

Paraganglioma of the organ of Zuckerkandl associated with a somatic *HIF2 α* mutation: A case report

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Abstract. Paragangliomas of the organ of Zuckerkandl (OZ-PGL) are rare tumors that, in >70% of cases, occur in association with succinate dehydrogenase complex iron sulfur subunit B (*SDHB*) or *SDHD* gene mutations. The aim of the current study was to determine whether a somatic genetic defect in the hypoxia-inducible factor 2 α (*HIF2 α*) gene was present in a case of sporadic OZ-PGL. A 32-year-old African female presented with uncontrolled hypertension during the first trimester of pregnancy. A diagnostic hysteroscopy was performed 3 months after delivery, precipitating a hypertensive crisis. Thereafter, the patient was diagnosed with noradrenaline-secreting OZ-PGL. A complete blood count identified mild normocytic anemia of an inflammatory origin. Surgical removal of the tumor resulted in normalization of plasma and urinary normetanephrine levels. Genetic testing for germline mutations (including large deletions) in the von Hippel-Lindau tumor suppressor, *SDHB*, *SDHC* and *SDHD*

genes was normal. However, a heterozygous missense mutation (c.1589Cys>Tyr) was detected in exon 12 of *HIF2 α* , which results in a substitution of alanine 530 with valine (Ala530Val) in the *HIF2 α* protein. A germline mutation was excluded based on the negative results of blood DNA testing. A three-dimensional homology model of Ala530Val was constructed, which showed impaired *HIF2 α* /VHL interaction and decreased *HIF2 α* ubiquitination. ¹H-high-resolution magic-angle-spinning nuclear magnetic resonance spectroscopy detected low succinate levels and high α and β glucose levels. To the best of our knowledge, the present case represents the first of its kind to associate a somatic *HIF2 α* gain-of-function mutation with OZ-PGL. It is therefore recommended that patients without germline *SDHx* mutations should be tested for *HIF2 α* mutations.

Introduction

In 1901, Emile Zuckerkandl first described the abdominal para-aortic paraganglia in fetal and newborn humans as a paired retroperitoneal organ located laterally to the abdominal aorta at the level of the inferior mesenteric aorta (1). This paraganglionic complex, known as the organ of Zuckerkandl (OZ), also includes smaller accessory paraganglia located anteriorly to the aorta between the lateral organs or below the aortic bifurcation (2). In 1903, Alfred Kohn established that the OZ commonly originated from chromaffin cells of the adrenal medulla (3), and it has later been established that it constitutes the largest accumulation of extrarenal chromaffin cells in mammals. In humans, the OZ reaches its maximal size at

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the age of ~ 3 years and subsequently regresses after reaching its peak by autophagy (4). The OZ is considered to be most important physiologically throughout the early gestational period, during which it secretes catecholamines into the fetal circulation, functioning as a homeostatic regulator of blood pressure (5). The OZ represents a site of origin for paragangliomas (PGLs) that preferentially secrete norepinephrine and induce symptoms of catecholamine excess (6). OZ-PGLs are rare tumors typically located close to the origin of the inferior mesenteric artery or between the proximal common iliac arteries (1). These lesions may occur sporadically or, in $\sim 70\%$ of cases, in association with succinate dehydrogenase complex iron sulfur subunit B (*SDHB*) or, less commonly, *SDHD* gene mutations (7). In addition, OZ-PGLs are particularly aggressive with high rates of metastatic spread (8). At least 150 cases of OZ-PGLs have been reported in the literature. They are strongly associated with an aggressive behavior, likely associated with the *SDHB* mutation status (7). Due to the rarity of this disease, not much is known about its natural history. A single-center retrospective study of 371 patients with either pheochromocytoma or sympathetic paraganglioma revealed only 21 cases of OZ-PGLs, 14 of which (66%) had metastases at diagnosis (9). To the best of our knowledge, the current case demonstrates that somatic *HIF2 α* [also known as endothelial PAS domain-containing protein 1 (*EPAS1*)] mutations may be associated with OZ-PGL for the first time.

Case report

In September 2014, a 32-year-old African woman native to Burkina Faso was referred to the hypertension unit of La Timone University Hospital (Marseille, France) for screening for secondary hypertension. Hypertension was initially noted during the first trimester of pregnancy. The patient went into premature labor at 22 weeks and a cesarean delivery was performed 15 days later; the baby did not survive and succumbed a few minutes after birth. Following delivery, the patient experienced persistent and uncontrolled hypertension despite taking nicardipine (60 mg/day) and labetalol (400 mg/day) for 3 months. A diagnostic hysteroscopy was performed 3 months later, precipitating a hypertensive crisis [systolic blood pressure (BP), 300 mmHg; normal, <140 mmHg]. Thereafter, the patient was referred to the hypertension unit of La Timone University Hospital for secondary hypertension screening in September 2014. There was no known family history of tumors, syncope or sudden death. At admission (weight, 51 kg; height, 163 cm; and body mass index, 19.2), the patient presented with headaches, recurring episodes of palpitations and sweating, chest tightness, and polyuria. Treatment with nicardipine and labetalol was replaced with verapamil (240 mg/day). Ambulatory 24-h BP monitoring was performed during treatment with verapamil and demonstrated that the patient maintained a BP of 155/96 mmHg. Prazosin (2.5 mg once per day) was subsequently administered to reduce blood pressure further until surgical intervention.

Additional laboratory tests identified highly elevated 24 h urinary normetanephrine levels [20,140 nmol/24 h; upper reference limit (URL), <1900 nmol/24 h] and normal metanephrine levels (380 nmol/24 h; URL, <1600 nmol/24 h). In addition, serum chromogranin A was observed to be elevated

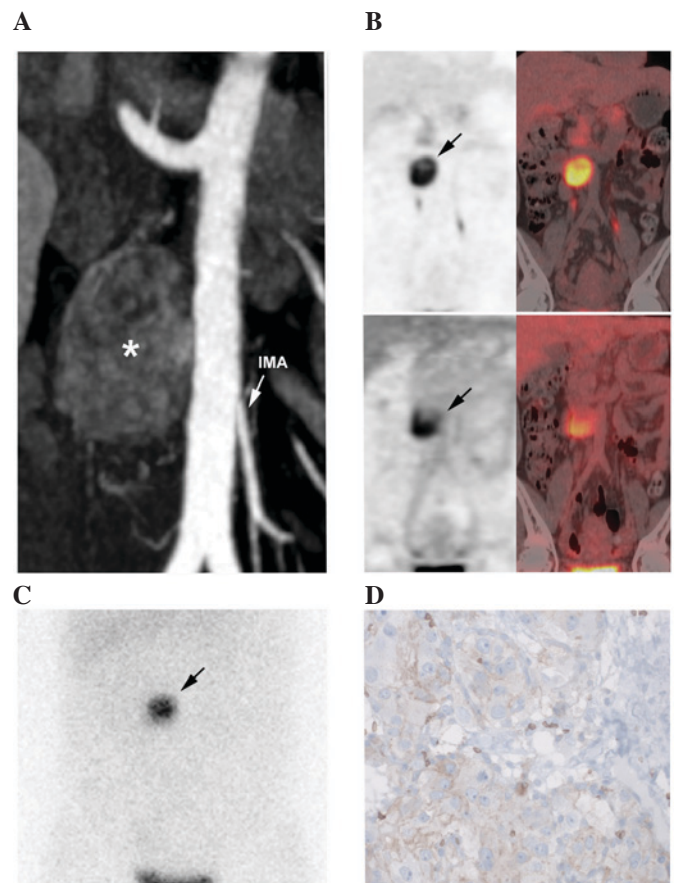


Figure 1. Imaging and pathological features of the OZ-PGL. (A) Contrast-enhanced CT (arterial phase) showing a 40-mm hypervascular and heterogeneous left para-aortic mass located at the level of the IMA (asterisk). (B) ^{18}F -FDOPA (upper image) and ^{18}F -FDG PET/CT (lower image) imaging showing a single tumor. (C) Iodine-123-metaiodobenzylguanidine scintigraphy also positively located the mass (planar anterior view). (D) Immunohistochemical analysis of the tumor demonstrated positive glucose transporter-1 immunostaining ($\sim 10\%$). CT, computed tomography; IMA, inferior mesenteric artery; ^{18}F -FDOPA, ^{18}F fluorine-L-dihydroxyphenylalanine; ^{18}F -FDG, ^{18}F -fluorodeoxyglucose; PET, positron emission tomography.

(223 $\mu\text{g/l}$; URL, <100 $\mu\text{g/l}$). A complete blood count revealed mild normocytic anemia (hemoglobin count, 108.0 g/l; normal hemoglobin count for female adults, 11.5-15.0 g/dl; mean corpuscular volume, 83.4 fl; normal mean corpuscular volume of adults, 80-100 fl) of an inflammatory origin with normal platelets and leukocytes. Diagnostic computed tomography (CT) revealed a 40-mm hypervascular, heterogeneous, left para-aortic mass located at the level of the inferior mesenteric artery (Fig. 1A). ^{18}F Fluorine-L-dihydroxyphenylalanine positron emission tomography/CT (Fig. 1B) and iodine-123-metaiodobenzylguanidine scintigraphy (Fig. 1C) confirmed the diagnosis of OZ-PGL without multifocal disease. The tumor also exhibited moderate heterogeneous ^{18}F -fluorodeoxyglucose uptake (Fig. 1B). In October 2014, complete surgical resection was performed. Histopathological analysis of the tumor tissue revealed typical PGL features, including a low Ki-67 index ($<1\%$) (monoclonal mouse antibody; clone, MIB-1; catalogue no., M7240; dilution, 1:100; Dako, Glostrup, Denmark). Genetic testing for germline mutations (including large deletions) in the von Hippel-Lindau tumor suppressor (*VHL*), succinate dehydrogenase complex iron sulfur subunit B (*SDHB*), *SDHC*

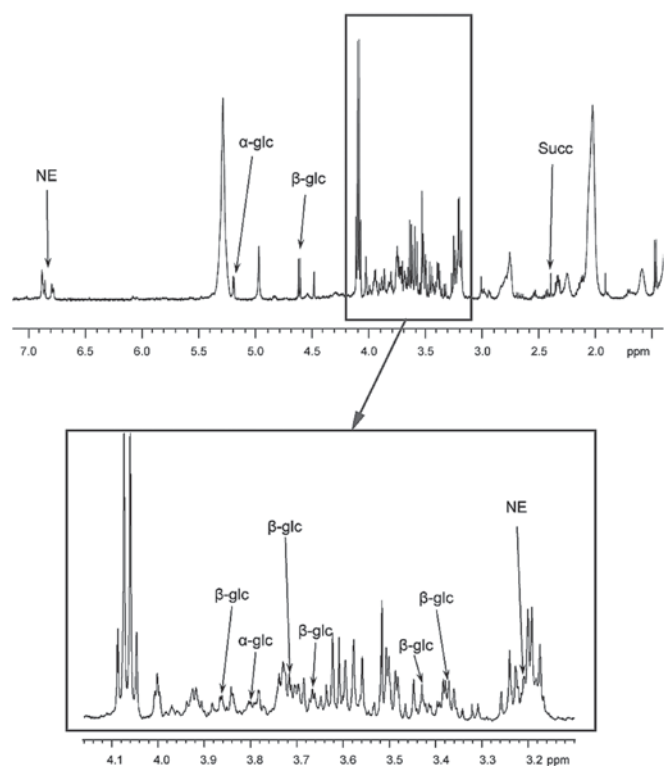


Figure 2. Results of HRMAS NMR spectroscopy (500 MHz) performed on tumor samples from the organ of Zuckerkindl paraganglioma. Partial metabolite assignment is indicated. The metabolic content may be directly compared as the spectrum intensity was normalized with respect to the weight of each examined sample. For display purposes, the amplitude of the lactate peak at 4.09 ppm has been cut out. The top image shows a representative spectrum with highly elevated levels of α -glc and β -glc. Spectrum regions ranging from 3.10-4.15 ppm are magnified in the lower box. Amongst catecholamines, only an NE signal was detected in all the examined tissue samples. Finally, the level of Succ was low, which confirmed the absence of a succinate dehydrogenase complex deficiency. HRMAS, ^1H -high-resolution magic-angle-spinning; NMR, nuclear magnetic resonance; α -glc, α -glucose; β -glc, β -glucose; NE, norepinephrine; Succ, succinate.

and *SDHD* genes was normal. Immunostaining demonstrated that the tumor cells were positive for SDHB. Further genetic testing revealed a heterozygous cysteine to tyrosine substitution at base 1589 (c.1589Cys>Tyr) in the *HIF2 α* coding sequence of the OZ-PGL, resulting in the replacement of alanine with valine at amino acid position 530 (Ala530Val). This leads to HIF2 α stabilization as described by a previous *in vitro* experiment (10). A germline *HIF2 α* mutation was excluded based on the negative results of blood DNA testing.

In order to assess the metabolic properties of the tumor, the present study performed ^1H -high-resolution magic-angle-spinning (HRMAS) nuclear magnetic resonance spectroscopy-based global metabolomic profiling on tumor samples. A one-dimensional proton spectrum (1.5-7.2 ppm) using a Carr-Purcell-Meiboom-Gill pulse sequence with water presaturation was acquired from each intact tissue sample. Low levels of succinate were detected, and according to our previous study (11) this excludes a SDH deficiency. Notably, the tumor also exhibited abnormally high levels of α - and β -glucose isomers as identified by HRMAS (Fig. 2). The patient is currently in remission, and regular clinical follow up occurred every 6 months with normal metanephrines. Conventional radiological imaging (MRI) and functional

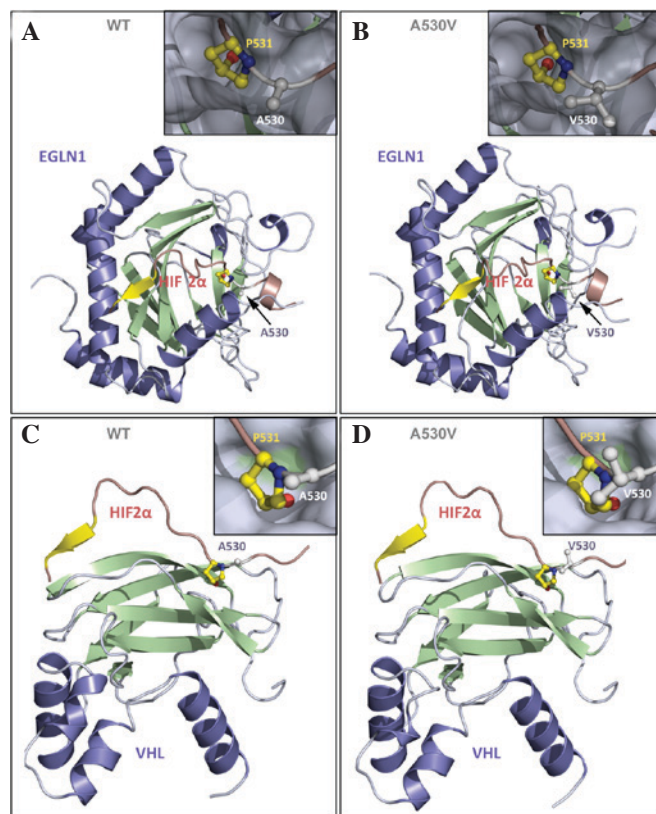


Figure 3. Representation of human HIF2 α in the presence of its binding partners EGLN1 and VHL. (A) WT HIF2 α (A560) interacting with EGLN1; (B) mutant Val560 interacting with EGLN1; (C) WT HIF2 α interacting with VHL; and (D) mutant Val560 interacting with VHL. HIF2 α (16 residues) is represented in red and yellow, and the interactive partners (EGLN1 or VHL) are represented in blue and green. (B and D) The inserts present a closer view of Pro531 and Ala530 (or Val530) from HIF2 α in the ball-and-stick representation to show the atomic details, while HIF-2 α partners, (A and B) EGLN1 or (C and D) VHL are shown as a grey surface showing that residues 530 and 531 bind to small pockets at the surface of the protein partner. Residue Ala530 is located in close proximity to residue Pro531, which is hydroxylated by EGLN1 and at the interface with the binding partners EGLN1 and VHL. Hydroxylation of Pro531 is required for interaction with VHL. It is anticipated that valine, which is a larger residue than alanine, increases steric hindrance at Pro531, resulting in a reduction in its accessibility to EGLN1 by inhibition of Pro531 hydroxylation; therefore, interaction with VHL and subsequent ubiquitination is prevented. Panel D is presented as a model, but VHL interaction should not occur in the Val530 mutant. HIF2 α , hypoxia inducible factor 2 α ; EGLN1, Egl-9 family hypoxia-inducible factor 1; VHL, von Hippel-Lindau tumor suppressor; WT, wild-type.

imaging (PET-FDOPA) at 1 year post-intervention were also normal.

Written informed consent was obtained from the patient for publication of the present case report and any accompanying images.

Discussion

To the best of our knowledge, the present case demonstrates, for the first time, that patients with somatic *HIF2 α* mutations may present with OZ-PGL.

Germline mutations in the *HIF2 α /EPAS1* gene have been previously associated with congenital polycythemia (12). A syndromic association has been reported between somatic gain-of-function mutations in *HIF2 α* and congenital

polycythemia, multiple PGL, duodenal somatostatinoma and ocular vascular abnormalities (for example, Pacak-Zhuang syndrome) (10,13-16). Mutations in *HIF2 α* have also been observed in apparently sporadic pheochromocytomas (PHEOs)/PGLs without polycythemia (17-19). In one study, mutations (exon 12) were identified in 2 cases of solitary PHEO and 1 para-adrenal PGL (18). In an additional study, 6/42 cases of apparently sporadic PHEOs were identified to have *HIF2 α* mutations (3 in exon 9 and 3 in exon 12) (17). *HIF2 α* protein stability is dependent on the hydroxylation of two specific proline residues (Pro405 and Pro531) located in the O₂-dependent degradation domain (10). Until present, all mutations described were known to be located in hot spots adjacent to hydroxylation sites (16). These specific mutations disturb *HIF2 α* prolyl hydroxylation and subsequent recognition by the VHL protein, resulting in the failure of *HIF2 α* degradation via ubiquitination (16). As mutant *HIF2 α* protein has a longer half-life compared with the wild-type protein, it has a targeted effect downstream of *HIF2 α* (10).

The mutation identified in the present study had previously been reported in a case of apparently sporadic PHEO/PGL (18). The mutation involved Ala530, which is located in close proximity to the second hydroxylation site (Pro531) and at the interface with VHL and Egl-9 family hypoxia-inducible factor 1 (EGLN1) client-proteins. Homology modeling was performed to outline the biological properties of the Val530 mutant (Fig. 3). These three-dimensional models were generated with IBM SPSS Modeler v14 (IBM SPSS, Armonk, NY, USA) using the crystal structures of *HIF1 α* in interaction with EGLN1 or VHL as templates. *HIF1 α* and *HIF2 α* exhibit a sequence identity of 65% in the region modeled, which guarantees (>50% identity) that the models are of a high quality. The model anticipates that valine, a larger residue than alanine, increases steric hindrance at Pro531, leading to: i) A reduction in its accessibility to EGLN1 by inhibition of Pro531 hydroxylation; and ii) impairment of *HIF2 α* /VHL interaction with decreased *HIF2 α* ubiquitination. The present study also identified a novel metabolomic pattern with low succinate and high glucose levels associated with *HIF2 α* mutation. Abnormally high levels of glucose may be explained by increased glucose uptake induced by *HIF2 α* stabilization (20).

In conclusion, to the best of our knowledge, the current study identified, for the first time, an association between somatic *HIF2 α* mutations and OZ-PGL. It is therefore recommended that patients with OZ-PGL in the absence of germline *SDHx* mutations should undergo testing for *HIF2 α* mutations.

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