

**NOETERIC ACUMEN INTO POLYCYTHEMIA, PARAGANGLIOMA, AND  
SOMATOSTATINOMA: PACAK ZHUNG SYNDROME**

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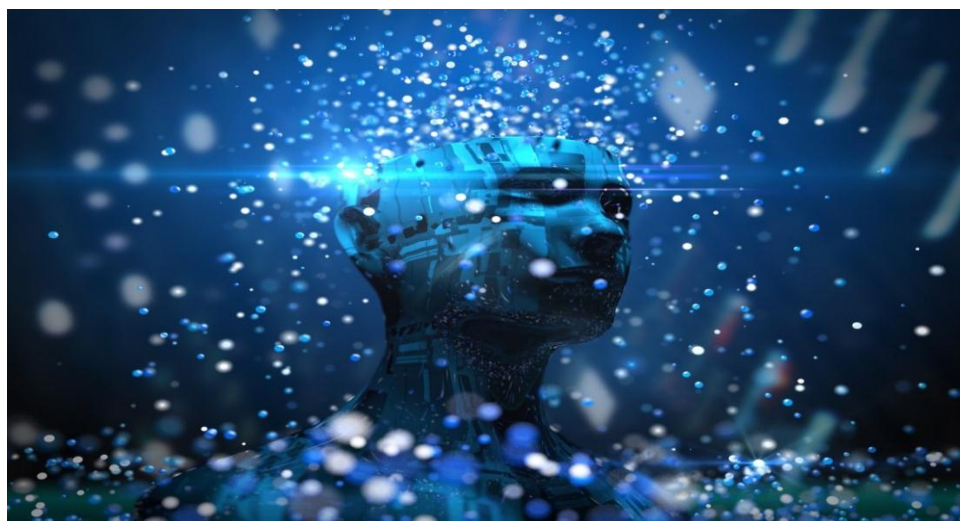
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**ABSTRACT**

A new syndrome of paraganglioma, somatostatinoma, and polycythemia has been discovered (known as Pacak–Zhuang syndrome). This new syndrome, with somatic HIF2A gain-of-function mutations, has never been reported in male patients. We describe a male patient with Pacak–Zhuang syndrome who carries a newly discovered HIF2A mutations. Somatic gain-of-function mutations in the gene encoding HIF-2a were reported in patients with paraganglioma and polycythemia and have been found exclusively in female patients. Through sequencing of the HIF2A using DNA from paraganglioma in 15-year-old male patient, we identified a novel mutation of HIF2A: a heterozygous C to A substitution at base 1589 in exon 12 of HIF2A. The mutation was not found in germline DNA from leukocytes. This study provides detailed information about the clinical aspects and course of 7 patients with this syndrome and brings into perspective these experiences with the pertinent literature. Six females and one male presented at a median age of 28 years (range 11–46). Two were found to have HIF2A somatic mosaicism. No relatives were affected. All patients were diagnosed with polycythemia before age 8 and before PGL/SOM developed. PGLs were found at a median age of 17 years (range 8–38) and SOMs at 29 years (range 22–38). PGLs were multiple, recurrent and metastatic in 100, 100 and 29% of all cases, and SOMs in 40, 40 and 60%, respectively. All patients had abnormal ophthalmologic findings and those with SOMs had gallbladder disease. Computed tomography (CT) and magnetic resonance imaging (MRI) revealed cystic lesions at multiple sites and hemangiomas in 4 patients (57%), previously thought to be pathognomonic for von Hippel-Lindau disease. The most accurate radiopharmaceutical to detect PGL appeared to be [18F]-fluorodihydroxyphenylalanine ([18F]-FDOPA). Therefore, [18F]-FDOPA PET/CT, not [68Ga]-(DOTA)-[Tyr3]-octreotate ([68Ga]-DOTATATE) PET/CT is recommended for tumor localization and aftercare in this syndrome. The long-term prognosis of the syndrome is unknown. However, to date no deaths occurred after 6 years of follow-up. Physicians should be aware of this unique syndrome and its diagnostic and therapeutic challenges.

**KEYWORDS:** Hypoxia-inducible factor, tumorigenesis, pheochromocytoma, paraganglioma, Pacak-Zhuang syndrome.



## BACKGROUND

A new syndrome involving somatic gain-of function mutations in the gene encoding hypoxia-inducible factor 2 $\alpha$  (HIF2A) was described by Pacak and Zhuang. There is increasing evidence of the role of hypoxia or pseudohypoxia in tumorigenesis, including pheochromocytoma (PHEO) and paraganglioma (PGL). (Pseudo)hypoxia leads to activation of hypoxia-inducible transcription factors (HIFs) and thus, promotes the transcription of hypoxia-responsive genes which are involved in tumorigenesis. Recently identified is a new syndrome consisting of multiple and recurrent PGLs or PHEOs, somatostatinoma, and congenital polycythemia, due to somatic hypoxia-inducible factor 2 $\alpha$  gene (HIF2A) mutations.

## INTRODUCTION

A new syndrome involving somatic gain-of function mutations in the gene encoding hypoxia-inducible factor 2 $\alpha$  (HIF2A) was described by Pacak and Zhuang. This syndrome, only presenting in females, is characterized by congenital or early onset polycythemia, multiple paragangliomas (PGLs)/pheochromocytomas (PHEOs), and duodenal somatostatinomas.<sup>[1,2]</sup> Pheochromocytomas (PHEOs) and paragangliomas (PGLs) are rare catecholamine-producing neuroendocrine tumors (NETs) arising in or outside the adrenal medulla. By definition, a PHEO is an intra-adrenal PGL. To date, it has been recognized that up to 35–40% of these tumors are hereditary, with about 19 causally linked mutated genes. Among these genes, much attention has been directed to those affecting hypoxia signaling pathways because many of the associated tumors express a so-called ‘pseudohypoxic signature’ and most of them converge on the hypoxia-signaling pathway. Highly conserved HIF proteins are composed of  $\alpha$  and  $\beta$  subunits. The HIF- $\beta$  subunit is constitutively expressed, whereas the  $\alpha$  subunits are hypoxia inducible and are associated with aggressive, treatment-refractory tumors. Under normoxic conditions, HIF-1 $\alpha$ , HIF-2 $\alpha$ , and HIF-3 $\alpha$  are hydroxylated primarily on specific prolyl residues, allowing for recognition by the von Hippel-Lindau (VHL) tumor-suppressor protein, ubiquitination, and rapid degradation through the proteasome.

Paraganglioma (PGL) and somatostatinoma are tumors arising from distinct types of neuroendocrine cells. PGLs arise from chromaffin or chromaffin-like cells that develop during embryogenesis from neural crest cells. As these neuroendocrine cells migrate, they populate the adrenal medulla and extra-adrenal paraganglia associated with paraxial sympathetic nerve fibers and branches of the vagus and glossopharyngeal nerves in the head and neck, including the carotid body. In contrast, somatostatinomas develop from enteric endocrine cells currently believed to arise from the endoderm. Despite their different origins, neuroendocrine cells of the paraganglia and GI tract share the ability to secrete specific peptides or amines, as do C cells of the thyroid

and neuroendocrine cells found in the lungs, pituitary gland, brain, and other tissues.

The occurrence of two distinct types of NETs in an individual patient is unusual, except in patients with hereditary syndromes such as von Hippel–Lindau (VHL) disease, neurofibromatosis 1 (NF1), mutations in the succinate dehydrogenase (SDH) subunits, and multiple endocrine neoplasia (MEN) types 1 and 2.

Under hypoxic conditions, prolyl hydroxylation of HIF- $\alpha$  proteins is reduced, resulting in their stabilization and, in turn, transcription of genes involved in the hypoxia response. HIF2A mutations disrupt the prolyl hydroxylation site of HIF-2 $\alpha$  and, as a result, inhibit its recognition by the VHL protein, leading to HIF-2 $\alpha$  stabilization.

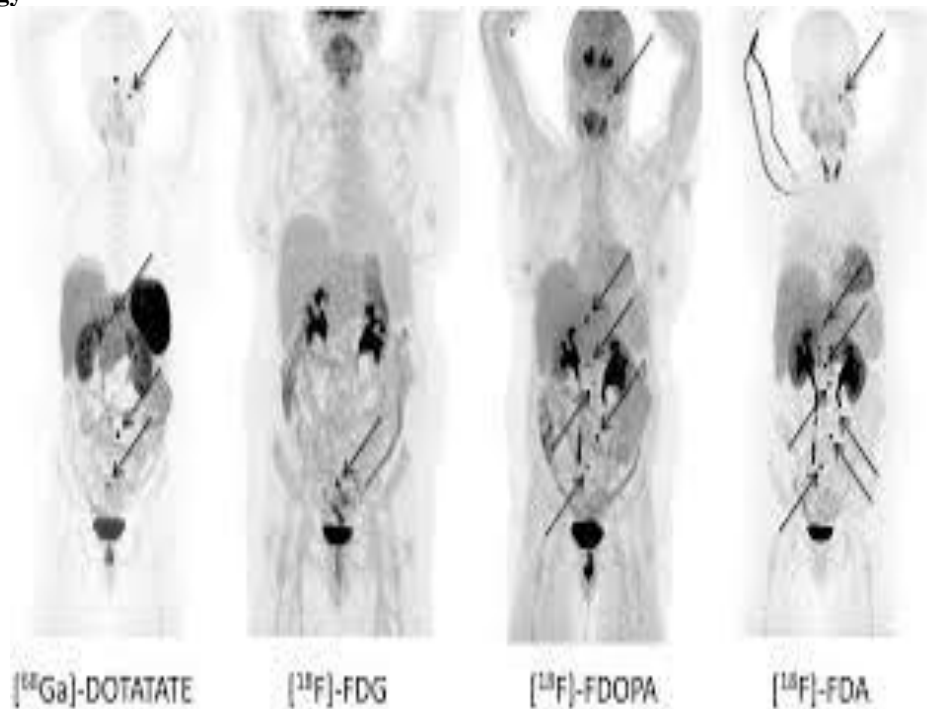
Thus, HIF-2 $\alpha$  mutations increase protein half-life and HIF-2 $\alpha$  activity. HIFs are transcription factors controlling energy, iron metabolism, erythropoiesis, and development. The occurrence of two distinct types of NETs in an individual patient is unusual, except in patients with hereditary syndromes such as von Hippel–Lindau (VHL) disease, neurofibromatosis 1 (NF1), mutations in the succinate dehydrogenase (SDH) subunits, and multiple endocrine neoplasia (MEN) types 1 and 2.4,5 In this study, we investigated the clinical and genetic characteristics of four female patients who presented to the National Institutes of Health and Tufts Medical Center with PGL, somatostatinoma, and polycythemia.

Congenital polycythemia is also associated with germline mutations in VHL and EGLN1. HIF2A germline mutations have also been previously reported in patients with familial polycythemia and in 1 patient presenting with multiple PGLs, all with mutations at hot spots in exon 12 of HIF2A. Nevertheless, the occurrence of PGL together with polycythemia is rare.

The clinical dyad/triad of PGLs and/or SOMs associated with polycythemia, also referred to as ‘Pacak–Zhuang syndrome’ may be regarded as a new tumor syndrome, similar to multiple endocrine neoplasia (MEN) syndromes, VHL disease, Carney–Stratakis syndrome, Carney triad, Cowden syndrome or the PHEO–PGL syndrome, among others.

From the first studies published by our group, additional new clinical phenotypes have been identified through the follow-up of previously described and newly diagnosed patients with this syndrome.

## A Very Rare Syndrome Till Now Seen In Females But First Time Occurred In Men Pathophysiology



HIF2A must occur in early life or during embryogenesis similar to the McCune Albright syndrome. It has been shown that HIF plays an important role in neural crest development and differentiation, and in the function of adrenal medulla and paraganglia. HIF-1 $\alpha$  is essential in the development of neural tube and cardiovascular system and high HIF-2 $\alpha$  expression was observed in developing paraganglia and HIF-2 $\alpha$  is necessary for catecholamine synthesis HIF-2 $\alpha$  is also considered the key regulator of erythropoiesis and this association has been demonstrated initially in four patients with polycythemia who were found to have activating germline HIF2A mutations. HIF-2 $\alpha$  stabilization and PGL-associated EPO production have been also found in patients with PHD2 and VHL mutations. HIF2A gain-of-function mutations in patients with the Pacak-Zhuang syndrome lead to reduced HIF-2 $\alpha$  hydroxylation and binding to the pVHL resulting in 4-6 times higher stability of mutant HIF-2 $\alpha$  compared to a wild-type. The clinical presentations of patients were consistent with HIF-2 $\alpha$  dysregulation. PGLs are found to have a typical noradrenergic biochemical phenotype, which reflects the involvement of HIF-2 $\alpha$  in the preferential norepinephrine synthesis. the strong positive immunohistochemical staining for HIF-2 $\alpha$  in patient's tumor tissues and increased tumor mRNA for HIF-2 $\alpha$  downstream genes indicate HIF2 $\alpha$  upregulation. degree of hypoxia and microenvironmental changes, including nutrition, may also play an important role. The HIF pathway has been found to be disrupted in some PHEOs and PGLs. depending on their genetic background and contributing to their development. PGL development and crosstalk within these pathways and HIF signaling pathway. Furthermore, it is of a great interest to find out whether

females found in Pacak-Zhuang syndrome are exclusively affected and if so, what pathogenic mechanism is involved in this sex selected process. In addition, erythropoietin is elevated in tumor tissue, however, it is not clear how this elevation occurs in early life and from which additional tissues, except tumor tissue, erythropoietin is derived from. Finally, it would be of interest to further study these patients whether they develop metastatic disease, other types of neuroendocrine and/or other abnormalities as the syndrome has been just discovered and more studies are needed to fully understand this disease.

In the Pacak-Zhuang syndrome, patients have somatic gain of function mutations in the genes encoding for HIF2A, leading to prolonged HIF-2 $\alpha$  activity and, thus, an increase in its half-life. While each patient has different nucleic acid changes, all patients are found to have a point mutation near the prolyl-sensing residue site, responsible for HIF-2 $\alpha$  hydroxylation.

### Risk Factors

- Elder age
- Malignant tumors present in the pancreas.

### Complications

1. Blood clots
2. Enlarged spleen (SPLEENOMEGALY)
3. Problems due to increase in increased levels of RBC.
4. Other blood disorders.
5. Mild diarrhoea
6. Steatorrhea
7. Loss of appetite
8. Jaundice

9. Fatigue
10. Gastrointestinal obstruction.

### Symptoms

- Anxiety
- Cholecystitis
- Diabetes
- Flushing
- Heart palpitations
- Hypertension
- Red cheeks
- Night sweats
- Fatigue
- Heat intolerance
- Nausea and vomiting

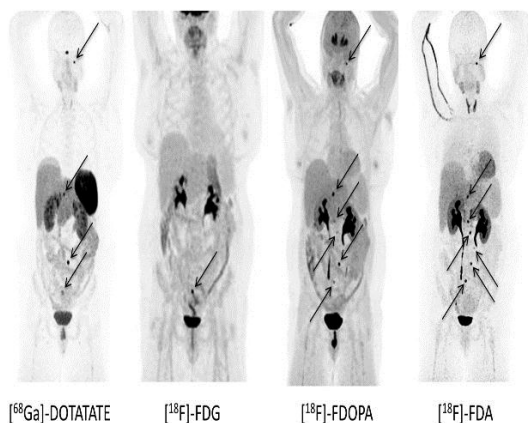
### Diagnosis

#### Laboratory Analyses

All laboratory analyses, mutation analysis, hydroxylation assays, real-time polymerase chain reaction, and chromatin immunoprecipitation.

#### Imaging Studies

Anatomical imaging using computed tomography (CT) and magnetic resonance imaging (MRI) of the neck, chest, abdomen, and pelvis along with positron emission tomography (PET)/CT studies using [18F]-fluorodeoxyglucose ([18F]-FDG), [18F]-fluorodopamine ([18F]-FDA), [18F]-fluorodihydroxyphenylalanine ([18F]-FDOPA), and [68Ga]-(DOTA)-[Tyr3]-octreotate ([68Ga]-DOTATATE) as radiopharmaceuticals. In addition, CT scans with negative enteric contrast were used to better detect tumors.



### Polycythemia

Patients with this syndrome usually have early onset of secondary polycythemia, most of them at birth. They are diagnosed with typical physical characteristics (red cheeks, flushing, swelling of the extremities), which are then confirmed through lab results that show abnormally increased erythropoietin levels.

### Tumors

Patients are initially suspicious for paragangliomas when they present with symptoms like hypertension, heart palpitations, headaches, and anxiety; somatostatinomas

usually induce diabetes and cholecystitis. From these symptoms, patients undergo biochemical testing from blood and urine samples as well as functional and anatomical imaging to confirm tumor presence.

### Other findings

Some patients with this syndrome have also been found to have ocular abnormalities, including bilateral fibrosis upon the optic disc and dilated capillaries.

### Treatment

The most common therapies for secondary polycythemia are phlebotomies and, for paraganglioma and/or somatostatinoma in this cohort of patients, surgery accompanied by antihypertensive medication.

### Conclusions and Future Therapeutic Options

The finding of the Pacak-Zhuang syndrome led to an important discovery of HIF-2 $\alpha$  mutation in PHEO/PGL. HIF-2 $\alpha$  signaling pathway appears to play one of the most important roles in PHEO/PGL pathogenesis and other cancers development and this designates HIF-2 $\alpha$  as an attractive and promising therapeutic target. The most promising therapeutic strategies are HIF pathway targeted therapies, especially on HIF-2 $\alpha$  inhibition. Currently, there are several agents affecting the HIF-1 $\alpha$  signaling [96]. Drugs selectively targeting HIF-2 $\alpha$  signaling have not been fully developed yet but are under investigation [99,100]. Moreover, it has been shown that HIF-1 $\alpha$  and HIF-2 $\alpha$  can activate target genes alternatively<sup>[26,56]</sup>, thus development of drugs targeting both HIF-1 $\alpha$  and HIF-2 $\alpha$  is of a great interest. Another therapeutic approach could be changing the balance of HIF $\alpha$  isoforms by modulating HIF signaling in tumors, since it was shown that HIF switches cells from HIF-1 $\alpha$  to HIF-2 $\alpha$  signaling.

Further investigations are needed to elucidate the other signaling pathways involved in PHEO/PGL development and crosstalk within these pathways and HIF signaling pathway. Finding novel diagnostic biomarkers associated with hypoxia and altered metabolic pathways should help to select patients who are likely to respond to a specific type of therapy (e.g. to the HIF signaling inhibitors) for personalized anti-cancer treatment. Based on this, multi-targeted therapeutic approaches, which should be more effective, can be used in PHEO/PGL treatment. Furthermore, it is of a great interest to find out whether females found in Pacak-Zhuang syndrome are exclusively affected and if so, what pathogenic mechanism is involved in this sex selected process. In addition, erythropoietin is elevated in tumor tissue, however, it is not clear how this elevation occurs in early life and from which additional tissues, except tumor tissue, erythropoietin is derived from. Finally, it would be of interest to further study these patients whether they develop metastatic disease, other types of neuroendocrine and/or other abnormalities as the syndrome has been just discovered and further more

studies are needed to know completely about this disease and clinical trial studies are still in process.

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