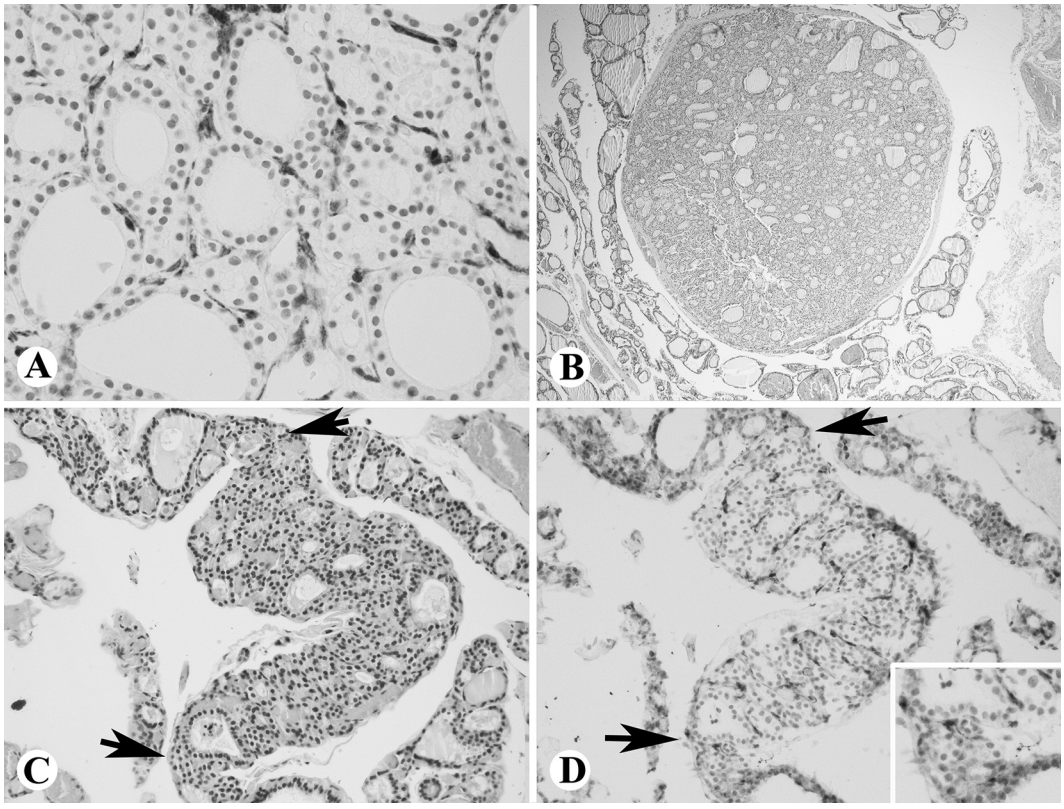

Figure 2. *PTEN immunohistochemistry in the follicular thyroid carcinoma and adenomatous nodules. A) PTEN immunohistochemical loss in the follicular carcinoma cells. B and C) H&E stained sections show multiple adenomatous nodules, some with fibrosis (B, 100×; C, 200×). D) PTEN immunohistochemical loss in the adenomatous nodule, while the adjacent thyroid parenchyma shows an intact reaction (arrows indicating the transition, 200×; inset at 400×).*

Finally, two variants of uncertain significance, c.2755G>A (p.Ala919Thr) and c.940C>T (p.Pro314Ser), were identified in *RECQL4*. The data from this test cannot definitively determine whether these variants are on the same or opposite chromosomes. The *RECQL4* gene is associated with autosomal recessive Rothmund-Thomson syndrome (RTS) (MedGen UID: 10819), RAPADILINO syndrome (MedGen UID: 336602) and Baller-Gerold syndrome (BGS) (MedGen UID: 120532). The RecQ family of DNA helicases is highly conserved throughout evolution and plays an important role in the maintenance of genomic stability in all organisms. The identification of *RECQL4* mutations is notable for clarifying an increased cancer risk and the risk of recurrence in the family. It has been previously shown that RTS patients with *RECQL4* mutations are at increased risk of osteosarcoma and lymphoma (7). The two variants reported here, c.2755G>A (p.Ala919Thr) and c.940C>T (p.Pro314Ser), have not been reported in those syndromes nor in thyroid cancer. Thus, the clinical significance of the variants identified in this gene are uncertain without a clear association with FTC and PTC.

In summary, we highlight a unique case of PHTS, which presented with multiple different thyroid gland disease processes including multifocal and bilateral adenomatous nodules (hamartomas) and two malignancies: FTC and PTC. Thyroid follicular nodular disease is common, but when seen in young patients who have innumerable, tight and well-formed nodules, the index of suspicion for possible familial syndrome association should be raised, with PTEN immunostaining employed as a potential screen for PHTS. To our knowledge, this is the first case report of *RECQL4* mutation in a PTEN-loss FTC and in the associated nodules that are part of PHTS. With further study, these mutations may shed light on the mechanism, prognosis, and potential treatment options in this familial disorder.

Conflicts of Interest

The Authors declare that they have no conflicts of interest regarding this case report.

Authors' Contributions

A.L. drafted the article; P.M.B. contributed to the treatment; Y.S.T. contributed to the patient care; L.D.T made the diagnosis and critically reviewed the article; M.X.K critically reviewed the article; and J.L. designed the study, made the diagnosis, collected and analyzed the data, wrote and finalized the article.

References

1 Suzuki H, Nishikawa D, Beppu S, Terada H, Sawabe M and Hanai N: Prognostic value of age and distant metastasis in differentiated thyroid carcinoma undergoing salvage surgery. Anticancer Res *40(2)*: 1127-1133, 2020. PMID: 32014964. DOI: 10.21873/anticanres.14053

2 Pilarski R: *PTEN* hamartoma tumor syndrome: a clinical overview. Cancers (Basel) *11(6)*: 844, 2019. PMID: 31216739. DOI: 10.3390/cancers11060844

3 Pilarski R, Stephens JA, Noss R, Fisher JL and Prior TW: Predicting PTEN mutations: an evaluation of Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome clinical features. J Med Genet *48(8)*: 505-512, 2011. PMID: 21659347. DOI: 10.1136/jmg.2011.088807

4 Barletta JA, Bellizzi AM and Hornick JL: Immunohistochemical staining of thyroidectomy specimens for PTEN can aid in the identification of patients with Cowden syndrome. Am J Surg Pathol *35(10)*: 1505-1511, 2011. PMID: 21921783. DOI: 10.1097/PAS.0b013e31822fbc7d

5 Harach HR, Soubeyran I, Brown A, Bonneau D and Longy M: Thyroid pathologic findings in patients with Cowden disease. Ann Diagn Pathol *3(6)*: 331-340, 1999. PMID: 10594284. DOI: 10.1016/s1092-9134(99)80011-2

6 Cao C, Poti SM, Ledgerwood LG and Lai J: Mixed HPV-related neuroendocrine carcinoma and HPV-related squamous cell carcinoma of the base of tongue in a patient with incidental identification of synchronous metastatic papillary thyroid carcinoma. Anticancer Res *41(7)*: 3639-3642, 2021. PMID: 34230161. DOI: 10.21873/anticanres.15153

7 Siitonen HA, Sotkasiira J, Biervliet M, Benmansour A, Capri Y, Cormier-Daire V, Crandall B, Hannula-Jouppi K, Hennekam R, Herzog D, Keymolen K, Lipsanen-Nyman M, Miny P, Plon SE, Riedl S, Sarkar A, Vargas FR, Verloes A, Wang LL, Kääriäinen H and Kestilä M: The mutation spectrum in RECQL4 diseases. Eur J Hum Genet *17(2)*: 151-158, 2009. PMID: 18716613. DOI: 10.1038/ejhg.2008.154

Received January 17, 2022
Revised February 4, 2022
Accepted February 7, 2022