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Brief Report

FLCN Variants in Parathyroid Carcinoma and Atypical Parathyroid Tumors

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Abstract

Parathyroid carcinoma (PC) and atypical parathyroid tumors (APT) are incompletely understood and pose challenges in definitive diagnosis. *FLCN* sequence variants have recently been linked to PC and APT. Inactivating mutations in the ubiquitously expressed *FLCN* tumor suppressor gene, encoding folliculin, cause Birt-Hogg-Dubé syndrome (BHD), a rare tumor predisposition syndrome. Germline inactivating *FLCN* variants, accompanied by somatic allelic loss, were reported in 2 unrelated patents with PC, both with clinical features, but no diagnosis, of BHD. Somatic frameshift variants of likely pathogenicity were reported in 1 patient with PC and 1 with APT. On the other hand, neither PC nor APT has been reported in sizeable BHD series. To better understand the frequency of *FLCN* variants in PC and APT, we analyzed a series of 10 patients with sporadic PC and 14 with APT by direct Sanger DNA sequencing. We identified no inactivating *FLCN* mutations in any of the PC or APT samples examined. A germline missense variant (p.Gly325Val), predicted as benign/tolerated, was seen in 1 PC and a synonymous variant in 1 APT. The absence of pathogenic mutations detected in our series of PC and APT further suggests that *FLCN* variants are rare in these tumors. Nevertheless, the potential roles of *FLCN* in the pathogenesis of PC and APT merits further consideration and study.

Key Words: parathyroid carcinoma, hyperparathyroidism, atypical parathyroid tumor, Birt-Hogg-Dube syndrome

Abbreviations: APT, parathyroid tumor; BHD, Birt-Hogg-Dube; NGS, next-generation sequencing; PC, parathyroid carcinoma; WES, whole-exome sequencing.

The molecular pathogenesis of parathyroid carcinoma (PC) and atypical parathyroid tumors (APT) are incompletely understood. APTs are defined as parathyroid lesions with atypical features, such as fibrous banding, trabecular growth pattern, increased mitotic activity, or other aberrant features, but lack the definitive evidence of malignancy required for unequivocal diagnosis of PC (ie, invasion into adjacent structures or metastasis). Thus, APTs are of uncertain malignant potential and long-term follow-up is advised [1]. However, few data on the recurrence rate or pathogenic drivers of APT exist [2].

The most common genetic alteration in PC is inactivation of the *CDC73* tumor suppressor gene, observed in a mean of around 35% of sporadic carcinomas across multiple studies but with considerable variation in frequency (13%-100%), likely because of differences in case selection and the potential inclusion of cases meeting some histopathologic criteria for carcinoma but with less definitive evidence for malignant clinical behavior [3-12]. *CDC73* mutations have been reported in approximately 5% of sporadic APT [2, 13, 14]. In both PC and APT, *CDC73* mutations may occasionally be germline, despite sporadic tumor presentation. The frequency of other genetic alterations in PC, such as amplifications of the cyclin D1/*CCND1* gene, or gain-of-function mutations in *PIK3CA* or *MTOR* [15] is unclear in APT [16]. Recently, *FLCN* variants have been linked to PC [17]. *FLCN*, located on chromosome 17, encodes folliculin, a ubiquitously expressed protein with roles in multiple cellular processes such as apoptosis and cell signaling. Inactivating *FLCN* germline mutations are the only known genetic basis of Birt-Hogg-Dubé syndrome (BHD), an autosomal dominant condition characterized by benign fibrofolliculomas, pulmonary cysts and spontaneous pneumothorax, and increased risk of renal cancer. Although benign parathyroid tumors have been reported [18-20], PC in the context of BHD had not previously been reported. Germline *FLCN* variants were found in 3 of 17 unrelated patients with PC negative for *CDC73* and *MEN1* mutation [17]. Identical variants in 2 of 3 patients had been previously reported in patients with BHD [21, 22]. Parathyroid tumor DNA sequencing revealed loss of the normal *FLCN* allele in 2 of the 3 patients. All 3 patients had additional clinical features suggestive of, or potentially consistent with, BHD, such as lung or kidney lesions, but no fibrofolliculomas and no established diagnosis of BHD before the study. An identical somatic *FLCN* inactivating variant was identified in 2 patients: 1 with metastatic PC and 1 with an APT. Prior to that report, no *FLCN* variants had been identified in next-generation sequencing (NGS) studies of PC, including more than 100 patients.

However, in 35% of cases subjected to NGS across the published literature, germline variants were specifically filtered out during data analysis. Interestingly, lower than expected

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Table 1. FLCN primer sequences

Exon	Forward primer (5′-3′)	Reverse primer (5′-3′)	Product size (bp)	Annealing temperature (° C)
4a	AGTCCATGGCACCCAGGTGC	TCTTCCGCCTGCTCACCCTG	265	55
4b	CACTCTCTTCTGCACGGAGG	ACTGCTCTCAGGTCCTCCTG	275	55
5	AGCTCAGATTTGCATAAACC	TGTGCAATGCTGGCTCCGAG	315	55
6a	ATTTGTGCCAGCTGACTCTG	TGATGAGGTAGATCCGGTCC	250	55
6b	ATCAAGGACAGCCTGGCCAG	CCTCAACCTCAGCACAGAGC	220	60
7	GTTGGCTGTGAACGAGTAGG	ACAAGCCAACCAATGTATCG	300	55
8	AACTGGTAGGATCAGGGGCT	TTCCTGCCAGGAGAGCAGAC	260	55
9	CCATGAAGTATCTTGGGCTG	AGGCTGTCAGTCACTTCCAG	400	60
10	ACACACAAATAACTTCATGG	TGGTGCACAGCGGTTCTGTG	300	55
11	GTTTGGGTAGTAGAGCATGG	GAGATCTGGTTCCACTTTGG	280	55
12	GACCTGGGATGAGCGGAGTG	GAGCAAGGGCAGGCGTTAGC	300	55
13	TGTAGCTGCAGACCGAGGTG	CCTCTTTTGGAAACAGCTCC	270	55
14	AGCTCCTGCTGGTGCCAAAG	GAAACTCAAGGGACAGTCCC	390	55

coverage levels of *FLCN* on whole-exome sequencing (WES), resulting in the failure to detect *FLCN* variants that had been identified by Sanger sequencing, was observed by Jha et al [17]. This raises the possibility that even somatic *FLCN* variants may have been missed in published WES studies. Thus, using Sanger sequencing because of its reportedly superior sensitivity for detecting variants in this particular gene, we sought to better understand the frequency of germline or somatic *FLCN* variants in PC and APT.

Methods

Patients and Samples

Ten primary, recurrent or metastatic PCs, 14 APTs and paired nontumor tissue or blood were obtained from patients who underwent surgery for primary hyperparathyroidism. All samples were obtained with informed consent in accordance with institutional review board-approved protocols. All patients were diagnosed per clinical routine with sporadic presentation; no patient was diagnosed preclinically in a surveillance program for known/suspected carriers of germline *CDC73* mutation. Tumors were categorized in accordance with stringent clinicopathologic criteria [1]. Apart from histologic diagnosis and sporadic presentation, cases were otherwise unselected with regard to *CDC73* mutation status or other criteria. Nine of the carcinoma samples had been previously subjected to WES [5]. Samples were flash frozen on surgical excision, with the exception of 1 PC and 1 APT, which were from formalin-fixed, paraffin-embedded tissue. Genomic DNA was extracted from each sample using either proteinase K digestion for surgical samples or sucrose gradient centrifugation for blood samples, followed by phenol-chloroform extraction and ethanol precipitation.

PCR and Sanger Sequencing

Primers were designed to cover the entire *FLCN* coding region and intron/exon boundaries (Table 1). PCR was performed in 20-µL reaction volumes, using template DNA at a concentration of 25 ng/µL. Each reaction contained 13 µL H_2O, 2 µL 10X PCR buffer, 200 µmol deoxynucleotide triphosphates, 1.5 mmol $MgCl_2$, 1 µM of each forward and reverse primer, and 0.2 µL of AmpliTaq Gold (Applied Biosystems/ThermoFisher, Waltham, MA, USA). The PCR reactions were performed by incubating at 95 °C for 10 minutes, followed by 35 cycles of 95 °C for 30 seconds, 55 °C or 60 °C for 30 seconds, 72 °C for 1 minute, and a final elongation step of 72 °C for 10 minutes. PCR products were run on 1.5% agarose gel to confirm amplification of the intended product. Then, remaining PCR product was enzymatically purified with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and sequenced by GENEWIZ (GENEWIZ, Inc, South Plainfield, NJ, USA). The results were assembled and compared to published reference data (ENSG00000154803) using Sequencher (Gene Codes, Ann Arbor, MI). Variants were confirmed with an independent PCR/sequencing reaction. The somatic/germline status of each identified variant was confirmed by PCR amplification and sequencing of nontumor, germline DNA from the same individual. The novelty of identified variants was assessed by the presence/absence in the Genome Aggregation Database [23] and predicted effects of known variants were evaluated using embedded *in silico* tools.

Results

We identified no inactivating *FLCN* variants in any of the 10 PC or 14 APT samples. A heterozygous, germline missense variant, c.973G > T, resulting in a p.Gly325Val change in the predicted protein, was found in 1 PC (Fig. 1). This is a very rare allele, appearing only once in the Genome Aggregation Database, and predicted by multiple *in silico* analyses to be benign/tolerated. In the absence of functional data, the variant is presently classified as a variant of uncertain significance [23]. The patient with this variant had metastatic parathyroid cancer diagnosed at age 40 years. Although not specifically assessed for clinical features suggestive of BHD, no evidence of pneumothorax or lung blebs was seen on multiple chest scans, and no skin lesions were noted. However, small, bilateral renal cysts were noted. A known synonymous variant (c.1233G > A, MAF = 0.007908 in the Genome Aggregation Database) was also observed in 1 APT but nontumor DNA was not available to determine germline/somatic status. Overall, across the present and previous study [17], likely pathogenic *FLCN* variants have been detected in 3 of 27 (11.1%) sporadic PCs and in 1 of 17 (5.9%) APTs.

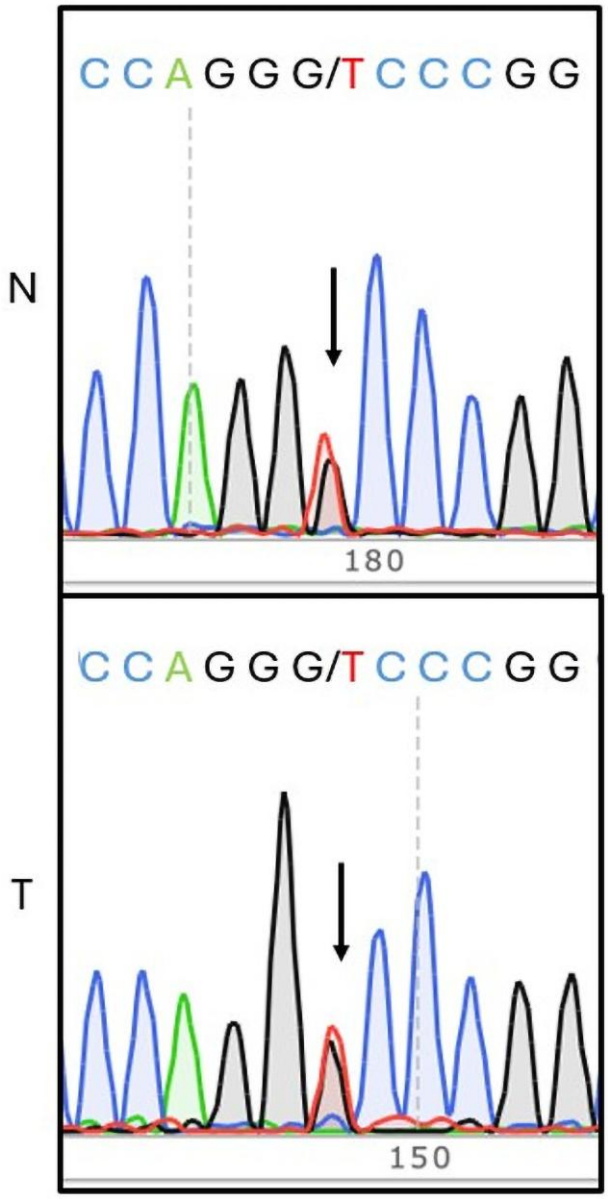

Figure 1. Identified *FLCN* missense variant. A heterozygous guanine to thymine transversion was identified at position 973 of the *FLCN* coding sequence (c.973G > T), resulting in a Gly325Val protein change, in 1 sporadic parathyroid carcinoma in this study. This variant was also present in nontumor germline DNA from the same individual. The top electropherogram shows the nontumor (N) DNA sequence; the bottom electropherogram shows the tumor sequence (T). Position of the variant is indicated by an arrow.

Discussion

We sought to determine the frequency of pathogenic germline and/or somatic *FLCN* variants in a series of sporadic PCs and ATPs by direct Sanger DNA sequencing, given prior information that coverage of this gene can be poor when using NGS technology. We identified 1 germline *FLCN* missense variant in 1 of 10 PCs and 1 synonymous variant in 1 of 14 APTs, neither of likely pathogenic importance, and suggesting that pathogenic *FLCN* variants are likely rare in PC and APT.

With the expansion of the number of NGS studies of PC in recent years, one might turn to such published studies to further understand the overall frequency of newly reported gene variants. In total, 133 PCs across 10 studies have been subjected to NGS, including whole exome, whole genome, and panel sequencing for which *FLCN* was included [3-12]. No pathogenic *FLCN* variants have been reported in prior WES studies of PC. Germline variants were intentionally and specifically filtered out during data analysis in 35% of cases across all studies. Indeed, the case in our series with an *FLCN* variant of uncertain significance had been included in a prior NGS study of somatic variants [5], and on reexamination of the data from that study, this variant had been present but not reported because of its germline status. Although germline variants could have been present but unreported in some NGS studies, somatic *FLCN* variants (that can often be present as pathogenic second hits in a classic tumor suppressor) would have been detected and reported. Finally, the lower than expected coverage levels of *FLCN* on WES reported by Jha et al, resulting in the failure to detect *FLCN* variants in 2 patients that had been identified by Sanger sequencing [17], raises the possibility that some *FLCN* variants may have been missed by prior published NGS studies. Such studies may also have missed potentially important alterations in other genes with lower coverage levels, an important consideration for interpreting data from published NGS studies and for design of future studies.

The link between parathyroid tumors and BHD is intriguing. Before Jha et al [17], only 3 patients with BHD across more than 600 families had been reported to have parathyroid tumors, all benign: 2 with parathyroid adenoma and 1 with multigland parathyroid disease [18-20]. This does suggest that parathyroid tumorigenesis is only a very rare manifestation of BHD. Genetic screening of a health care system population revealed pathogenic/likely pathogenic *FLCN* truncating mutations in 0.03% of unrelated individuals, 68.6% of whom had classical BHD related manifestations but only 11.4% had a diagnosis of BHD [24]. Taken together, these observations suggest that the clinical spectrum of BHD outside of the classical familial presentation is incompletely understood and thus raises the possibility that BHD-associated primary hyperparathyroidism could be more prevalent than currently believed. The rare nature of PC poses a limitation to these studies, as sample numbers are often low. Although our study did not reveal any inactivating *FLCN* variants, the presence of such variants could be enriched to some extent when *CDC73*-mutation positive patients are excluded, as in Jha et al [17].

Folliculin is associated with both mTOR and AMPK pathways. These pathways are both essential in maintaining cellular homeostasis, and dysregulation of mTOR and AMPK pathways have been linked to several cancers; therapeutic agents targeting them have been and continue to be developed. Examining such agents for efficacy in treating *FLCN*-mutation positive patients with PC and BHD would be an important consideration [25].

The preponderance of evidence suggests that pathogenic *FLCN* mutations are quite rare in sporadic PC and APT. However, given the potential clinical implications, such as the benefits of diagnosing BHD for the prevention of other severe manifestations and the possibility of future precision medicine therapeutic approaches, germline *FLCN* testing and/or additional screening for BHD-related lesions in patients with PC, especially in the absence of confirmed *CDC73* germline mutation, merits further study.

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Disclosures

The authors declare no conflicts of interest.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

1. Erickson LA, Mete O, Juhlin CC, Perren A, Gill AJ. Overview of the 2022 WHO classification of parathyroid tumors. *Endocr Pathol*. 2022;33(1):64-89.
2. Cetani F, Marcocci C, Torregrossa L, Pardi E. Atypical parathyroid adenomas: challenging lesions in the differential diagnosis of endocrine tumors. *Endocr Relat Cancer*. 2019;26(7):R441-R464.
3. Kasaian K, Wiseman SM, Thiessen N, *et al*. Complete genomic landscape of a recurring sporadic parathyroid carcinoma. *J Pathol*. 2013;230(3):249-260.
4. Yu W, McPherson JR, Stevenson M, *et al*. Whole-exome sequencing studies of parathyroid carcinomas reveal novel PRUNE2 mutations, distinctive mutational spectra related to APOBEC-catalyzed DNA mutagenesis and mutational enrichment in kinases associated with cell migration and invasion. *J Clin Endocrinol Metab*. 2015;100(2):E360-E364.
5. Pandya C, Uzilov AV, Bellizzi J, *et al*. Genomic profiling reveals mutational landscape in parathyroid carcinomas. *JCI Insight*. 2017;2(6):e92061.
6. Kang H, Pettinga D, Schubert AD, *et al*. Genomic profiling of parathyroid carcinoma reveals genomic alterations suggesting benefit from therapy. *Oncologist*. 2019;24(6):791-797.
7. Clarke CN, Katsonis P, Hsu TK, *et al*. Comprehensive genomic characterization of parathyroid cancer identifies novel candidate driver mutations and core pathways. *J Endocr Soc*. 2019;3(3):544-559.
8. Cui M, Hu Y, Bi Y, *et al*. Preliminary exploration of potential molecular therapeutic targets in recurrent and metastatic parathyroid carcinomas. *Int J Cancer*. 2019;144(3):525-532.
9. Kutahyalioglu M, Nguyen HT, Kwatampora L, *et al*. Genetic profiling as a clinical tool in advanced parathyroid carcinoma. *J Cancer Res Clin Oncol*. 2019;145(8):1977-1986.
10. Hu Y, Zhang X, Wang O, *et al*. The genomic profile of parathyroid carcinoma based on whole-genome sequencing. *Int J Cancer*. 2020;147(9):2446-2457.
11. Teleanu MV, Fuss CT, Paramasivam N, *et al*. Targeted therapy of advanced parathyroid carcinoma guided by genomic and transcriptomic profiling. *Mol Oncol*. 2023;17(7):1343-1355.
12. Jo SY, Hong N, Lee S, *et al*. Genomic and transcriptomic profiling reveal molecular characteristics of parathyroid carcinoma. *Exp Mol Med*. 2023;55(5):886-897.
13. Chen Y, Song A, Nie M, *et al*. Clinical and genetic analysis of atypical parathyroid adenoma compared with parathyroid carcinoma and benign lesions in a Chinese cohort. *Front Endocrinol (Lausanne)*. 2023;14:1027598.
14. Saponaro F, Pardi E, Mazoni L, *et al*. Do patients with atypical parathyroid adenoma need close follow-up? *J Clin Endocrinol Metab*. 2021;106(11):e4565-e4579.
15. Brewer K, Costa-Guda J, Arnold A. Molecular genetic insights into sporadic primary hyperparathyroidism. *Endocr Relat Cancer*. 2019;26(2):R53-R72.
16. Pardi E, Poma AM, Torregrossa L, *et al*. Whole-exome sequencing of atypical parathyroid tumors detects novel and common genes linked to parathyroid tumorigenesis. *J Clin Endocrinol Metab*. 2024;110(1):48-58.
17. Jha S, Welch J, Tora R, *et al*. Germline- and somatic-inactivating FLCN variants in parathyroid cancer and atypical parathyroid tumors. *J Clin Endocrinol Metab*. 2023;108(10):2686-2698.
18. Mikesell KV, Kulaylat AN, Donaldson KJ, Saunders BD, Crist HS. A rare soft tissue tumor masquerading as a parathyroid adenoma in a patient with birt-hogg-dube syndrome and multiple cervical endocrinopathies. *Case Rep Pathol*. 2014;2014:753694.
19. Vinit J, Friedel J, Bielefeld P, Muller G, Goudet P, Besancenot JF. Birt-Hogg-Dube syndrome and multiple recurrent tumors]. *Rev Med Interne*. 2011;32(3):e40-e42.
20. Chung JY, Ramos-Caro FA, Beers B, Ford MJ, Flowers F. Multiple lipomas, angiolipomas, and parathyroid adenomas in a patient with Birt-Hogg-Dube syndrome. *Int J Dermatol*. 1996;35(5):365-367.
21. Nahorski MS, Reiman A, Lim DH, *et al*. Birt Hogg-Dube syndrome-associated FLCN mutations disrupt protein stability. *Hum Mutat*. 2011;32(8):921-929.
22. Schmidt LS, Nickerson ML, Warren MB, *et al*. Germline BHD-mutation spectrum and phenotype analysis of a large cohort of families with Birt-Hogg-Dube syndrome. *Am J Hum Genet*. 2005;76(6):1023-1033.
23. Chen S, Francioli LC, Goodrich JK, *et al*. A genomic mutational constraint map using variation in 76,156 human genomes. *Nature*. 2024;625(7993):92-100.
24. Savatt JM, Shimelis H, Moreno-De-Luca A, *et al*. Frequency of truncating FLCN variants and Birt-Hogg-Dube-associated phenotypes in a health care system population. *Genet Med*. 2022;24(9):1857-1866.
25. Ramirez Reyes JMJ, Cuesta R, Pause A. Folliculin: a regulator of transcription through AMPK and mTOR signaling pathways. *Front Cell Dev Biol*. 2021;9:667311.