Novel insights into the polycythemia-paraganglioma-somatostatinoma syndrome

Roland Därr1, Joan Nambuba1, Jaydira Del Rivero1, Ingo Janssen1, Maria Merino2, Milena Todorovic3, Bela Balint4, Ivana Jochmanova1,5, Josef T Prchal6, Ronald M Lechan7, Arthur S Tischler8, Vera Popovic9, Dragana Miljic10, Karen T Adams1, F Ryan Prall10, Alexander Ling11, Meredith R Golomb12, Michael Ferguson13, Naris Nilubol14, Clara C Chen15, Emily Chew16, David Taieb17, Constantine A Stratakis18, Tito Fojo19, Chunzhang Yang20, Electron Kebebew14, Zhengping Zhuang20 and Karel Pacak1

1Section on Medical Neuroendocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA
2Laboratory of Pathology, National Institutes of Health, Bethesda, Maryland, USA
3Institute of Hematology, Clinical Center of Serbia and Medical Faculty University of Belgrade, Belgrade, Serbia
4Institute of Transfusion and Hemobiology of Military Medical Academy and Institute for Medical Research, University of Belgrade, Belgrade, Serbia
151st Department of Internal Medicine, Faculty of Medicine, Pavol Jozef Safarik University in Kosice, Kosice, Slovakia
16Division of Hematology, University of Utah, Salt Lake City, Utah, USA
17Tupper Research Institute and Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, Tufts Medical Center, Boston, Massachusetts, USA
18Department of Pathology and Laboratory Medicine, Tufts Medical Center, Boston, Massachusetts, USA
19Institute of Endocrinology, Clinical Center of Serbia, Medical Faculty, University Belgrade, Belgrade, Serbia
20Department of Ophthalmology, Eugene and Marilyn Glick Eye Institute, Indiana University School of Medicine, Indianapolis, Indiana, USA
21Department of Radiology and Imaging Sciences, Clinical Center, National Institutes of Health, Bethesda, Maryland, USA
22Division of Child Neurology, Department of Neurology, Indiana University School of Medicine, Indianapolis, Indiana, USA
23Riley Hospital for Children at Indiana University Health, Indianapolis, Indiana, USA
24Endocrine Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA
25Division of Nuclear Medicine, Department of Radiology and Imaging Sciences, Clinical Center, National Institutes of Health, Bethesda, Maryland, USA
26Division of Epidemiology and Clinical Applications, National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA
27Department of Nuclear Medicine, La Timone University Hospital & CERIMED & Inserm UMR1068 Marseille Cancérology Research Center, Institut Paoli-Calmettes, Aix-Marseille University, Marseille, France
28Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA
29Medical Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA
30Neuro-Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Abstract

Worldwide, the syndromes of paraganglioma (PGL), somatostatinoma (SOM) and early childhood polycythemia are described in only a few patients with somatic mutations in the hypoxia-inducible factor 2 alpha (HIF2A). This study provides detailed information about the clinical aspects and course of 7 patients with this syndrome and brings

Key Words
- pheochromocytoma
- paraganglioma
- somatostatinoma
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 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.

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 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 (Crona et al. 2013, Pacak & 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, Taieb 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 (Zhuang et al. 2012, Pacak et al. 2013, Yang 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 & 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 this 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, see section on supplementary data given at the end of this article).

**Laboratory analyses**

All laboratory analyses, mutation analysis, hydroxylation assays, real-time polymerase chain reaction and chromatin immunoprecipitation were performed, as described previously (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 described previously, 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 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 or biochemically. In the case of patient No. 4, the diagnosis of SOM was confirmed.

### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Visual Acuity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Optic disc fibrosis (bilateral)</th>
<th>Posterior pole (macular) changes</th>
<th>Peripheral retinal changes</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RE: 20/16 LE: 20/16</td>
<td>Present</td>
<td>Vascular tortuosity with dilated veins Few arteriolar narrowing and subtle retinal pigmentary changes</td>
<td>Bilateral peripheral scattered retinal pigment epithelial (RPE) changes</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>RE: 20/32 LE: 20/25</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Bilateral cataract (posterior subcapsular)</td>
</tr>
<tr>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>RE: 20/25 LE: 20/25</td>
<td>Present</td>
<td>Absent</td>
<td>Bilateral peripheral retinal neovascularization present; LE: single hemangioblastoma-like lesion similar to VHL in the inferior temporal retina</td>
<td>Myelinated nerve fiber layer in LE</td>
</tr>
<tr>
<td>4</td>
<td>RE: 20/20 LE: 20/20</td>
<td>Present</td>
<td>Absent</td>
<td>Bilateral temporal vasculature anomalies similar to familial exudative vitreoretinopathy/ retinopathy of prematurity-like appearance with U-turning blood vessels</td>
<td>Bilateral enlarged blind spot</td>
</tr>
<tr>
<td>5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>RE: 20/25 LE: 20/32</td>
<td>Present</td>
<td>Bilateral macular edema&lt;sup&gt;b&lt;/sup&gt; with retinal hard exudate</td>
<td>Absent</td>
<td>LE with optic nerve head drusen and amblyopia</td>
</tr>
<tr>
<td>6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>RE: 20/20 LE: 20/63</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>RE: 20/250 LE: 20/20</td>
<td>Present</td>
<td>Macular edema&lt;sup&gt;c&lt;/sup&gt; with hard exudate RE only</td>
<td>Absent</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Visual acuity: measured on a LogMAR Visual Acuity Chart (Early Treatment Diabetic Retinopathy Study visual acuity chart) at 4m, best corrected;  
<sup>b</sup>Intra-vitreal injection of ranibizumab was administered;  
<sup>c</sup>Intra-vitreal injection of bevacizumab was administered;  
<sup>d</sup>See also Pacak et al. (2014).  
LE, left eye; RE, right eye; VHL, von Hippel–Lindau.
based solely on elevated levels of somatostatin along with retroperitoneal abdominal nodules on imaging studies, but without histological confirmation of the diagnosis to date as 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 Fig. 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 before 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 [18F]-FDOPA PET/CT (according to our chosen ‘gold standard’, see Supplementary Table 2) closely followed by [18F]-FDA PET/CT, as exemplified by the images of patients No. 2 and 4 (Fig. 3 and Supplementary Fig. 2). In contrast, overall sensitivities and positive predictive values obtained were considerably lower for [18F]-FDG PET/CT and [68Ga]-DOTATATE PET/CT, respectively.

Table 2 Anatomical and functional imaging characteristics of patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>CT</th>
<th>MRI</th>
<th>[18F]-FDG</th>
<th>[18F]-FDOPA</th>
<th>[18F]-FDA</th>
<th>[68Ga]-DOTATATE*</th>
<th>[123I]-MIBG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>R adrenal, one abdominal lesion, bilateral renal cysts</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
</tr>
<tr>
<td>2</td>
<td>22–24</td>
<td>Multiple retroperitoneal lesions and jugular foramen lesion</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>Multiple abdominal lesions, asc. aortic aneurysm</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>ND</td>
<td># #</td>
<td># #</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>Two abdominal lesions</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>Mesenteric adenopathy of uncertain significance</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>Abdominal lesions, pancreatic cyst, liver lesion on MRI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>R adrenal</td>
<td>ND</td>
<td>+++</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Not initially performed in previous studies, [68Ga]-DOTATATE, a new functional imaging modality, was included as further means of disease localization in 5 out of 7 patients; [68Ga]-DOTATATE PET/CT was not performed in the teenage girl and complete series is also lacking for patient No. 4; [123I]-MIBG scintigraphy was less accurate compared with PET/CT imaging, and was used for eligibility to MIBG treatment after approval of [68Ga]-DOTATATE for our protocol only.

Based on the images of patients No. 2 and 4 (Fig. 3 and Supplementary Fig. 2), anatomical and functional imaging modalities allow the detection of PGL.

### Table 2 Anatomical and functional imaging characteristics of patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>CT</th>
<th>MRI</th>
<th>[18F]-FDG</th>
<th>[18F]-FDOPA</th>
<th>[18F]-FDA</th>
<th>[68Ga]-DOTATATE*</th>
<th>[123I]-MIBG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>R adrenal, one abdominal lesion, bilateral renal cysts</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
</tr>
<tr>
<td>2</td>
<td>22–24</td>
<td>Multiple retroperitoneal lesions and jugular foramen lesion</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>Multiple abdominal lesions, asc. aortic aneurysm</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>ND</td>
<td># #</td>
<td># #</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>Two abdominal lesions</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>Mesenteric adenopathy of uncertain significance</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>Abdominal lesions, pancreatic cyst, liver lesion on MRI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>R adrenal</td>
<td>ND</td>
<td>+++</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Not initially performed in previous studies, [68Ga]-DOTATATE, a new functional imaging modality, was included as further means of disease localization in 5 out of 7 patients; [68Ga]-DOTATATE PET/CT was not performed in the teenage girl and complete series is also lacking for patient No. 4; [123I]-MIBG scintigraphy was less accurate compared with PET/CT imaging, and was used for eligibility to MIBG treatment after approval of [68Ga]-DOTATATE for our protocol only.

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 [18F]-FDOPA PET/CT (according to our chosen ‘gold standard’, see Supplementary Table 2) closely followed by [18F]-FDA PET/CT, as exemplified by the images of patients No. 2 and 4 (Fig. 3 and Supplementary Fig. 2). In contrast, overall sensitivities and positive predictive values obtained were considerably lower for [18F]-FDG PET/CT and [68Ga]-DOTATATE PET/CT, respectively.

### Table 2 Anatomical and functional imaging characteristics of patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>CT</th>
<th>MRI</th>
<th>[18F]-FDG</th>
<th>[18F]-FDOPA</th>
<th>[18F]-FDA</th>
<th>[68Ga]-DOTATATE*</th>
<th>[123I]-MIBG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>R adrenal, one abdominal lesion, bilateral renal cysts</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
</tr>
<tr>
<td>2</td>
<td>22–24</td>
<td>Multiple retroperitoneal lesions and jugular foramen lesion</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>Multiple abdominal lesions, asc. aortic aneurysm</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>ND</td>
<td># #</td>
<td># #</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>Two abdominal lesions</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>Mesenteric adenopathy of uncertain significance</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>Abdominal lesions, pancreatic cyst, liver lesion on MRI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>R adrenal</td>
<td>ND</td>
<td>+++</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Not initially performed in previous studies, [68Ga]-DOTATATE, a new functional imaging modality, was included as further means of disease localization in 5 out of 7 patients; [68Ga]-DOTATATE PET/CT was not performed in the teenage girl and complete series is also lacking for patient No. 4; [123I]-MIBG scintigraphy was less accurate compared with PET/CT imaging, and was used for eligibility to MIBG treatment after approval of [68Ga]-DOTATATE for our protocol only.

Based on the images of patients No. 2 and 4 (Fig. 3 and Supplementary Fig. 2), anatomical and functional imaging modalities allow the detection of PGL.

### Table 2 Anatomical and functional imaging characteristics of patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>CT</th>
<th>MRI</th>
<th>[18F]-FDG</th>
<th>[18F]-FDOPA</th>
<th>[18F]-FDA</th>
<th>[68Ga]-DOTATATE*</th>
<th>[123I]-MIBG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>R adrenal, one abdominal lesion, bilateral renal cysts</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
</tr>
<tr>
<td>2</td>
<td>22–24</td>
<td>Multiple retroperitoneal lesions and jugular foramen lesion</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>Multiple abdominal lesions, asc. aortic aneurysm</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>ND</td>
<td># #</td>
<td># #</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>Two abdominal lesions</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>Mesenteric adenopathy of uncertain significance</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>Abdominal lesions, pancreatic cyst, liver lesion on MRI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>R adrenal</td>
<td>ND</td>
<td>+++</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Not initially performed in previous studies, [68Ga]-DOTATATE, a new functional imaging modality, was included as further means of disease localization in 5 out of 7 patients; [68Ga]-DOTATATE PET/CT was not performed in the teenage girl and complete series is also lacking for patient No. 4; [123I]-MIBG scintigraphy was less accurate compared with PET/CT imaging, and was used for eligibility to MIBG treatment after approval of [68Ga]-DOTATATE for our protocol only.

Based on the images of patients No. 2 and 4 (Fig. 3 and Supplementary Fig. 2), anatomical and functional imaging modalities allow the detection of PGL.

### Table 2 Anatomical and functional imaging characteristics of patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>CT</th>
<th>MRI</th>
<th>[18F]-FDG</th>
<th>[18F]-FDOPA</th>
<th>[18F]-FDA</th>
<th>[68Ga]-DOTATATE*</th>
<th>[123I]-MIBG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>R adrenal, one abdominal lesion, bilateral renal cysts</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
</tr>
<tr>
<td>2</td>
<td>22–24</td>
<td>Multiple retroperitoneal lesions and jugular foramen lesion</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>Multiple abdominal lesions, asc. aortic aneurysm</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>ND</td>
<td># #</td>
<td># #</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>Two abdominal lesions</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>#</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>Mesenteric adenopathy of uncertain significance</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>Abdominal lesions, pancreatic cyst, liver lesion on MRI</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>R adrenal</td>
<td>ND</td>
<td>+++</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
An overview of the current literature on patients with HIF2A gain-of-function mutations and a summary of pertinent information about the position and type of mutations, presence of mosaicism, mode of inheritance, phenotypic expression and gender distribution are given in Supplementary Table 3. 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 among 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 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 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, Gale et al. 2008, Percy et al. 2008a,b, Furlow et al.
indicating that the link between polycythemia and EPO in patients with this 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 this syndrome with or without established SOMs (Comino-Mendez et al. 2013, Taieb et al. 2013, Buffet et al. 2014, Toyota 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 with 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, Taieb et al. 2016, Zhuang 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 this 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 a median age of onset of which is nearly two decades earlier (at 29 years), a lower rate of malignancy (despite early metastases in regional lymph nodes), and the preferential duodenal localization compared with 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 this 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 this 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 diseases (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 this syndrome in our series was [18F]-FDOPA, and in line with recent recommendations for localization of NETs with a low Ki-67 Index (van Essen et al. 2014). [18F]-FDG appeared to perform best in patients with mainly metastatic NET (Supplementary Table 2). In contrast, accuracy of [68Ga]-DOTATATE was low. This finding points to substantial differences in tumor biology among patients with HIF2A and SDHB mutations, where [18F]-FDG PET/CT and now [68Ga]-DOTATATE PET/CT provide superior results (Timmers et al. 2007, 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, Taieb et al. 2016).

Although limited by a small number of patients in this case series, our findings call for several recommendations (Neumann & 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 as no specific therapies are currently known; (f) PGLs are predominantly norpinephrine-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 or SOM associated with polycythemia, and discuss these experiences in the context of the pertinent literature. We conclude that due to its multifaceted 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 data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/ERC-16-0231.

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.

Funding
This work was supported by the Intramural Research Program of the National Institutes of Health; the Eunice Kennedy Shriver National Institute of Child Health and Human Development; the National Institute of Neurological Disorders and Stroke; and the German Research Foundation (DFG) (grant number DA 1630/1-1 to R D).

Author contribution statement

References
right direction. Journal of the National Cancer Institute 105 1270–1283. (doi:10.1093/jnci/djt201)
Yang C, Hong CS, Prchal JT, Balint MT, Pacak K & Zhuang Z 2015
Somatic mosaicism of EPAS1 mutations in the syndrome of
paraganglioma and somatostatinoma associated with polycythemia.
*Human Genome Variation* 2 15053. (doi:10.1038/hgv.2015.53)
Zhuang Z, Yang C, Lorenzo F, Merino M, Fojo T, Kebebew E, Popovic V,
Stratakis CA, Prchal JT & Pacak K 2012 Somatic HIF2A gain-of-
function mutations in paraganglioma with polycythemia. *New England
Zhuang Z, Yang C, Ryska A, Ji Y, Hou Y, Graybill SD, Bullova P,
Lubensky IA, Klöppel G & Pacak K 2016 HIF2A gain-of-function
mutations detected in duodenal gangliocytic paraganglioma.
*Endocrine-Related Cancer* 23 L13–L16. (doi:10.1530/ERC-16-0148)

Received in final form 23 September 2016
Accepted 27 September 2016
Accepted Preprint published online 27 September 2016