Association Between Parafibromin Expression and Presence of Brown Tumors and Jaw Tumors in Patients with Primary Hyperparathyroidism: Series of Cases with Review of the Literature

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Data Collection B
Statistical Analysis C
Data Interpretation D
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Case series
Patients: **Male, 57-year-old • Male, 42-year-old • Female, 45-year-old • Male, 42-year-old**
Final Diagnosis: **Brown tumor • hyperparathyroidism**
Symptoms: **Brown tumor • hypercalcemia**
Medication: **—**
Clinical Procedure: **—**
Specialty: **Endocrinology and Metabolic**

Objective: **Rare disease**
Background: Brown and jaw tumors are rare entities of poorly understood etiology that are regarded as end-stage of bone remodeling in patients with long-lasting and chronic hyperparathyroidism. Jaw tumors are mainly diagnosed in jaw tumors syndrome (HPT-JT syndrome) and are caused by mutation in the *CDC73* gene, encoding parafibromin, a tumor suppressing protein. The aim of this work is to present 4 cases of patients in whom the genetic mutation of the *CDC73* gene and clinical presentation coexist in an unusual setting that has not yet been described.
Case Reports: We present cases of 4 patients with primary hyperparathyroidism. Three were diagnosed with brown tumors (located in long bones, ribs, iliac, shoulders) and 1 with brown and jaw tumors. Expression of parafibromin in affected parathyroid tissues were analyzed. In patients without positive parafibromin staining, we searched for *CDC73* mutation using next-generation sequencing. Parafibromin staining was positive in 1 patient with brown tumors and was negative in 2 individuals with brown tumors and 1 with brown and jaw tumors. *CDC73* mutation was detected in two-thirds of patients (60%) with negative staining for parafibromin and brown tumors. *MEN1* mutation was found in the patient with brown tumor and positive staining for parafibromin.
Conclusions: Patients with hyperparathyroidism and coexistence of brown tumors or jaw tumors might have decreased expression of parafibromin in parathyroid adenoma tissue, which might be caused by *CDC73* mutation and suggest a genetic predisposition. Further research on much larger study groups is needed.

Keywords: **CDC73 Protein, Human • Hedgehogs • Hyperparathyroidism**

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Background

Primary hyperparathyroidism is one of the most common endocrinopathies, with a prevalence of up to 1.1% in Europe. About 80% of cases involve a single adenoma arising as a result of a mutation or loss of heterozygosity in the nucleus of a single parathyroid cell [1,2]. The other 20% of cases result from congenital mutations in all nucleated cells [3-7].

Bone tumors are very rare entities that can coexist with prolonged primary hyperparathyroidism. They are believed to represent the end-stage of the bone remodeling process. One type is the jaw tumor, which, in patients with primary hyperparathyroidism, is known as hyperparathyroidism-jaw tumor (HPT-JT) syndrome. It is caused by mutation in the *CDC73* gene, which encodes parafibromin, a well-known tumor suppressor [8-12]. Jaw tumors, such as ossifying fibroma or fibrous dysplasia tumors, show similarities to brown tumors [8-19]. HPT-JT syndrome diagnostic criteria includes parathyroid tumors, ossifying fibromas of the mandible and maxilla (no other location is mentioned), cystic and neoplastic renal abnormalities, and hyperplastic and neoplastic uterine involvement. Our team has observed the presence of brown tumors within long and flat bones in patients with HPT-JT syndrome. This was the factor that encouraged our team to investigate a connection between the presence of brown tumors (in patients with or without lesions in jaw or maxilla) and abnormal parafibromin expression or *CDC73* mutation.

Mutations in the *CDC73* gene occur in 71% of malignant parathyroid cancers and are correlated with the coexistence of other malignancies, as well as with recurrent hyperparathyroidism [20-30]. Parafibromin, encoded by the *CDC73* gene, consists of 531 amino acids. It creates a scaffold for the polymerase II-associated factor enzymatic complex, which is responsible for the communication with transcription activators, regulation of histone modifying factors, and effect on extension of the chromatin pattern [31]. It suppresses cell cycle progression through the inhibition of the cyclin D1 promoter and c-myc oncogene (which may explain the occurrence of hyperparathyroidism) [32-36] and/or through the activation of apoptosis [23,37-39].

The role of parafibromin is important and complex. The connection with the Wnt, NOTCH, and Hedgehog (Hh) pathways indicates its role in embryogenesis, in the regulation of gene expression, and in carcinogenesis, in which the Wnt and Hh pathways are closely related to this process and the inhibition of oncogenesis (the role of parafibromin is poorly understood in this process) [38-44].

In an animal model, a 50% reduction in the expression of the C fragment of the final parafibromin did not stimulate carcinogenesis. This may indicate a correlation between uncontrolled cell proliferation within the N-terminal fragment and its effect on the regulation of the Wnt-NOTCH-Hh pathways. A comparison of immunohistochemical tests in adenomas and parathyroid carcinomas revealed no differences in the expression of Wnt/Hh pathway components interacting with parafibromin [45].

Within the parafibromin molecule, 3 binding sites of the entire nuclear localization signal (NLS) have been located. The first seems to play a key role (position 125-139), the second a supportive role (position 76-92), and the third, which is within the final fragment C (position 393-409), is probably involved in the activation of the polymerase I-associated factor (PAF1) enzymatic complex due to homology with the primary molecule Cdc73 found in yeast [46].

The nuclear attachment sites of parafibromin determine its regulatory effect on cell proliferation. Therefore, the intact molecule is necessary not only to create a scaffold for the PAF1 enzymatic complex and the Wnt-NOTCH-Hh metabolic pathways, but also to attach the entire complex to the cell nucleus. Leucine-rich fragments of parafibromin may also function as sequences necessary for transport from the cytoplasm to the nuclear export sequences of the nucleus, but its exact location has not been determined [46,47].

In this study, we wanted to determine whether the presence of brown tumors with or without lesions in the jaw or maxilla can be associated with abnormal parafibromin expression or *CDC73* mutation in a group in which hyperparathyroidism was detected at an early stage by outpatient screening. We present the cases of 4 patients with rare symptoms of hyperparathyroidism, namely brown tumors. In 3 patients no parafibromin expression was present (in 2 patients *CDC73* gene mutation was confirmed), and in 1 patient there was *MEN1* gene mutation.

Case Reports

Patient Selection Criteria

The inclusion criteria were (1) clinical and biochemical symptoms of primary hyperparathyroidism and (2) presence of brown tumor or jaw tumor confirmed by MIBI SPECT/CT.

The exclusion criteria were (1) secondary and tertiary hyperparathyroidism; (2) malabsorption (eg, celiac disease); (3) previous bariatric surgery; (4) renal failure (eGFR <60 mL/min./1.73m2); (5) parathyroid hormone (PTH)-independent hypercalcemia; and (6) malignant diseases with metastatic bone lesions.

Case Presentations

Patient 1

A 57-year-old man, who was diagnosed with hyperparathyroidism at age of 29, presented with a history of nephrolithiasis, nephrocalcinosis, and 2 hypercalcemic crises in the course of an adenoma of the right upper parathyroid gland that was treated surgically in 1993 and an adenoma of the left lower parathyroid gland that was treated surgically in 2013. The patient did not report a history of smoking, alcohol use, or substance abuse. In the course of making the differential diagnosis, brown tumors in the right humerus and both tibias were found. The patient's main medical problem was recurrent hyperparathyroidism along with nephrocalcinosis, recurrent nephrolithiasis, brown tumors, and osteoporosis. He had an increasing proliferation index of parathyroid cells (2% in 1993, 7.3% in 2013). The patient had coexisting kidney cysts and hypertension. His medications were vitamin D 2000 units daily, amlodipine 10 mg daily, and alendronate sodium 70 mg once a week.

The following tests were performed. An imaging examination was done with MIBI SPECT/CT. Immunohistochemical staining and genetic tests were conducted following the parathyroidectomy. The removed parathyroid glands were stained against parafibromin using immunohistochemical methods, anti-parafibromin mouse monoclonal antibody, primary parafibromin antibody, and secondary antibody. Subsequently, genetic tests were performed for mutations in the following genes: *CDC73*, *MEN1*, *RET*, *CDKN1B*, and *CASR*.

Parafibromin staining was negative within the cell nuclei. A very weak cytoplasmic expression of parafibromin was observed. In genetic testing, a *CDC73/HRPT2* gene germline mutation was confirmed. Transcription was inhibited in the 616th nucleotide. This caused protein synthesis to stop at the point of 202 amino acids, resulting in loss of the C-terminal fragment.

The summary of the clinical problem was a genetically determined form of primary hyperparathyroidism in the course of mutation of the CDC73 gene encoding the parafibromin protein.

Patient 2

A 42-year-old White man who was diagnosed with hyperparathyroidism at the age of 36 years and had a medical history of nephrolithiasis, recurrent cysts of long bones (resection of the left fifth metacarpal bone cyst in November 2014), osteopenia, brown tumors, kidney cysts, and chronic obstructive pulmonary disease was admitted to the Endocrinology Department owing to the persistence of high calcium and PTH levels. The patient received 1000 units vitamin D per day. The patient

reported a history of smoking (20 cigarettes per day) and no alcohol or other substance abuse.

In a SPECT/CT examination, a lesion of the left lower parathyroid gland with a possible infiltration of the sternothyroid muscle was diagnosed. Because of the suspicion of a proliferative process, the patient was urgently referred for surgical treatment. Following the parathyroidectomy, immunohistochemical staining and genetic tests were conducted. The removed parathyroid glands were stained against parafibromin using immunohistochemical methods, anti-parafibromin mouse monoclonal antibody, primary parafibromin antibody, and secondary antibody. Subsequently, genetic tests were performed for mutations in the following genes: *CDC73*, *MEN1*, *RET*, *CDKN1B*, and *CASR*.

Very weak nuclear expression of parafibromin (intensity score <1) with negative cytoplasmatic staining were observed. The proliferation index of parathyroid cells was 1.5%. In genetic testing, the *CDC73/HRPT2* gene mutation was confirmed. The protein synthesis was stopped at the point of 414 amino acids, which was caused by deletion of the 14/17 exon on the *CDC73* gene (*HRPT2*) within 1240 nucleotides.

The summary of the clinical problem was a genetically determined form of primary hyperparathyroidism in the course of mutation of the CDC73 gene encoding the parafibromin protein.

Patient 3

A 45-year-old White woman who was first diagnosed with hyperparathyroidism at the age of 36 years and who had congenital craniofacial development disorder and eyeball hypoplasia, an 8-year history of nephrolithiasis (that was treated with extracorporal shockwave lithotripsy), complicated by ureteral stenosis and secondary hydronephrosis (that was also treated surgically in 2014), thrombosis of the iliac veins and inferior vena cava, uterine fibroids, left ovary cyst and spleen cysts, colon polyps, and very low level of vitamin D (<3 ng/dL) was referred to the Endocrinology Department because of uncontrolled hyperparathyroidism. The patient was taking enoxaparin 60 mg twice a day. In the course of the diagnostic process, multiple brown tumors of the long, flat bones, mandible, and maxilla were found. During hospitalization, the patient had a transverse fracture of the right femur, which occurred while changing the position of the body. It was fracture in the site of the brown tumor and a pathological fracture of the left patella.

In a SPECT/CT examination, a 3-cm lesion of the right lower parathyroid gland was diagnosed, and the patient was referred for surgery. Following the parathyroidectomy, the removed parathyroid glands were stained against parafibromin using immunohistochemical methods, anti-parafibromin mouse

monoclonal antibody, primary parafibromin antibody, and secondary antibody. Subsequently, genetic testing was performed for mutations in the following genes: *CDC73*, *MEN1*, *RET*, *CDKN1B*, and *CASR*.

Parafibromin staining within the cell nuclei was negative. No mutations were detected within the *CDC73* gene.

The summary of the clinical problem was that because of the clinical features of the HPT-JT syndrome, a test for mutation of the *CDC73* gene was performed. There were no abnormalities within the *CDC73* gene detected. Nevertheless, the staining for parafibromin was negative.

Patient 4

A 42-year-old White man who was first diagnosed with hyperparathyroididsm at the age of 30 years and who had severe skeletal deformities was referred to the Endocrinology Department owing to hypercalcemic crisis secondary to hyperparathyroidism. The patient's concomitant disorders were non-secreting neuroendocrine tumors of the pancreas, macroprolactinoma, and pituitary insufficiency. His previous medical history included nephrolithiasis, complicated with hydronephrosis. The patient reported no history of smoking, alcohol use, or substance abuse. He was taking levothyroxine 75 ug and hydrocortisone 15 mg daily in selected doses, and Cabergoline 0.5 mg once a week. In the course of the diagnostic process, brown tumors in the long bones were detected. The presence of a focal lesion in the pancreas and pituitary microprolactinoma resulted in suspicion of MEN1 or MEN4 syndrome. Because of fulfilling the diagnostic criteria of MEN syndrome, the patient was referred for a subtotal parathyroidectomy. Immunohistochemical staining and genetic tests were conducted following the parathyroidectomy. The removed parathyroid glands were stained against parafibromin using immunohistochemical methods, anti-parafibromin mouse monoclonal antibody, primary parafibromin antibody, and secondary antibody. Positive staining in the nucleus and cytoplasm was detected.

Subsequently, genetic tests were performed for mutations in the following genes: *CDC73*, *MEN1*, *RET*, *CDKN1B*, and *CASR*. No mutations were detected within the *CDC73* gene. A *MEN1* mutation was confirmed.

The summary of the clinical problem was that, in view of the clinical suspicion of MEN syndrome, tests for mutation of *MEN* and *CDKN1B* genes were performed and confirmed MEN1 syndrome. Numerous brown tumors and the experience of researchers to date with their occurrence in *CDC73* mutations were the reasons for performing additional research on this gene. No mutation of *CDC73* was revealed. The staining for parafibromin was positive.

The samples of parafibromin staining for all patients are presented in **Figure 1**. The clinical features in the group of patients with brown/jaw tumors are presented in **Table 1**. The data concerning parafibromin staining and genetic tests in the study group are summarized in **Tables 2 and 3**.

Discussion

Today, due to the common screening of calcium levels in outpatient clinics, brown tumors are rarely observed, except for in patients with HPT-JT syndrome, which is caused by a mutation of the *CDC73* gene.

In our study, we presented 4 cases of patients in whom the genetic mutation of the *CDC73* gene and clinical presentation coexisted in an unusual setting. To the best of our knowledge, this is the first case report describing this occurrence.

In our patients, genetic testing and parafibromin staining were performed. The complete coding sequence of the *CDC73* gene was analyzed using next-generation sequencing with the NextSeq500 instrument (Illumina, San Diego, CA, USA). Also, *MEN1*, *RET*, *CDKN1B*, and *CASR* genes were analyzed. Immunohistochemical assessment of parafibromin expression was carried out on 3-μm tissue sections using anti-parafibromin mouse monoclonal antibody (Parafibromin (2H1): sc-33638, Santa Cruz Biotechnology, Inc, Dallas, TX, USA), recognizing an N-terminal fragment of 87 to 100 amino acids.

Genetic testing enabled not only confirmation of the presence of the *CDC73/HRPT2* gene mutation, but also determination of the exact location of lost nucleotides, which allowed for identification of the possible location of the active NLS nucleus attachment site. In the present study, we could assess their presence or loss as a result of damage to the parafibromin molecule. Tests allowed us to determine the pathogenicity of the mutation and, due to sequencing of the entire gene, to assess the degree of shortening of this protein. To the best of our knowledge, this is the first study proposing nuclear export sequence localization for parafibromin.

Pathogenic mutations of the *CDC73* gene were found in 2 of 3 patients with brown tumors (patients 1 and 2). Genetic analysis confirmed damage to the parafibromin molecule in 3 of 4 patients.

In patient 1, transcription was inhibited in the 616th nucleotide. This caused protein synthesis to stop at the point of 202 amino acids, resulting in loss of the C-terminal fragment. From a clinical point of view, this is due to the inability of attachment of the PAF2 enzyme complex to the cell nucleus within the conservative NLS (393-409). In spite of having other NLS,
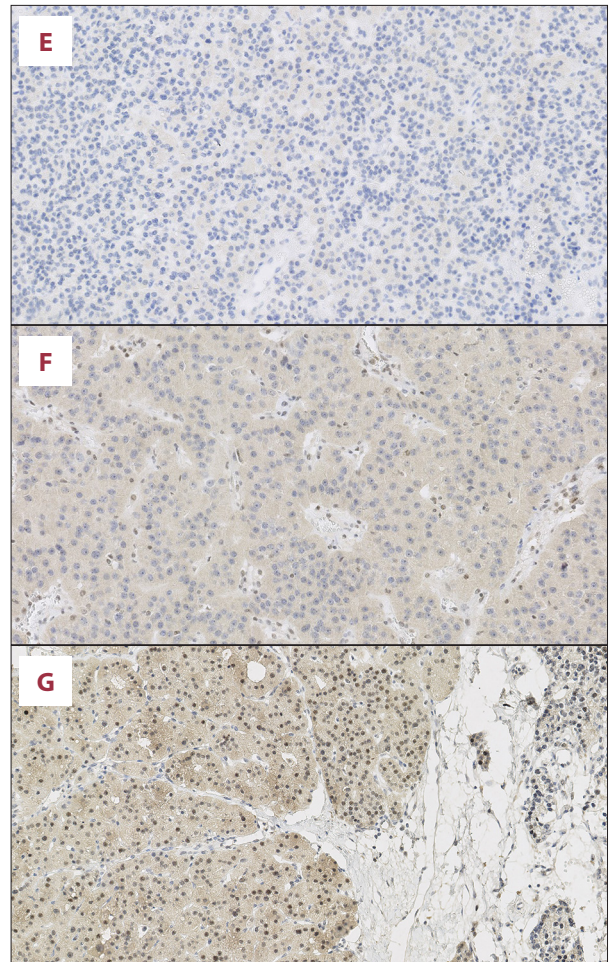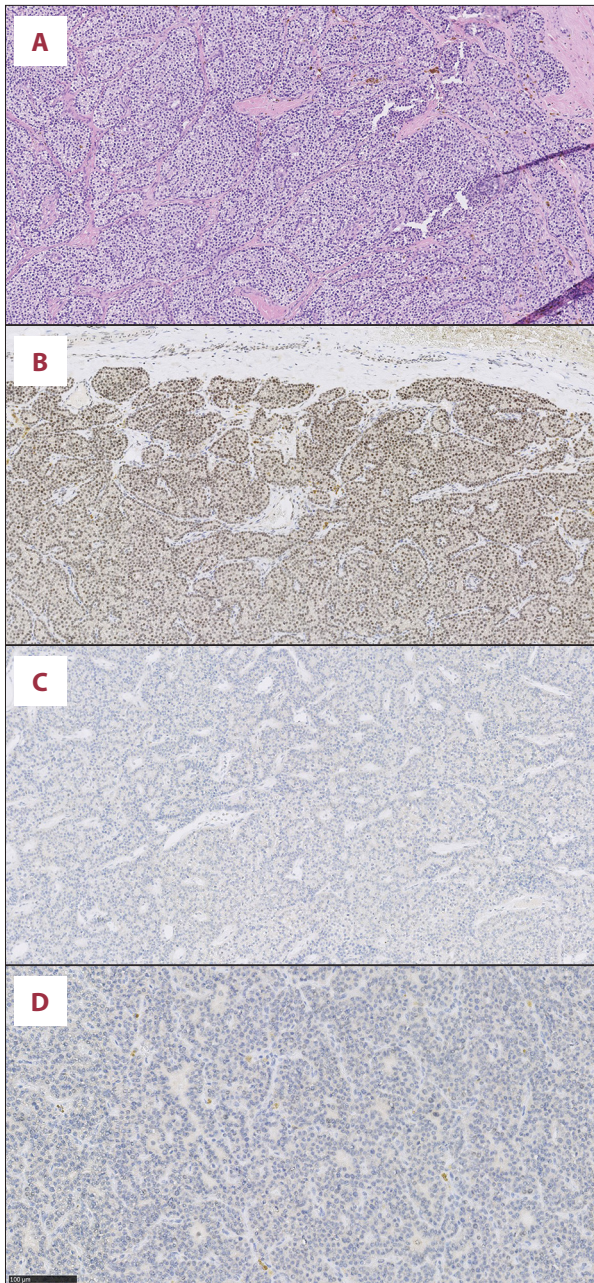
Figure 1. Staining for parafibromin in group of patients with brown tumors. (**A**) Physiological hematoxylin eosin staining of healthy parathyroid gland. (**B**) Physiological parafibromin staining of healthy parathyroid gland. (**C**) Primary hyperparathyroidism and brown tumors in ribs in patient 2. (**D**) Primary hyperparathyroidism and multiple brown tumors in ribs, pelvis, lower limbs, and mandibula in patient 3. (**E**) Primary hyperparathyroidism and brown tumor in sternum in patient 4. (**F**) Recurrent primary hyperparathyroidism and multiple brown tumors in lower and upper limbs in patient 1. (**G**) Primary hyperparathyroidism, multiple brown tumors in lower and upper limbs, and severe bone deformities in patient 4.

staining of parathyroid cells obtained from parafibromin histopathological preparations showed that this protein was not present in the cell nucleus. At the same time, staining in the cytoplasm was observed, which may suggest loss of the nuclear export sequence site responsible for the transport of parafibromin from the cytoplasm into the cell nucleus.

In patient 2, protein synthesis was stopped at the point of 414 amino acids, which was caused by deletion of the 14/17 exon on the *CDC73* gene (*HRPT2*) within 1240 nucleotides. This abnormality also led to the loss of the final C fragment, without concomitant loss of NLS. Staining of parathyroid cells against

parafibromin showed its very weak expression within the cell nucleus and complete lack of staining in the cytoplasm. This may suggest the preserved transport of parafibromin to the cell nucleus as well as accelerated metabolism of this protein.

In the case of the classic form of HPT-JT syndrome in patient 3, there was no *CDC73* mutation and no parafibromin in parathyroid cells. This may suggest other mechanisms were regulating its expression, which have already been described in the

Table 1. Clinical features in group of patients with brown and jaw tumors.

ID	Brown tumor	Jaw tumor	CDC73/ HRPT2 mutation	Staining for parafibromin in nucleus	PTH [pg/mL] 15-65	Ca [mmol/L] 2.15-2.6	Pi [mmol/L] 0.81-1.45	Duration of hyperpara-thyroidism	MEN1, CDKN1B, RET mutations	Other
1	Long bones, shoulders	Not present	Germinal (pathogenic)	Negative	NID	NID	NID	>10 years	–	Nephrolithiasis. Kidney cysts
2	Ribs	Not present	Germinal (pathogenic)	Very weak	768.7	3.18	0.64	2 years	RET (not pathogenic)	Kidney cysts. Nephrolithiasis. Coexistence of RET mutation of unknown pathogenicity
3	Iliac, ribs, long bones	Present	Absent	Negative	1678	3.27	0.92	Unknown but >1 year	RET (not pathogenic)	Pathological fractures, uterus, ovary and spleen cysts. Nephrolithiasis. Coexistence of RET mutation of unknown pathogenicity
4	Long bones	Not present	Absent	Positive	2226	3.09	0.79	2 years	MEN1 (pathogenic)	Nephrolitiasis, microadenoma of pituitary gland, NET of pancreas

NID – no initial data.

Table 2. Results of the staining against parafibromin and selected clinical features of the patients.

Patient ID	Staining				Other data	
	Nuclear		Cytoplasmic	Control staining	Clinical data	Genetic data
	Proportion score	Intensity score	Cytoplasmic staining	Staining in other cells	Medical condition	CDC73 mutation
1	0	0	Yes	Present	PHPT and brown tumors	CDC73 germline
2	0	<1	No	Present	PHPT and brown tumors	CDC73 germline
3	0	0	No	ND	HPTJT	Absent
4	5	4	Yes	Present	PHPT and brown tumors	MEN1 germline

Proportion scoring was as follows: **0** – no cells are positive; **1** – ≤1% of cells are positive; **2** – 1-10% of are positive; **3** – 11-33% of cells are positive; **4** – 34-66% of cells are positive; **5** – 67-100% of cells are positive.
Intensity scoring was as follows: **0** – negative; **1** – weak; **2** – intermediate; **3** – strong.

literature [48]. In this patient, neither cytoplasmic nor parathyroid cell nuclear staining was detected, indicating a huge probability that no parafibromin molecules longer than 100 amino acids were present.

Patient 4 had positive staining for parafibromin in the probed parathyroid tissue. In 2 cases, the loss at least of 1 of the 3 attachment points to the cell nucleus and the binding site to the PAF1 polymerase complex was observed. The subsequent

Table 3. Results of genetic testing.

ID	Gene	Warrant	Localization	Amino acid change	Zygosity of warrant	Appearance in Europe	Mutation	Pathogenicity
1	CDC73/HRPT2	ENST00000367435 c.616delA	Ekson7_17	pThr206fs	Heterozygotic	No data	Germline	Pathogenic
2	CDC73/HRPT2	ENST00000367435 c.1240_1255del	Ekson_14/17	pPro414fs	Heterozygotic	No data	Germline	Pathogenic
3	–	–	–	–	–	–	No germline and no somatic mutation	–
4	MEN1	NM_130799 c791T>C	Ekson5_10	pLeu264Pro	Heterozygotic	No data	Germline	Potentially Pathogenic Clinically Pathogenic

staining demonstrated the absence of parafibromin in the cell nucleus and presence in the cytoplasm. This may indicate that shortening the molecule to 202 amino acids results in the loss of the nuclear export sequence necessary to translocate this protein into the nucleus. Loss of the final C fragment, but with the NLS left intact, allowed for its attachment to the DNA strand, but in this case (patient 2), the staining was very weak.

Failure to detect the *CDC73/HRPT2* mutation in 3 patients with the HPT-JT syndrome phenotype may have resulted from promoter mutations in the untranslated fragment, a deletion that was undetectable by PCR, inhibition of transcription by overexpression of some factors (WT1), or other reasons requiring further research (epigenetic dysregulations) [49].

The *CDC73* mutation is associated with the coexistence of cancers of the genitourinary organs and other malignant tumors; therefore, detecting new predictive factors can contribute to the prevention of tumor development [23,26,28].

In 1 patient, advanced bone changes and skeletal deformity with hyperparathyroidism and normal staining for parafibromin were detected. In this patient, mutation in the *MEN1* gene was confirmed, which can suggest a multifactorial mechanism of brown tumor development in patients with primary hyperparathyroidism.

Crosstalk between hyperparathyroidism, high expression of the *SHH* gene, and parafibromin dysfunction may explain the improvement of bone lesions after parathyroidectomy in patients with brown tumors [23,26,28].

Due to the suspected association between an increased risk of the *CDC73* mutation in patients with hyperparathyroidism and brown tumors, we recommend the exclusion of parathyroid biopsies from diagnostic procedures in such patients, because the

CDC73 mutation is a known risk factor of parathyroid carcinoma. We think that a biopsy in this group of patients can lead to the dissemination of parathyroid cells, likely causing parathyreomatosis.

Germinal mutation of *CDC73/HRPT2* is associated with the development of cysts (often), teratomas (rarely), and Wilms tumor (extremely rarely) in kidney, reproductive organ, lung, and intestinal tumors [25-28]. Abnormal *CDC73* expression in other tumors can cause them to be more aggressive [28]. The presence of focal lesions in patients with a congenital *CDC73* mutation or an occurrence of other congenital causes of disturbance of parafibromin expression requires oncological supervision.

It has been proposed that parafibromin is an important modulator and regulator of gene expression dependent on the Hh-Wnt-NOTCH pathways [40-42]. We think that the change in protein structure can lead to dysregulation of the Wnt and Hh pathways and, through this mechanism, promote carcinogenesis. Our team suspects that patients with brown tumors may have a higher risk of the mutation of *CDC73* and therefore may also have impairments in the regulation of the aforementioned pathways. In our patients with negative staining for parafibromin, we did not find other histological features of parathyroid carcinoma.

We hope our study can be a driver toward discussion and cooperation leading to studies in a larger groups of patients, and that these studies derive stronger statistically and clinically significant conclusions.

Conclusions

In patients with brown tumors, a genetic etiology of the disease should be considered.

The association of parafibromin expression changes with malignancies (parathyroid, genitourinary system, lung, skin, and pancreas) can change the approach to such patients, who should be diagnosed more broadly than patients with classic primary hyperparathyroidism.

We hope our case study opens the discussion on the significance of brown tumors as a warning against the potential risk of parathyroid cancer or other malignant comorbidities.

Further research of the topic is necessary with multi-source cooperation and data collection.

Nuclear export sequence location for parafibromin has been proposed.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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