Polycythemia and Paraganglioma With a Novel Somatic HIF2A Mutation in a Male

Recently, 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 mutation. Congenital polycythemas have diverse etiologies, including germline mutations in the oxygen-sensing pathway. These include von Hippel–Lindau (Chuvash polycythemia), prolyl hydroxylase domain–containing protein-2, and hypoxia-inducible factor-2α (HIF-2α). Somatic gain-of-function mutations in the gene encoding HIF-2α 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. This mutation is undoubtedly associated with increased HIF-2α activity and increased protein half-life, because it affects the vicinity of the prolyl hydroxylase target residue, proline 531. To our knowledge, this is the first report describing Pacak–Zhuang syndrome with somatic gain-of-function mutation in HIF2A in a male patient. Congenital polycythemia of unknown origin should raise suspicion for the novel disorder Pacak–Zhuang syndrome, even in male patients. Pediatrics 2014;133:e1787–e1791
Polycythemia is characterized by a raised hemoglobin (Hgb) level and elevated red cell mass. It is commonly associated with variable serum erythropoietin (EPO) levels. Hypoxia is a crucial stimulus for EPO production. Studies of hypoxic regulation of EPO led to the discovery of the master hypoxia transcription factor, hypoxia-inducible factor (HIF).\(^1\) EPO synthesis is controlled by the HIF complex, dimers composed of hypoxia-inducible \(\alpha\) subunits (HIF-1\(\alpha\), HIF-2\(\alpha\), and HIF-3\(\alpha\)) and the constitutively expressed \(\beta\) subunit. Under normoxic conditions, HIF-\(\alpha\) subunits are hydroxylated on specific prolyl residues by prolyl hydroxylase domain (PHD) protein, allowing recognition by the von Hippel–Lindau (VHL) tumor suppressor protein, ubiquitination, and rapid degradation through the proteasome.\(^1\) Aberrant function of the oxygen-sensing pathway, which controls EPO production, appears to be a major cause of polycythemia, with mutations being described in VHL and PHD2.\(^2\) A recent study of the HIF2A in patients with polycythemia uncovered mutations located near the hydroxylacceptor Pro-531.\(^3\)

Paragangliomas are catecholamine-producing neuroendocrine tumors derived from chromaffin or chromaffinlike cells that develop during embryogenesis from neural crest cells.\(^1,4,5\) Mutations in SDHA, SDHB, SDHC, SDHD, SDHAF2, TMEM127, MAX, PHD2, and VHL have been implicated in the pathogenesis of these neuroendocrine tumors.\(^1,4,5\) These mutations indirectly lead to HIF stabilization and aberrant expression of hypoxia-related genes.

Recently, somatic gain-of-function mutations in HIF2A were reported in patients with multiple paragangliomas, pheochromocytomas, and somatostatinoma associated with polycythemia (Pacak–Zhuang syndrome).\(^4,7\) These mutations lead to significant stabilization of HIF-2\(\alpha\) and hence a gain-of-function phenotype, resulting in the upregulation of various HIF-2\(\alpha\)-related genes, including EPO.\(^5,7\)

Interestingly, it has been postulated that these somatic mutations, currently found only in female patients, probably occur during early (postzygotic) development in neural crest precursor cells that spread to different locations and eventually develop specific tumors. In the current report, the patient presented with paragangliomas and polycythemia associated with a novel somatic HIF2A mutation, a clinical situation that has not been previously described in male patients.

### CASE REPORT

A 15-year-old boy exhibited cyanosis since infancy and subsequently received a diagnosis of polycythemia based on an erythrocyte count of 7.64 \(3\times 10^{12}/L\), Hgb level of 23.1 g/dL, hematocrit of 64.3\%, and EPO of 60.3 mU/mL (upper reference limit [URL], 32.8 mU/mL). He had been treated with infrequent phlebotomies. There was no family history of neuroendocrine tumor or polycythemia. At age 15, he started to experience headaches, palpitations, diaphoresis, night sweats, fatigue, heat intolerance, nausea, and vomiting. He was found to have blood pressure of 180/120 mm Hg. Plasma norepinephrine, epinephrine, and dopamine levels were 50 457 pg/mL (URL <450), 104 pg/mL (URL <100), and 117 pg/mL (URL <20), respectively. Multiple paraaortic masses were discovered by computed tomography (CT) scan and were substantiated by \(^{123}\)I-metaiodobenzylguanidine scintigraphy (Fig 1). Three solid, encapsulated, red-brown tumors were resected. Histopathologic examination confirmed the diagnosis of multiple paragangliomas (Fig 2). The masses were composed of large spindle cells and round cells with prominent nuclei and granular cytoplasm (Fig 2A). The tumor cells were arranged in nests or showed a pattern of diffuse growth and appeared to be embedded in a richly vascularized stroma (Fig 2A). In addition, immunohistochemical analysis showed positive staining for chromogranin A and synaptophysin (Fig 2 B and C). After surgery, plasma level of norepinephrine decreased to 1055 pg/mL, and EPO level remained slightly elevated (48.6 mU/mL),

![FIGURE 1](https://example.com/figure1.png)
with the presence of polycythemia (Hgb, 15.9 g/dL; hematocrit, 52%). No tumor was detected on CT scan or by 131I-MIBG scintigraphy. His blood pressure is currently within normal range without medications. He has been in good condition for the last 21 months after surgery.

To identify the gene responsible for paraganglioma and polycythemia, we sequenced both germline DNA from leukocytes and DNA from the tumors. Figure 3A shows genomic DNA sequencing of nucleotides 1581 to 1597 in exon 12 of HIF2A. All 3 tumors were found to have a heterozygous C to A substitution at base 1589 in exon 12 of HIF2A (Fig 3A). A similar mutation was not found in germline DNA from leukocytes (Fig 3A). The C1589A mutations resulted in substitution of alanine 530 in the HIF-2α protein with glutamic acid. To explore the effect of the mutation in terms of HIF-2α protein stabilization, HIF-2α levels in tumors were examined. Immunohistochemical analysis by anti-HIF-2α antibody (Novus Biologicals, Littleton, CO) showed positive staining for HIF-2α (Fig 3B). Western blotting analysis by anti–HIF-2α antibody (Abcam, Cambridge, MA) revealed that the protein level of HIF-2α was higher in tumor than the patient’s peripheral mononuclear cells as internal control (Fig 3C). Other genes of the HIF pathway (PHD2, VHL, SDHB, and SDHD) and those causing polycythemia by different mechanisms (JAK2, EPOR) had no mutations.

**DISCUSSION**

We report a novel somatic HIF2A Ala530Glu missense mutation in a patient with Pacak–Zhuang syndrome. Whether the novel Ala530Glu mutation leads to HIF-2α stabilization must be confirmed. PHD hydroxylates specific proline in HIF-α subunits in the context of a strongly conserved LXXLAP sequence motif (where X indicates any amino acid and P indicates the hydroxylacceptor proline). This site-specific proline hydroxylation allows recognition by the VHL protein, a component of an E3 ubiquitin ligase complex that targets hydroxylated HIF-α for degradation by ubiquitin–proteasome pathway. Mutation of Pro531 has been

**FIGURE 2**

Histopathologic findings in samples obtained from the patient. A, Microscopic image of the tumor (hematoxylin and eosin) reveals typical patterns of a paraganglioma with an organoid arrangement (Zellballen) of tumor cells with pink granular cytoplasm and with nuclear pleomorphism and vascular invasion. B, Immunocytochemical image showing cytoplasmic expression of chromogranin A by tumor cells. C, Immunocytochemical image showing synaptophysin by tumor cells.
previously shown to result in aberrant stabilization of HIF-2α and thus upregulate genes downstream of HIF transcription.8 Ala530 is located in the vicinity of the primary hydroxylation site of the HIF-2α. Both Ala530Thr and Ala530Val mutant peptides affect prolyl hydroxylation and VHL protein binding, resulting in reduced HIF-2α degradation but intact transcriptional activity and activation of genes downstream of HIF-2.7 Therefore, Ala530Glu mutation is also likely to affect the conformation of the HIF-2α hydroxylation domain and in turn the stabilization of HIF-2α.

Interestingly, in the present patient, the tumor resections decreased plasma level of norepinephrine, whereas the EPO level (48.6–58.2 mU/mL) remained slightly elevated, with the presence of polycythemia. These observations suggest that EPO production not only may be related to the presence of paragangliomas but also can be derived from other neural crest cells. Those neural crest cells could also harbor HIF2A gain-of-function mutations, because a postzygotic event could have generated a somatic HIF2A mutation in multiple cells within the same tissues. Furthermore, although the most recent CT scan performed after surgery did not show any remaining or relapsed tumors, this finding does not exclude the presence of small residual paragangliomas. The patients described by Pacak et al6 had enlarging multiple tumors after surgery and developed not only paragangliomas but also somatostatinomas. Four patients presented with pheochromocytomas and paragangliomas associated with polycythemia.9,10 In the present case, after surgery, the patient had a persistent although much lower elevation of plasma norepinephrine (904–1055 pg/mL). Therefore, he should be screened for the presence of somatostatinomas and pheochromocytomas, as well as residual and recurrent paragangliomas. Furthermore, it would be important to investigate whether other sources and mechanisms of EPO production are also involved.

HIFs have been found to promote key steps in tumorigenesis, including angiogenesis,
metabolism, proliferation, metastasis, and differentiation.\textsuperscript{1,5,6} The pheochromocytomas and paragangliomas of the first mentioned cluster are characterized by a pseudohypoxic phenotype such as inappropriate stabilization of HIF-\(\alpha\) subunits under normoxia.\textsuperscript{1,5,6}

Under normoxia, hydroxylation of HIF-\(\alpha\) by PHD designates them for VHL-dependent ubiquitylation and subsequent degradation.\textsuperscript{1} Accordingly, VHL mutations can promote HIF-\(\alpha\) stabilization and induce pseudohypoxia.\textsuperscript{1,5} Similarly, succinate dehydrogenase dysfunction causes HIF stabilization by succinate or reactive oxygen species accumulation-mediated PHD inhibition.\textsuperscript{1,5} In aggregate, these studies have shown changes in the regulation of, but not mutations in, HIF-\(\alpha\) subunits in various tumors. Additional studies are needed to elucidate how the mutations in HIF-2\(\alpha\) contribute to tumorigenesis.

Whether Pacak–Zhuang syndrome exists only in female patients is unclear. Several unique female-related molecular mechanisms, such as hormone and gender-dependent copy number variations, may contribute to various HIF-2\(\alpha\) signaling and tumorigenesis in the particular tumors associated with polycythemia preferentially in female patients.\textsuperscript{10}

Studying a larger number of patients with polycythemia or paragangliomas who carry HIF2A mutations could help to solve this new phenotypic puzzle. Our report highlights the diagnostic and management challenges in patients with Pacak–Zhuang syndrome. The appearance of abdominal tumors in patients with preexisting polycythemia should raise suspicion of this new syndrome, and those presenting with paragangliomas associated with polycythemia should be screened for HIF2A mutations, even in male patients.

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