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Novel insights into the polycythemia-paraganglioma-somatostatinoma syndrome

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Author contributions

RD, JN, and KP designed the study with JDR, IJ, MM, IJ, KTA, AL, CCC, EC, CY, EK, ZZ. KP, MT, BB, JTP, RML, AST, VP, DM, FRP, MRG, MF, NN, DT, CAS, TF were involved in data collection, analysis, interpretation, and review, in the preparation of the manuscript, as well as subsequent revisions.

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Abstract

The syndrome of paraganglioma (PGL), somatostatinoma (SOM), and early childhood polycythemia in patients with somatic mutations in the hypoxia-inducible factor 2 alpha (*HIF2A*) gene is described in only a few patients worldwide. The present study provides detailed information about the clinical aspects and course of 7 patients with this syndrome and brings these experiences into perspective 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 secondary 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 PGLs were primarily norepinephrine producing. All patients had abnormal ophthalmologic findings and those with SOMs had gallbladder disease. Computed tomography (CT) and magnetic resonance imaging 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 follow-up. Physicians should be aware of this unique syndrome and its diagnostic and therapeutic challenges.

Keywords

Pheochromocytoma; paraganglioma; somatostatinoma; polycythemia; *HIF2A* mutation

Introduction

Pheochromocytomas (PHEOs) and paragangliomas (PGLs) are rare catecholamine-producing neuroendocrine tumors (NETs) arising in or outside the adrenal medulla, respectively (Lenders *et al*. 2005). By definition, a PHEO is an intraadrenal PGL. To date, it has been recognized that up to 35%–40% of these tumors are hereditary, with about 19 causally linked mutated genes (Crona *et al*. 2013; Pacak and Wimalawansa 2015). 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 (Jochmanova *et al*. 2013).

Germline mutations in the von Hippel-Lindau (VHL) gene trigger overexpression of hypoxia inducible factor (HIF) proteins and cause VHL disease, which may predispose to various tumors such as multiple PHEOs/PGLs, hemangioblastomas of the retina and central nervous system, as well as kidney cysts, renal cell carcinoma, and polycythemia, among others (Haase 2009; Taïeb *et al*. 2016). Mutations in another HIF-regulating protein gene, *prolyl hydroxylase domain protein 2* (PHD2) and, more recently, PHD1, which hydroxylate HIF and enable its VHL-mediated degradation, have been associated with secondary polycythemia and multiple PGLs (Yang *et al*. 2014). Nevertheless, the occurrence of PGL together with polycythemia is rare (Dionne *et al*. 2006).

Somatic mutations in *HIF2A* (*EPAS1*), affecting PHD hydroxylation and subsequent VHL degradation, were recently recognized to cause a syndrome consisting of PGL and/or somatostatinoma (SOM) associated with polycythemia in females (Pacak *et al*. 2013). Since then, more patients with the syndrome have been described carrying the mutations in this gene. Nevertheless, at present, the triad of PGL, SOM, and polycythemia has been exclusively found in females. Furthermore, normal tissues genomic DNA mosaicism of *HIF2A* mutations has recently been detected in two out of four initial patients specifically presenting with this syndrome (Yang *et al*. 2015).

The clinical dyad/triad of PGLs and/or SOMs associated with polycythemia, also referred to as “Pacak-Zhuang syndrome” (Toyoda *et al*. 2014), 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 (Gaal and de Krijger 2010; Ni *et al*. 2012). 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.

In the present study, we provide detailed clinical information on 7 patients with the PGL, SOM, and polycythemia syndrome carrying the somatic *HIF2A* mutation, including a teenage boy, diagnosed and followed at the NIH for 6 years. New clinical information particularly pertains to aspects of genetics, tumor imaging, organ involvement, disease progression, and patient outcomes. We also searched the literature for studies and reports on related tumor syndromes and diseases with activating mutations of the *HIF2A* gene and discuss our findings in the context of this other data. As a result, we propose strategies for diagnosis and therapy of patients with PGL, SOM, and polycythemia syndrome.

Materials and Methods

Patient Evaluation

Patients were evaluated under protocol (00-CH-0093) approved by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Institutional Review Board. All patients provided written informed consent. We carefully reviewed all patients' demographics, clinical manifestations, biochemical and hematological profiles, radiographic findings, and outcomes based on frequent follow-ups and very close interactions with outside physicians (Supplementary Table 1).

Laboratory Analyses

All laboratory analyses, mutation analysis, hydroxylation assays, real-time polymerase chain reaction, and chromatin immunoprecipitation were performed, as previously described (Pacak *et al.* 2013).

Imaging Studies

Anatomical imaging using computed tomography (CT) and magnetic resonance imaging (MRI) of the neck, chest, abdomen, and pelvis were performed as previously described, along with positron emission tomography (PET)/CT studies using [^{18}F]-fluorodeoxyglucose ([^{18}F]-FDG), [^{18}F]-fluorodopamine ([^{18}F]-FDA), [^{18}F]-fluorodihydroxyphenylalanine ([^{18}F]-FDOPA), and [^{68}Ga]-(DOTA)-[Tyr3]-octreotate ([^{68}Ga]-DOTATATE) as radiopharmaceuticals (Supplementary Table 2) (Janssen *et al.* 2015). In addition, CT scans with negative enteric contrast were used to better detect duodenal tumors, as performed in our previous studies (Pacak *et al.* 2013).

Results

Gender, mutation status with evidence of mosaicism of *HIF2A*, ages at the latest outpatient visit, and ages at diagnosis of polycythemia, occurrence of PHEO/PGL, SOM and number of lesions, recurrence of lesions, as well as surgical and other treatment modalities for individual patients in chronological order are shown in Fig. 1. None of the family members had any history of polycythemia, PHEO/PGL, or SOM. Detailed clinical presentations are described in Supplementary Table 1. Patients received regular follow-up at the NIH for a median of 6 years (range 1–11) since their first tumor resection. The median age of patients at the time of their last follow-up at the NIH was 28 years (range 11–46).

Polycythemia was detected in patients in early childhood (median 2 years, range from birth to 7 years). Erythropoietin (EPO) levels were an average of 5 times above the upper limit of normal in all patients. For patient number (No.) 3, EPO levels are plotted together with levels of hemoglobin and hematocrit over the course of the patient's visits at the NIH (Fig. 2). There was no long-term normalization of EPO levels even after surgery in any patient.

PGL developed later in life at a median age of 17 years (range 8–38) in all patients. PGLs were predominantly of the norepinephrine-producing biochemical phenotype. After surgery, levels of normetanephrine dropped significantly (not shown). Patients were found to have multiple (100%), recurrent (100%), and metastatic (29%) PGLs, with adrenal involvement in four cases.

Five of 7 patients had a manifestation of SOM at a median of 29 years (range 22–38). No patient was found to have SOM diagnosed before the occurrence of PGL. Patient No. 5, an 11-year-old girl, and No. 7, a 17-year-old-boy have not yet developed SOM. SOM was confirmed either histologically and/or biochemically. In the case of patient No. 4, the diagnosis of SOM was based solely on elevated levels of somatostatin along with retroperitoneal abdominal nodules on imaging studies, but without histological confirmation of the diagnosis to date since the patient has refused to have an operation. For patient No. 6, we found elevated levels of gastrin that remained high after surgery, as well as slightly elevated levels in vasoactive intestinal peptide for patient No. 3.

In four patients, we had histological proof of SOM. We found solitary (40%), multiple (40%), recurrent (40%), and metastatic SOMs in 3 cases (60%). All patients with SOM were diagnosed with gallbladder disease, with four having chronic cholecystitis and two, cholelithiasis, usually in early adulthood at a median of 29 years (range 19–39). Two patients, No. 3 and 6, were diagnosed with noninsulin-dependent diabetes at the ages of 26 and 38, respectively, which may be regarded as manifestation of PGL (Lenders *et al.* 2005), and which resolved in patient No. 3 after her second surgery (Supplementary Table 1).

All patients received an ophthalmology consultation because of known ocular complications occurring with this syndrome (Pacak *et al.* 2014). The results are summarized in Table 1. We observed optic disc fibrosis in all patients (Supplementary Figure 1). Three patients had macular changes and three peripheral retinal changes. For patient No. 5, ophthalmologic changes were noticed even before polycythemia was diagnosed and prior to the occurrence of PGL.

Anatomical and functional imaging characteristics are summarized in Table 2. On anatomical imaging, we found cystic lesions in 4 out of 7 patients. Cysts were localized to the kidneys, breasts, lungs, pericardium, cervix, and pancreas. In addition, patient No. 4 was found to have a liver hemangioma. Overall, the most suitable functional imaging modality that detected most of tumor lesions was [^{18}F]-FDOPA PET/CT (according to our chosen "gold standard", see Supplementary Table 2) closely followed by [^{18}F]-FDA PET/CT, as exemplified by the images of patients No. 2 and 4 (Fig. 3 and Supplementary Figure 2). In contrast, overall sensitivities and positive predictive values obtained were considerably lower for [^{18}F]-FDG PET/CT and [^{68}Ga]-DOTATATE PET/CT, respectively.

Supplementary Table 3 provides an overview of the current literature on patients with *HIF2A* gain-of-function mutations and summarizes pertinent information about position and type of the mutations, presence of mosaicism, mode of inheritance, phenotypic expression, and gender distribution. Apart from the 7 patients described in the present investigation, 56 additional patients with activating *HIF2A* mutations were identified in prior published studies and reports between the years 2008 and 2016: one additional female patient with Pacak-Zhuang syndrome, 7 patients (3 females, 4 males) with polycythemia and PHEO/PGL but without SOM, 16 patients (12 females, 2 males) with PHEO/PGL including 2 females with gangliocytic PGL (GPGL) without polycythemia or SOM, 2 patients (1 female, 1 male) with central nervous system hemangioblastomas without polycythemia, PHEO/PGL or SOM, and 28 patients (15 females, 12 males, no record of gender in 1) with isolated sporadic or familial polycythemia plus one female offspring with inherited *HIF2A* mutation, but without polycythemia. One other female patient without any signs of organic disease was found to be a carrier of an activating *HIF2A* mutation, which she passed to her 50 year-old son who had polycythemia and PHEO/PGL. There was a remarkable partial overlap of position and type of mutations between patients with PHEO/PGL with or without polycythemia, and patients with Pacak-Zhuang syndrome. Identical mutations were also described in patients with isolated sporadic and familial polycythemia. However, there was no overlap of mutations in these latter two groups of patients compared with the former, indicating mutation specific fundamental differences in downstream signaling pathways of HIF2A leading to either isolated polycythemia or PHEO/PGL with or without polycythemia or Pacak-Zhuang syndrome.

Discussion

This report provides comprehensive new information on a 6-year follow-up of 7 patients carrying activating somatic *HIF2A* mutations who presented with the syndrome of PGL and/or SOM associated with polycythemia (Pacak-Zhuang syndrome), and brings these experiences into perspective with the pertinent literature. The involvement of the downstream HIF2A signaling pathway in the pathophysiology of this new syndrome is strongly supported by its association with distinct *HIF2A* mutations as well as by the occurrence of closely related clinical phenotypes described in some reports of patients with VHL syndrome (Karasawa *et al.* 2001; Haase 2009), and of polycythemia and PGL in patients with mutated *PHD1* and *PHD2* (Yang *et al.* 2014).

As a hallmark of the syndrome in all of our patients, polycythemia was diagnosed either at birth or in early childhood, and always before PHEO/PGL and/or SOM, which is typically discovered after the development of PHEO/PGL. All patients were found to have eye involvement at manifestation of the disease (Pacak *et al.* 2014). Furthermore, EPO levels were elevated in all patients and did not return to normal after surgical tumor removal, making paraneoplastic EPO production less likely. Mosaicism with *HIF2A* gain-of-function mutations in EPO-producing renal interstitial cells or hepatocytes (Haase 2013) leading to continuous stimulation of EPO synthesis may be a reasonable alternative explanation. Although, in our series, mosaicism was only found in two patients (Patients No. 1 and 3; see also Yang *et al.* 2015). On the other hand, there is evidence from studies in patients with familial or sporadic polycythemia harboring *HIF2A* gain-of-function mutations to suggest

that polycythemia does not necessarily rely on increased EPO (Martini *et al*. 2008; Percy *et al*. 2012; Perrotta *et al*. 2013), indicating that the link between polycythemia and EPO in patients with Pacak-Zhuang syndrome may be less direct than intuitively expected. Other genetic abnormalities, including the timing of when *HIF2A* mutations occur, may further contribute to the full spectrum of this disease as in other hereditary syndromes.

In our patients PHEOs/PGLs were detected at a median age of 17 years, some 15 years after the diagnosis of polycythemia, and similar time intervals have been reported in other studies on patients with Pacak-Zhuang syndrome with or without established SOMs (Taïeb *et al*. 2013; Comino-Mendez *et al*. 2013; Buffet *et al*. 2014; Toyoda *et al*. 2014), as well as in one patient with familial polycythemia and PGL (Lorenzo *et al*. 2013). Together, these observations are in support of the concept that activating *HIF2A* mutations predispose to, but may not be sufficient for, the development of PHEOs/PGLs (Lorenzo *et al*. 2013). Of note, sporadic PHEOs/PGLs and other solid tumors with activating somatic *HIF2A* mutations occurring in the adult age do not appear to be preceded or accompanied by polycythemia, despite largely overlapping genetic changes compared to patients with Pacak-Zhuang syndrome or familial polycythemia with PHEO/PGL (Favier *et al*. 2012; Comino-Mendez *et al*. 2013; Toledo *et al*. 2013; Welander *et al*. 2014; Taïeb *et al*. 2016). It is therefore conceivable, that patients with Pacak-Zhuang syndrome and familial polycythemia with PHEO/PGL may harbor additional, yet unidentified tumor promoting aberrations, which are not present in sporadic PHEOs/PGLs later in life, the most obvious phenotypic reflections of which are polycythemia and increased EPO. Another explanation may be due to the precise timing of when an activating *HIF2A* mutation occurs during embryogenesis. It is anticipated that deep sequencing may allow a more definitive answer.

Considerations such as the above may also explain the distinct clinical features of SOMs in the patients described in the present study and by others (Buffet *et al*. 2014), which resemble those seen in conjunction with hereditary tumor syndromes, specifically neurofibromatosis type 1 (von Recklinghausen disease-NF1), MEN type 1, and VHL disease. Similarities include the nearly two decades earlier age of onset, lower rate of malignancy (despite early metastases in regional lymph nodes), and the preferential duodenal localization compared to sporadic SOMs (Klöppel *et al*. 2004; Marini *et al*. 2009), with the notable exception of VHL disease, where SOMs are predominantly found in the pancreas (Hammel *et al*. 2000). However, sporadic SOMs and SOMs associated with NF1, MEN1 or VHL gene mutations are clearly distinguished from SOMs in patients with Pacak-Zhuang syndrome by their substantially lower incidence and equal gender distribution. Therefore, even assuming the presence of an acquired genetic predisposition, the observation of a highly efficient and exclusive expression of SOMs in women remains a puzzling feature of Pacak-Zhuang syndrome.

Tumor location and biochemical phenotype of our patients fit well into the entity of “pseudohypoxic” cluster 1 tumors, as is the case for *VHL*, *PHD*, and for patients harboring *succinate dehydrogenase* (*SDHx*) mutations (Eisenhofer *et al*. 2011; Jochmanova *et al*. 2013; Richter *et al*. 2013). As in these patients (Neumann *et al*. 2002), *HIF2A* gain-of-function mutations may manifest with head and neck PGL. This was the case in patient No. 2 with an undefined skull-based lesion (Fig. 3).

Patients with *SDHB* mutations are especially prone to malignant disease (Gimenez-Roqueplo *et al.* 2003). In contrast, expression of tumor promoting genes seems to be less pronounced in patients with activating *HIF2A* mutations (Favier *et al.* 2012), resulting in a less aggressive PHEO/PGL phenotype. Indeed, none of our patients with metastatic PGLs thus far required chemotherapy or repeated radiotherapy, and none have died even 11 years after the first surgery. Therefore, these patients can be reassured that their overall outlook is favorable, although recurrent disease at multiple sites is highly probable and will require repeated surgeries, beginning at a young age.

The best functional imaging tracer for localization of tumors within Pacak-Zhuang syndrome in our series was [^{18}F]-FDOPA, and in line with recent recommendations for localization of NETs with a low Ki-67 Index (van Essen *et al.* 2014). [^{18}F]-FDG appeared to perform best in patients with mainly metastatic NET (Supplementary Table 2). In contrast, accuracy of [^{68}Ga]-DOTATATE was low. This finding points to substantial differences in tumor biology between patients with *HIF2A* and *SDHB* mutations, where [^{18}F]-FDG PET/CT and now [^{68}Ga]-DOTATATE PET/CT provide superior results (Timmers *et al.* 2007; Timmers *et al.* 2009; Janssen *et al.* 2015). The demonstration of cystic and hemangiomatous lesions in all of our patients but the two teenage subjects suggests shared phenotypic features in patients harboring mutated *VHL* (Haase 2009; Taïeb *et al.* 2016).

Although limited by a small number of patients in this case series, our findings call for several recommendations (Neumann and Eng 2009): a) consider genetic testing for *HIF2A* mutations in congenital polycythemia; b) screen for PHEO/PGL starting from the age of about eight; c) perform yearly measurement of plasma or urine metanephrines; d) perform regular (every 1–2 years) whole body or at least abdominal imaging, MRI in children; e) consider a surgical approach since no specific therapies are currently known; f) PGLs are predominantly norepinephrine-producing and, therefore, patients should preferably be on an α-adrenoceptor blockade; g) screen for SOM starting from about the age of 20; h) measure somatostatin levels in all patients with *HIF2A* mutations; i) in all SOMs larger than 1 cm metastatic potential is high and therefore, attempt early endoscopic or surgical removal; j) use a negative enteric contrast CT scan or endoscopic examination for SOMs; k) base follow-up for SOMs on the measurement of plasma somatostatin levels and imaging; l) search for other, especially gastrin- but also other neurohormone-secreting NETs.

In summary, we present the most updated follow-up of 7 patients with *HIF2A* mutations and Pacak-Zhuang syndrome consisting of PHEO/PGL and/or SOM associated with polycythemia, and discuss these experiences in the context of the pertinent literature. We conclude that due to its multi-facetted manifestations an interdisciplinary approach to the patient with this syndrome is imperative in order to allow for the best possible outcome. Current treatment options are exclusively surgical.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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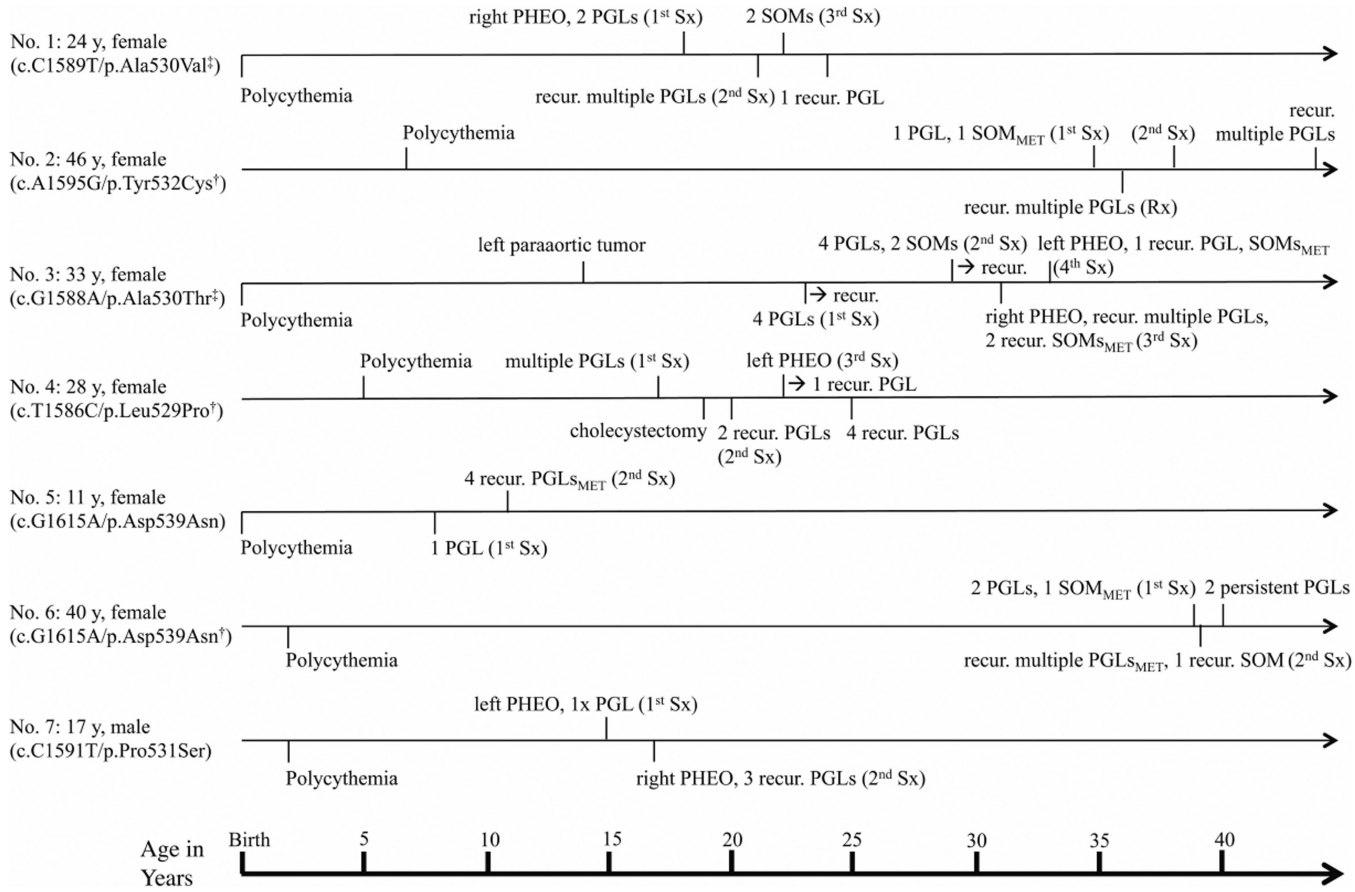

Figure 1.

Ages at diagnosis of polycythemia, pheochromocytoma (PHEO)/paraganglioma (PGL) and somatostatinoma (SOM) for individual patients in chronological order. Ages at latest outpatient visit, gender, mutation status with (‡) and without (†) mosaicism of *HIF2A*, numbers (multiple = >3), sites (right, left) and recurrence (recur.) of lesions, presence of metastatic disease ($_{MET}$), and number of surgical interventions (1^{st} – 4^{th} Sx, etc.) and/or radiotherapy (Rx) are indicated.

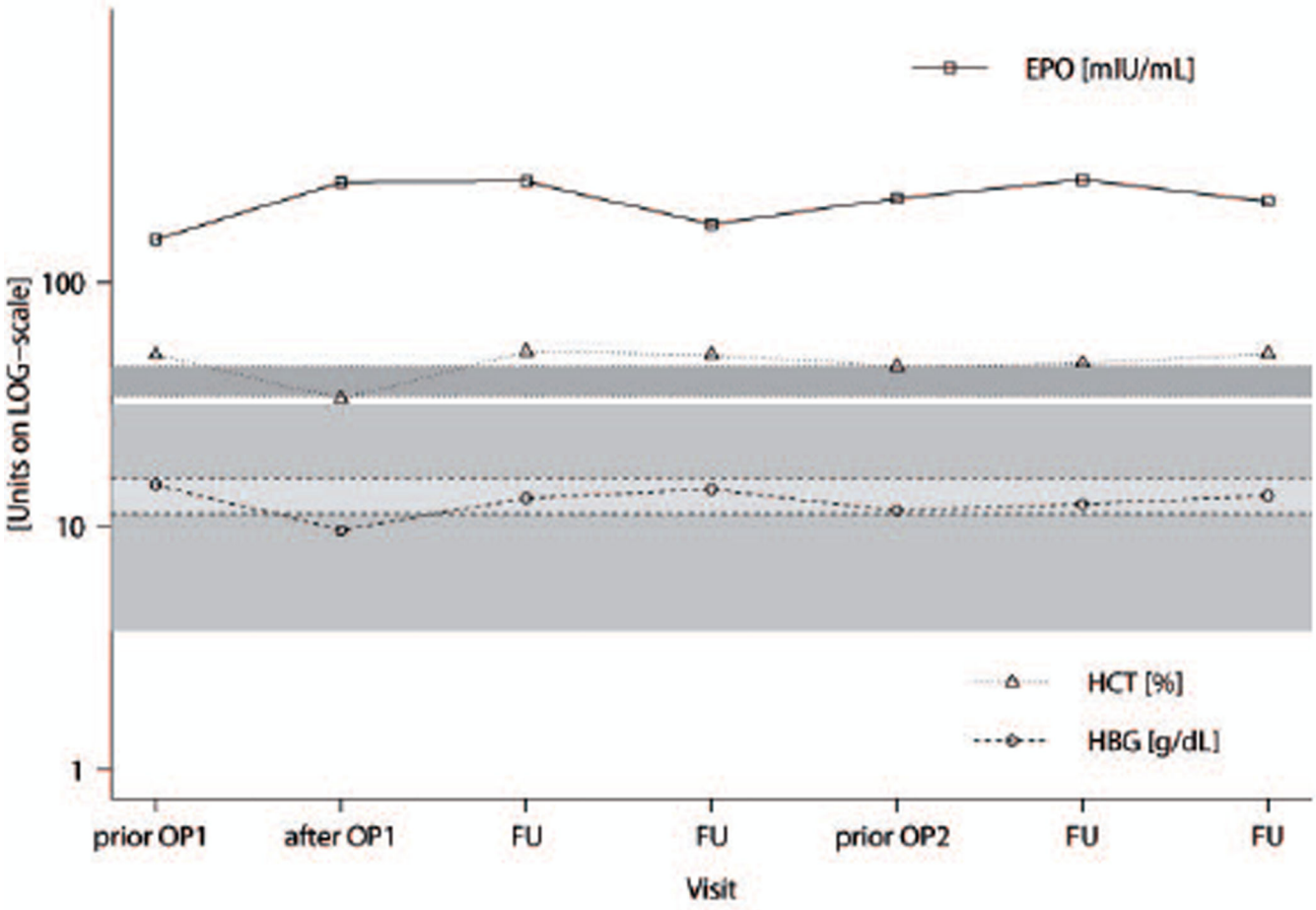

Figure 2.

Erythropoietin (EPO), hemoglobin (HGB), and hematocrit (HCT) levels over the course of several visits for patient No. 3, starting from first presentation at NIH, prior to and after surgeries (prior OP1, after OP1, and prior OP2), and during intermediate follow-up visits (FU) before the second surgery at the NIH. Respective upper and lower reference intervals are indicated for EPO, HGB, and HCT in gray, light gray, and dark gray, respectively. EPO levels did not normalize after surgeries, strongly refuting EPO production by tumor tissue.

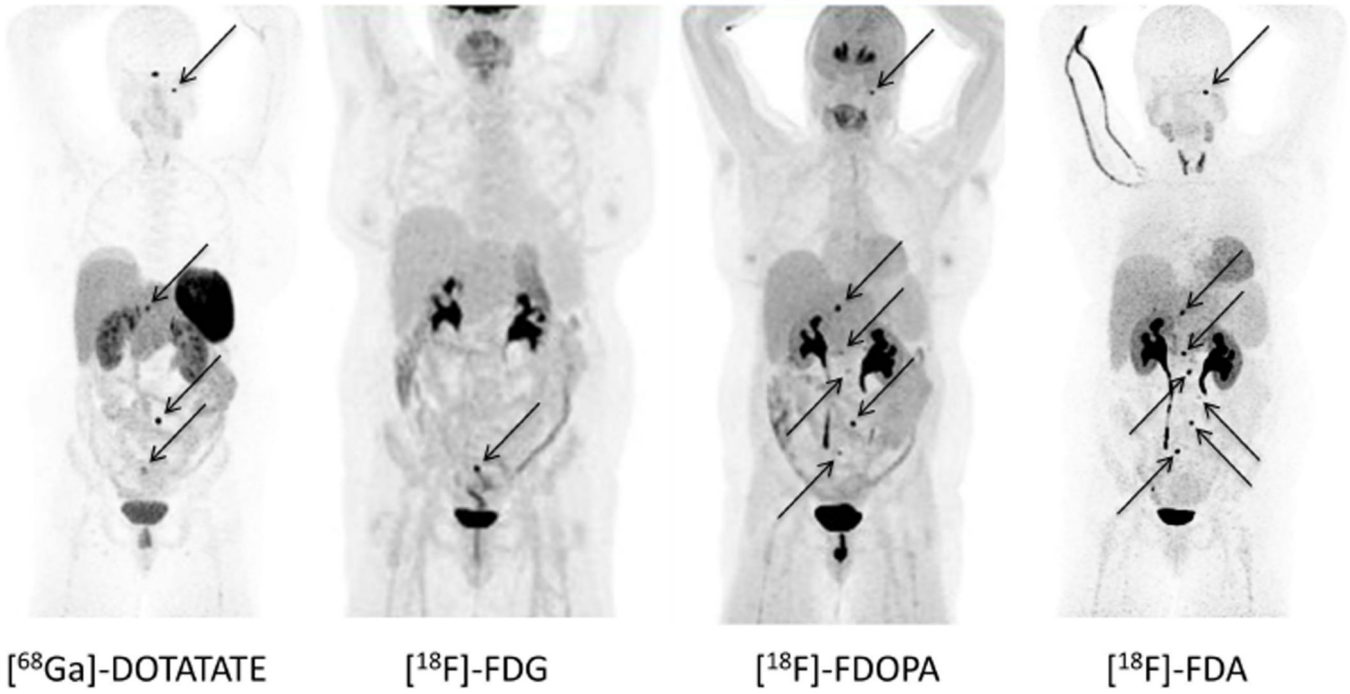

Figure 3.

Functional imaging with [^{68}Ga]-DOTATATE, [^{18}F]-FDG, [^{18}F]-FDOPA, and [^{18}F]-FDA PET/CT in patient No. 2. Radiotracer uptake is similar in [^{18}F]-FDA PET/CT and [^{18}F]-FDOPA, showing 5 and 6 retroperitoneal lesions, respectively. On [^{68}Ga]-DOTATATE PET/CT only 3 retroperitoneal lesions could be identified. All of these radiopharmaceuticals showed an additional lesion at the jugular foramen (see black arrow), which was negative with [^{18}F]-FDG. [^{18}F]-FDG PET/CT showed only one of the retroperitoneal lesions. All retroperitoneal lesions are also indicated with black arrows.

Table 1

Findings on ophthalmology consultation

Patient	Visual Acuity*	Optic Disc Fibrosis (bilateral)	Posterior Pole (Macular) Changes	Peripheral Retinal Changes	Other
1	RE: 20/16 LE: 20/16	Present	Vascular tortuosity with dilated veins Few arteriolar narrowing and subtle retinal pigmentary changes	Bilateral peripheral scattered retinal pigment epithelial (RPE) changes	
2	RE: 20/32 LE: 20/25	Present	Absent	Absent	Bilateral cataract (posterior subcapsular)
3◊	RE: 20/25 LE: 20/25	Present	Absent	Bilateral peripheral retinal neovascularization present; LE: single hemangioblastoma-like lesion similar to VHL in the inferior temporal retina	Myelinated nerve fiber layer in LE
4	RE: 20/20 LE: 20/20	Present	Absent	Bilateral temporal vasculature anomalies similar to familial exudative vitreoretinopathy / retinopathy of prematurity like appearance with U-turning blood vessels	
5◊	RE: 20/25 LE: 20/32	Present	Bilateral macular edema** with retinal hard exudate	Absent	Bilateral enlarged blind spot
6◊	RE: 20/20 LE: 20/63	Present	Absent	Absent	LE with optic nerve head drusen and amblyopia
7◊	RE: 20/250 LE: 20/20	Present	Macular edema*** with hard exudate RE only	Absent	

*Visual Acuity: measured on a LogMAR Visual Acuity Chart (Early Treatment Diabetic Retinopathy Study visual acuity chart) at 4 meters, best corrected

**Intravitreal injection of Ranibizumab was administered

***Intravitreal injection of Bevacizumab was administered

◊see also Pacak *et al*. 2014

Abbreviations: LE=left eye; RE=right eye; VHL=von Hippel-Lindau;

Table 2

Anatomical and functional imaging characteristics of patients

Patient	Age	CT	MRI	[^{18}F]-FDG	[^{18}F]-FDOPA	[^{18}F]-FDA	[^{68}Ga]-DOTATATE*	[^{123}I]-MIBG**
1	18	R adrenal, one abdominal lesion, bilateral renal cysts		+	+ + +	+ + +	ND	# #
	22–24	ND	pericaval focus, breast cysts	+	+ +	+ +	+ + (+)	ND
2	44 – 46	multiple retroperitoneal lesions and jugular foramen lesion		+	+ + +	+ + +	+ +	ND
		lung cysts	cysts in cervix					
3	29	multiple abdominal lesions, asc. aortic aneurysm		+ +	+ + +	+ + +	ND	# # #
	29–31	multiple abdominal lesions, R>L adrenal lesion pericardial cyst, physiologic R pelvic cyst on MRI		+ +	+ + +	ND	ND	# #
	33	L adrenal, 6 abdominal lesions		+ + +	+ +	+ +	+ +	ND
4	25	2 abdominal lesions	ND	+	+ + +	+ +	ND	ND
		hemangioma of the liver since age 20						
5	9	mesentericadenopathy of uncertain significance		-	ND	ND	ND	ND
	11	ND	5 abdominal lesions	+ +	+ + +	ND	ND	ND
6	39	abdominal lesions, pancreatic cyst, liver lesion on MRI		+ +	+ + (+)	+ + (+)	ND	ND
	39–40	ND	abdominal lesions	+ (+)	+ +	+ +	+ +	ND
7	17	ND	R adrenal	ND	+ + +	ND	-	ND

*Not initially performed in previous studies, [^{68}Ga]-DOTATATE, a new functional imaging modality, was included as further means of disease localization in five out of 7 patients; [^{68}Ga]-DOTATATE PET/CT was not performed in the teenage girl and complete series is also lacking for patient No. 4;

**[^{123}I]-MIBG scintigraphy was less accurate compared to PET/CT imaging, and was used for eligibility to MIBG treatment after approval of [^{68}Ga]-DOTATATE for our protocol only.

Abbreviations: asc.=ascending; CT=computed tomography; L=left; MRI=magnetic resonance imaging; ND=Not Done; R=right; positron emission tomography (PET)/CT using: [^{18}F]-FDG=[^{18}F]-fluorodeoxyglucose, [^{18}F]-FDOPA=[^{18}F]-fluorodihydroxyphenylalanine, [^{18}F]-FDA=[^{18}F]-fluorodopamine, and [^{68}Ga]-DOTATATE=[^{68}Ga]-(DOTA)-[Tyr3]-octreotate as radiopharmaceuticals, with "-", "+", "+ +", and "+ + +" signifying "no", "weak", "moderate", and "good" uptake; [^{123}I]-MIBG=[^{123}I]-metaiodobenzylguanidinescintigraphy, with "# #" and "# # #" signifying "moderate" and "strong" uptake;

Because the pancreatic cyst of patient No. 6 developed in the interval between two surgeries and was fading to disappearance on imaging over the course of several months, there is the possibility that this cyst was iatrogenically induced by the first surgery. Patients also presented with adnexal cysts but, because of time dependent changes, these were regarded as physiological or not part of the cystic lesions related to the syndrome. For instance, patient No. 3 had been diagnosed with polycystic ovary syndrome (PCOS) in the past and we could identify a persisting pelvic cyst on MRI, patient No. 4 had an enlarged ovarian cyst (30×28 mm) at the age of 11, and patient No. 6 had been operated on for polycystic ovaries at the age of 31.