Familial SDHB gene mutation in disseminated non-hypoxia-related malignant paraganglioma treated with $^{90}$Y-$^{177}$Lu-DOTATATE

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SUMMARY Familial paraganglioma may be related to mutations in succinate dehydrogenase (SDH) enzyme complex genes. Among patients with hereditary paraganglioma, SDH subunit B (SDHB) gene mutations are associated with the highest morbidity and mortality related to a higher malignancy rate. We report a family with the c.689G>A (p.Arg230His) mutation in the SDHB gene identified in two family members, a father and his daughter. While the 14-year-old daughter had no evidence of clinical disease, recurrent and later disseminated $^{131}$I-metaiodobenzylguanidine uptake-negative head and neck paraganglioma with multiple bone metastases developed in the father who underwent peptide receptor radionuclide therapy with $^{90}$Y-$^{177}$Lu-dodecane tetraacetic acid octreotate (DOTATATE) at the time of the genetic diagnosis. This treatment was repeated 6 years later due to disease progression and the patient, who is currently 49 years old, remains alive and in good overall clinical condition at 8 years of follow-up after the original presentation at our unit. The growing armamentarium of imaging methods available for such patients may inform decision making regarding choice of the optimal treatment approach, potentially contributing to improved outcomes.

Keywords somatostatin receptor imaging, succinate dehydrogenase, catecholamine-producing tumor, positron emission tomography/computed tomography

1. Introduction

Paragangliomas, along with pheochromocytomas, are rare catecholamine-producing neuroendocrine tumors originating from cells derived from the neural crest. According to the World Health Organization (WHO) classification, pheochromocytomas occur in the adrenal medulla, while paragangliomas are extraadrenal tumors of sympathetic or parasympathetic origin, which can occur in the paravertebral ganglia, mediastinum, abdomen, pelvis, head or neck.

They typically present as painless, gradually enlarging masses with slow growth and no specific clinical features until symptoms of catecholamine overproduction or a mass effect. In addition to variable location, they can be solitary or multiple, sporadic or hereditary, and benign or malignant. They may be of sympathetic or parasympathetic origin, and secreting or non-secreting hormones. Multiple tumors are more common in hereditary compared to sporadic cases.

Location in the upper part of the body above the diaphragm, particularly within the neck or head, is typical for parasympathetic paragangliomas. These tumors may also have specific names related to their site, including glomus jugulare for jugular location, glomus tympanicum for tympanic paraganglia and chemodectoma for carotid body paraganglia. Most of them are benign but sometimes they have a malignant nature with multiple distant metastases, usually to the cervical lymph nodes, lungs, bones, and liver.

The estimated combined annual incidence of pheochromocytoma/paraganglioma is 0.8 per 100,000 person-years, with approximately 500 to 1,600 cases annually in the United States (1). The prevalence of pheochromocytoma/paraganglioma among hypertensives in general outpatient clinics is 0.2-0.6%. Pheochromocytoma is found in nearly 5% of patients with an incidentally discovered adrenal mass on imaging (2).

In most reports that included large groups of patients, about 30% or more of paragangliomas were hereditary

(3,4). They are associated with germ-line or more rarely somatic mutations of one of five succinate dehydrogenase (SDH) enzyme complex genes, functioning as tumor suppressor genes, or occur as part of multineoplasia syndromes including von Hippel-Lindau disease (VHL), neurofibromatosis type 1, multiple endocrine neoplasia type 2A and 2B, and rare Carney-Stratakis triad. More recently, Myc-associated factor X (MAX) gene, hypoxia-inducible factor (HIF)-1α gene, and TMEM127 gene mutations were also identified in individuals with paragangliomas (5). An autosomal dominant inheritance with incomplete penetrance and variable expression is typical for the hereditary forms (1).

The most common genetic defects in patients with paragangliomas are mutations of SDH complex genes. Depending on the specific gene, there are 5 types of paraganglioma syndromes (PGL1 to 5 syndromes), which have been linked to mutations of various complex SDH genes. The most commonly mutated gene in patients with familial paraganglioma syndromes is the one coding for SDH subunit D (SDHD).

Malignancy in paragangliomas is rather rare, as only about 10% of paragangliomas are malignant but this rate is higher in hereditary compared to sporadic cases and it is closely related to the specific mutated gene. The highest morbidity and mortality has been reported, related to a higher (21-79%) malignancy rate, in carriers of SDH subunit B (SDHB) gene mutations (3,6).

Despite great progress, imaging of paragangliomas may remain challenging. Detection of distant metastases is crucial for the diagnosis of a malignant form and leads to a change in the therapeutic strategy.

In the present study, we present the clinical course and imaging study results that formed our decision making regarding the choice of the optimal treatment approach in a male patient with the hereditary, malignant form of paraganglioma associated with the Arg230His mutation in the SDHB gene (PGL4 syndrome).

2. Materials and Methods

2.1. The patient

A Caucasian male, currently 49 years old, presented first at the age of 25 years with right carotid paraganglioma. He was treated surgically in 1996, and a local recurrence was diagnosed 12 years later. For that reason, he was operated on again in 2008 and paraganglioma was again confirmed in the histopathological diagnosis. In October 2012, a large tumor was found in the right temporal region and the patient underwent right temporal craniectomy. The pathology report described a 5 × 4 × 1 cm tumor with an adherent dura mater area sized 5 × 3 cm, in the cross-section appearing creamy-brown, partly calcified, and invading the dura mater, with the Ki-67 index of 10%, corresponding to the WHO G2 grade. Immunohistochemical staining was positive for chromogranin A (CgA), protein S100 and synaptophysin, confirming the neuroendocrine nature of the tumor cells.

In November 2012, the patient was admitted for further evaluation to the Department of Internal Diseases, Hypertension and Angiology, Medical University of Warsaw. He did not have any symptoms suggesting catecholamine overproduction. Physical examination revealed pale skin, tachycardia, and systolic murmur at the base of the heart. Blood pressure was normal with no orthostatic hypotension. Laboratory tests showed mild microcytic anemia with low serum iron concentration. Transferrin and ferritin levels were in the normal range, as were thyroid hormones and thyroid-stimulating hormone. Echocardiography showed no abnormalities. Ambulatory blood pressure monitoring showed normotension with preserved normal circadian rhythm. Neck ultrasonography revealed focal recurrence.

Catecholamine testing showed elevated 24-hour urinary unfractionated metanephrines (1,326 µg/24h, reference range 100-1,000 µg/24h), while 24-hour urinary excretion of norepinephrine (70 µg/24h, reference range 23-105 µg/24h), epinephrine (6.9 µg/24h, reference range 4-20 µg/24h) and dopamine (307 µg/24h, reference range below 450 µg/24h) was within the normal range. Plasma CgA level was elevated more than 6-fold above the upper reference limit (641.6 ng/mL, reference range 0-94 ng/mL).

2.2. Genetic testing

Genomic DNA was extracted from venous blood and polymerase chain reaction (PCR) was used to amplify the eight exons of the SDHB gene, four exons of the SDHD gene, three exons of the VHL gene, and exons 10, 11, 13, 14, 15, 16 of the RET gene. Primer sequences and PCR conditions are listed in Table 1. PCR products were purified with NucleoFast 96 PCR kit (Macherey-Nagel, Düren, Germany). The sequencing of PCR products was conducted using BigDye 3.1 chemistry and ABI3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s protocol.

3. Results and Discussion

We found the c.689G>A (p.Arg230His) mutation in exon 7 of the SDHB gene (reference sequence NM_003000.2) in the patient and in one of his two daughters who was 14 years old at that time. The affected daughter was asymptomatic, had no tumors found in whole body magnetic resonance imaging (MRI) performed in January 2014, and her catecholamine testing results were normal. There was no family history of neoplasms and carotid body tumors.

Somatostatin receptor imaging with [68Ga]Ga-dodecane tetracetic acid octreotate (DOTATATE)
poset tim emission tomography/computed tomography (PET/CT) demonstrated an increased uptake in the right neck and regional lymph nodes as well disseminated foci in bones with high somatostatin receptor overexpression (maximum standardized uptake values [SUVmax] up to 57) (Figure 1A).

Due to poor results of chemotherapy in paraganglioma cases, radionuclide therapy was considered. Both peptide receptor radionuclide therapy (PRRT) with radiolabelled somatostatin analogues and $^{[131}I$metaiodobenzylguanidine (mIBG) therapy have become established methods for the treatment of disseminated paraganglioma. To choose the best treatment option, $^{[131}I$mIBG scan was performed but it showed no uptake at the site of metastases (Figure 1B).

On the basis of several studies in the literature that have shown that $^{[18}F$FDOPA PET/CT is an excellent imaging tool in head and neck parangliomas with the sensitivity approaching 100% (7), $^{[18}F$FDOPA PET/CT was performed to exclude metastases without somatostatin expression and it showed uptake in the same sites as $^{[68}Ga$Ga-DOTATATE PET/CT (Figure 1C).

Due to the high somatostatin receptor expression on $^{[68}Ga$Ga-DOTATATE PET/CT, a multidisciplinary team consisting of an oncologist, a nuclear medicine
In follow-up $[^{68}\text{Ga}]\text{Ga-DOTATATE}$ PET/CT at 3, 6 and 12 months after the therapy, stable disease was observed with decreasing SUVmax up to 38, with no new metastatic foci. The patient was able to resume work.

The disease was stable in follow-up imaging until July 2017 but in February 2019, follow-up $[^{68}\text{Ga}]\text{Ga-DOTATATE}$ PET/CT scanning revealed multiple small new foci in bones, including the skull, ribs, spine (Th9, L2 and L4 vertebrae), pelvis, and femurs, mostly of mixed osteolytic-osteosclerotic nature on CT. Two additional PRRT treatment sessions with $[^{90}\text{Y}]\text{Y/DOTATATE}$ and $[^{177}\text{Lu}]\text{Lu-DOTATATE}$ were performed in March and June 2019, with amino-acid infusion for nephroprotection. The total injected activity was 7.4 GBq (200 mCi), with 3.7 GBq (100 mCi) per session.

Follow-up PET/CT with $[^{68}\text{Ga}]\text{Ga-DOTATATE}$ in October 2019 and November 2020 demonstrated stable diffuse lesions in bones, largest in the spine (L5 and sacrum) with decreasing SUVmax up to 35, along with stable lesions in the right neck with lower somatostatin receptor expression (postoperative site at the right common carotid artery and group II lymph nodes, SUVmax values up to 17 in November 2020). The patient did not have anemia prior to the second treatment (hemoglobin level of 13.6 g/dL in 2018), and at the time of PRRT sessions in March and June 2019, hemoglobin level was 13.1 and 12.3 g/dL. As of April 2021, the patient remained in a good overall clinical condition. Follow-up $[^{68}\text{Ga}]\text{Ga-DOTATATE}$ PET/CT scans are shown in Figure 2.

In our patient, shortly after the disease recurred for the second time and temporal bone metastases were found, we have recognized a hereditary, malignant form of PGL4 syndrome with the c.689 G>A (p.Arg230His) mutation in exon 7 of the SDHB gene. The disease involved the carotid body (at the first disease presentation in 1997) and the temporal bone (with adherent dura mater), with dissemination to the long bones, skull, ribs and spine. Hormonal studies revealed only slight elevation of urinary metanephrines but not epinephrine, norepinephrine or dopamine. When it turned out that the disease is generalized, with multiple bone metastases, there was no option for resection. Metastatic paragangliomas and pheochromocytomas are also relatively radioresistant, as compared to bone metastases in breast cancer and lymphoma lesions (8). The patient underwent surgical treatment, however, when two metastatic tumors located within the spinal canal exerted a serious mass effect with myelopathy and an attempt of tumor embolization was ineffective in a critical moment of the disease. Resection of these two metastatic tumors resulted in a prompt and effective relief of neurological symptoms. This approach is in accordance with the recommendations and may also possibly improve survival, although there are no definitive data to support this (9).
Both PRRT with radiolabelled somatostatin analogues and $^{[131]}$I$m$IBG therapy have become established methods for treatment of disseminated paraganglioma (10). The efficacy and safety of most commonly used agents, $^{[90]}$Y$Y$-DOTATATE and $^{[177]}$Lu$Lu$-DOTATATE, in malignant paraganglioma has been described in a number of case reports and patient series. One of the largest recent series reported in the literature included 30 patients with inoperable or metastatic pheochromocytomas and paragangliomas (including 27 paragangliomas). Best tumor response was partial response in 7 (23%) patients and stable disease in 20 (67%) patients, while progressive disease was observed in 3 (10%) patients. The median progression-free survival was 91 months in patients with parasympathetic paragangliomas, 13 months in patients with sympathetic paragangliomas and 10 months in patients with metastatic pheochromocytomas. Grade 3/4 subacute haematotoxicity occurred in 6 (20%) of patients (11). In a recent systematic review, overall 12 studies were included, for a total of 201 patients with advanced (inoperable and metastatic) pheochromocytoma and paraganglioma who were treated with PRRT. A disease control rate of 84% was reported, and treatment-related adverse effects were minimal, with grade 3/4 neutropenia, thrombocytopenia, lymphopenia and nephrotoxicity observed in up to 11% of patients. Similar tumor response rates were noted for $^{90}$Y- and $^{177}$Lu-based agents (12). However, severe adverse reactions following $^{[177]}$Lu$Lu$-DOTATATE treatment were also reported in patients with paraganglioma, including catecholamine crisis and tumor lysis crisis and tumor lysis syndrome (13), as was marked progression of metastatic paraganglioma following initial partial response to PRRT (14).

PET/CT-based visualization of metastatic foci with very high uptake of $^{[68]}$GaGa-DOTATATE, with their confirmation by $^{[18]}$FDOPA PET/CT, was the reason for choosing this treatment method in the reported case. $^{[123]}$I$m$IBG scintigraphy may be suboptimal in patients with special genotypic features such as those with VHL and SDHB gene mutation-related paraganglioma (7).

Systemic chemotherapy is recommended for unresectable and rapidly progressive pheochromocytoma/paraganglioma and in patients with high tumor burden or multiple bone metastases. A critical appraisal of the reports evaluating chemotherapy reveals, however, that these studies predominantly involved patients with retroperitoneal sympathetic catecholamine-secreting tumors and pheochromocytoma (15,16). Both the location and the parasympathetic origin of neck and head paraganglioma suggest cautious interpretation of these results in relation to hereditary malignant paraganglioma.

Recent years brought hope for a new effective chemotherapeutic temozolomide, used alone or in combination with other agents including thalidomide, capecitabine, gemcitabine, paclitaxel, and docetaxel (17-19). Temozolomide may be particularly useful in hereditary paraganglioma with SDHB gene mutation, which is associated with hypermethylation of the promoter for O-6-methylguanine-DNA methyltransferase (17). Experience with the drug is still limited, however, as in a recent case report and literature review, only 26 cases of metastatic pheochromocytoma/paraganglioma treated with temozolomide were identified globally (20).

In our patient, genetic testing revealed PGL4 syndrome associated with the Arg230His mutation in the SDHB gene. The mutation was reported previously (21), including in familial cases, although to date, there are only a few families bearing the Arg230His mutation described in the literature (22-25).

In one of these reports, the Arg230His mutation was identified in the context of high-altitude hypoxia-related paraganglioma in two members of the same family living in Guadalajara, Mexico, at over 1500 m above sea level (23). More than 40 years ago, it was noted that high altitude is associated with an increased incidence of paraganglioma (26). Many years later, the link between aberrant cellular oxygen sensing (pseudo-hypoxia) and development of tumors of sympathetic and parasympathetic origin has become a newly

![Figure 2. Somatostatin receptor imaging with $^{[68]}$GaGa-DOTATATE – response to treatment.](image-url)
investigated hypothesis (27). Molecular data showed that mutations in the genes coding for SDH subunits result in accumulation of succinate and inhibition of HIF-1 hydroxylases leading to stabilization of HIF-1 (28,29). HIF-1 and 2 are transcription factors that activate several genes that promote adaptation and survival under hypoxic conditions. They control energy, iron metabolism, erythropoiesis and development. Paragangliomas harboring mutations in SDH genes as well as the VHL gene are characterized by HIF stabilization, dysregulation and overexpression (30).

Our patient, unlike the two Mexican patients cited above, is a resident of Warsaw, located approximately 100 m above sea level, and has not had a history of living at a high altitude (besides two one-week holiday visits for skiing in the Italian Alps at about 1,800 m above sea level). He also has not had any conditions associated with frequent hypoxia episodes, such as sleep apnea syndrome, asthma or chronic obstructive pulmonary disease, or cyanotic heart disease. The only condition in his medical history, which could be associated with tissue hypoxia was iron deficiency anemia, identified at the time of diagnosis of disseminated disease.

In conclusion, we reported a family with the c.689G>A (p.Arg230His) mutation in the SDHB gene, with recurrent and later disseminated $^{[18]F}$FDG and $^{[18]F}$FDOPA PET/CT images are reproduced courtesy of Prof. Janusz Braziewicz, head of the Department of Nuclear Medicine with Positron Emission Tomography Unit, Holy Cross Cancer Centre in Kielce, Poland.

Acknowledgements

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Funding: None.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References


Received March 10, 2021; Revised April 17, 2021; Accepted May 18, 2021.

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Released online in J-STAGE as advance publication June 4, 2021.