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Malignant paraganglioma and somatotropinoma in a patient with germline *SDHB* mutation—genetic and clinical features

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Abstract

Background Pituitary adenomas and paragangliomas/pheocromocytomas are rare endocrine tumours, which can be sporadic or familial. During many years their coexistence in the same individual was considered a coincidental finding. However, an association between these two entities was recently demonstrated, with the possible involvement of *SDHx* genes.

Case report We describe a 57-year-old female patient, who was under surveillance since 1997 for a malignant paraganglioma with vertebral bone metastasis, and harboured a germline frameshift mutation in exon 6 of *SDHB* gene [c.587–591DelC]. Seventeen years later, she was diagnosed with acromegaly and underwent transesphenoidal endoscopic resection of a somatotropinoma. Three months after surgery she started treatment with lanreotide for residual disease. Despite initial good response, she developed resistance to first generation of somatostatin analogues and treatment had to be switched to pegvisomant. In the immunohistochemical staining, the pituitary adenoma was positive for SDHA expression, while SDHB showed an heterogeneous staining pattern, with areas markedly positive and others with positive and negative cells.

Conclusions Our findings provide useful data for understanding the link between paragangliomas/pheocromocytomas and somatotropinomas. While we confirm the well-established link between *SDHB* mutations and paragangliomas/pheocromocytomas, particularly with malignant paragangliomas, the preservation—at least partially—of SDHB expression in the somatotropinoma tissue does not allow drawing definite conclusions about the involvement of the *SDHB* mutation in pituitary adenoma.

Keywords Paraganglioma · Somatotropinoma · *SDHB* gene · Resistance to Somatostatin Analogs

Introduction

Pituitary adenomas (PA) and paragangliomas/pheocromocytomas (PGL/Pheo) are two rare endocrine tumours. There are reports of their coexistence in the same individual, but during many years it was considered a coincidental finding. Recently, Xekouki et al. confirmed an association between these two entities and designated it as "3PAs" [1]. This association is mostly due to *SDHx* defects, although mutations in other genes implicated both on familial PA and PGL/Pheo pathogenesis can also occur [1, 2].

Since 2012, when the first PA associated with a *SDHD* mutation was reported in a family with multiple PGL/Pheo [3], the literature describes a total of 72 patients harbouring both PA and PGL/Pheo [2]. Although <0.3% of all PA are associated with *SDHx* mutations [4], the identification of particular features that differentiate them from sporadic cases is crucial.

Here, we report a patient with malignant PGL and somatotropinoma associated with a germline *SDHB* mutation, and its clinical evolution, particularly in terms of aggressiveness and pharmacological therapy response.

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Case report

A 57-year-old female patient was under surveillance since 1997 for a malignant PGL (vertebral and lymph nodes metastasis), which were detected during evaluation for headache, palpitations, and resistant hypertension. Biochemical results showed elevated urinary dopamine 711 µg/24 h (reference values (RV) 64–400 µg/24 h), norepinephrine 3161 µg/24 h (RV 23–105 µg/24 h), and normetanephrine 3546 µg/24 h (RV 88–444 µg/24 h); epinephrine and metanephrine were in the normal reference range. Abdominal computerized tomography disclosed a 5.2 cm mass in the left adrenal region, with corresponding uptake on ^{123}I-MIBG-scintigraphy. No other areas of radiotracer uptake were found on ^{123}I-MIBG-scintigraphy. Patient underwent laparoscopic surgery for tumour removal. Intraoperatively, the lesion was found to be independent of the left adrenal gland. Pathology and immunohistochemistry examination of the mass confirmed PGL diagnosis. Post-operatively there was remission of symptomatology and normalization of urinary catecholamine and metanephrine levels. Few months after surgery, genetic testing demonstrated that the patient carried a germline frameshift mutation in exon 6 of *SDHB* gene [c.587–591DelC] (Fig. 1). Genomic DNA from peripheral blood leukocytes was obtained using a standard salt-precipitation method. The entire coding region and exon-flanking regions of the *SDHB* gene were amplified by polymerase chain reaction and visualised in agarose gels. Sanger sequencing was performed using BigDye Terminator Sequencing kit, after enzymatic purification of the PCR products. Sequencing samples were then ran on an automated sequencer.

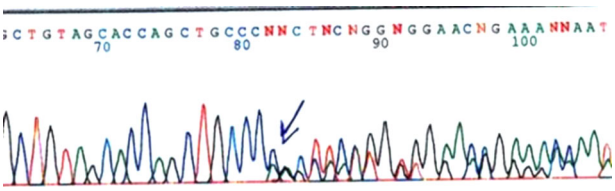

Fig. 1 Genomic DNA analysis from peripheral blood leukocytes, showing a germline frameshift mutation in exon 6 of *SDHB* gene [c.587–591DelC] (arrow)

When questioned the patient refered that she had a cousin with past history of Pheo diagnosed at 13 years of age and died with metastatic disease. Later an uncle of the patient had been diagnosed with Pheo and died in another institution (heredogram is shown in Fig. 2), but we did not have material to test for the *SDHB* mutation. The remaining carriers are being followed in our institution through regular urinary metanephrine measurement.

Patient was kept under surveillance with no signs of disease during 9 years, but in 2006 routine analysis showed an elevation of urinary norepinephrine 442 µg/24 h (RV 15–80 µg/24 h) and normetanephrine 2974 µg/24 h (RV < 390 µg/24 h). Abdominal CT and ^{123}I-MIBG-cintigraphy confirmed local recurrence of the previous PGL and a second surgery was performed, after which there was a progressive reduction on biochemical results. After 1 year, a bone metastasis in D11 vertebral body was diagnosed, after she complained of back pain. Analytical evaluation showed new elevation of urinary norepinephrine and normetanephrine [norepinephrine 773 nmol/24 h (RV 89–473 nmol/24 h) and normetanephrine 3521 nmol/24 h (RV 480–2424 nmol/24 h)]; epinephrine and dopamine were under the normal range, while metanephrine was elevated

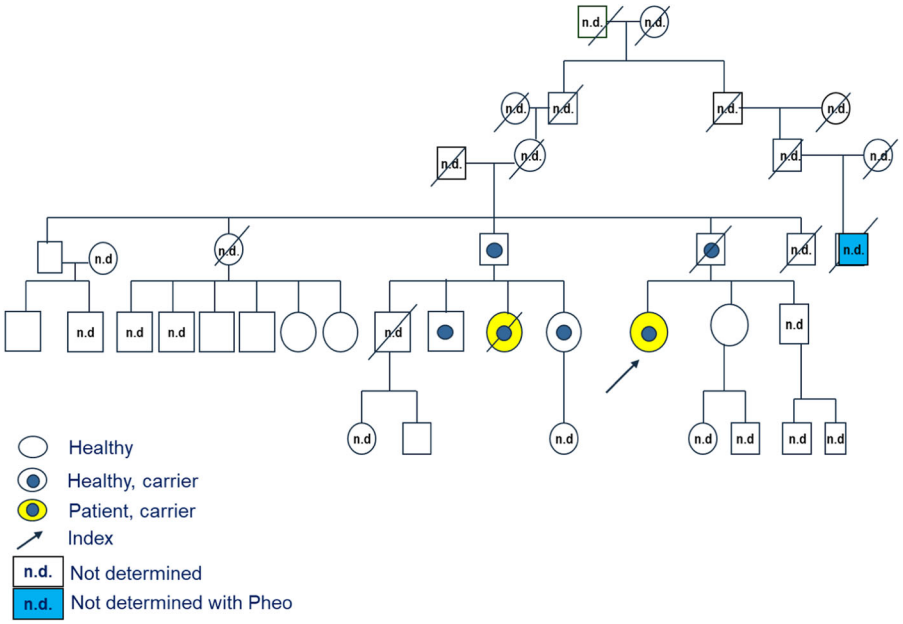

Fig. 2 Heredogram

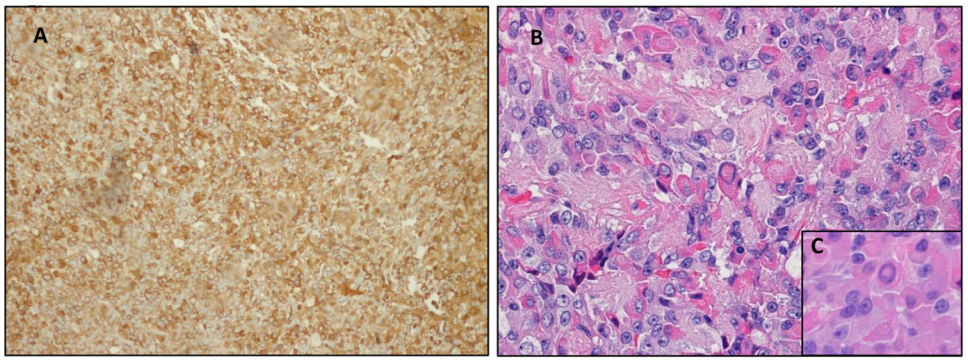

Fig. 3 Pituitary adenoma. **a** Immunohistochemical staining for GH. **b** Hematoxilin–Eosin staining 40×, with the presence of a nuclear pseudoinclusion. **c** Hematoxilin–Eosin staining 100×, pointing out the nuclear peudoinclusion

(2404 nmol/24 h, RV 264–1729 nmol/24h). Surgical excision of bone metastasis was initially tried, with improvement of the clinical symptoms and normalization of the analytical parameters. A new metastasis to a lymph node (25 × 21 mm) at the level of the right renal hilum was diagnosed and surgically removed in 2009. As elevated urinary metanephrines and D11 vertebral hyperfixation in ^{123}I-MIBG-cintigraphy persisted, two treatments with ^{131}I-MIBG were performed, followed by spine conventional radiotherapy. After these treatments, the patient presented a good clinical response, with regression of the vertebral lesion on imagiological evaluation. Since 2013, she has been under surveillance and the levels of urinary catecholamines and metanephrines remain normal.

In 2014, during follow-up, she referred increase of her hands/feet and she was diagnosed with acromegaly in the dependency of a pituitary macroadenoma—increased IGF-1 216 ng/mL (RV 71-284 ng/mL), no growth hormone suppression after 75 g OGTT; and pituitary MRI showed a probable pituitary adenoma with 11.8×14.3×11.1 mm). She underwent transsphenoidal endoscopic resection and pathology and immunohistochemistry confirmed the diagnosis of somatotropinoma (Fig. 3a).

Three months after surgery, the patient presented with residual disease, not suitable for surgical re-intervention, and it was decided to start medical therapy with lanreotide. There was a good initial response to treatment (Table 1), but about 12 months later the patient presented resistance, with biochemical worsening. Treatment with octreotide was attempted, but it also failed to achieve disease control, and therefore, treatment had to be switched to pegvisomant.

After the acromegaly diagnosis of the index patient, the follow-up *SDHB* mutation carriers also included the measurement of GH and IGF-1. Until now, we have no clinical or biochemical evidence of disease in the carriers.

Considering the rare association of these two diseases in the same patient, we hypothesized that the two tumours could be related. To investigate this possibility, we performed genetic analysis of the *SDHB* mutation in PA DNA using Sanger sequencing. We also analysed the pattern of SDHB and SDHA immunohistochemical staining in the PA, and also in primitive PGL and vertebral metastasis. The presence of the wild-type and mutant alleles, as evidenced in the electropherogram of the *SDHB* mutation, does not suggest LOH in the PA (Fig. 4), although it should be noted that Sanger sequencing is not the most adequate method to assess loss of heterozigoty (LOH).

SDHA and SDHB subunits' immunohistochemical staining was performed using mouse monoclonal antibodies specific for SDHA and SDHB. Signal detection was performed with a labelled streptavidin–biotin immunoperoxidase detection system. Tissue sections were counterstained with haematoxylin and the presence or absence of immunostaining in tumour cells was evaluated and is shown in Fig. 5. In the immunohistochemistry analysis, the initial PGL and the vertebral metastasis were both positive for SDHA and negative for SDHB expression (Fig. 5A1, A2 and Fig. 5C1, C2). In the PA, we found positivity for SDHA (Fig. 5E1) expression and a heterogeneous staining pattern for SDHB, with areas within the tumour where cells were markedly positive (Fig. 5E2) and other areas where positive and negative cells coexist (Fig. 5E3).

In the immunohistochemical analysis, negative and positive controls were used simultaneously to ensure specificity and reliability of the staining process. For the negative controls we omitted the primary antibody from the immunohistochemistry. As positive internal control, we used normal cells in the PGL section, outside the tumour bed (pointed out in the Fig. 5B1, B2) and normal cells in the vertebral metastasis section, outside the metastasis area (pointed out in the Fig. 5D1, D2).

Discussion

Succinate dehydrogenase (SDH) is a multimeric four-subunit enzyme, which is bound to the inner mitochondrial membrane, where it participates in the Krebs cycle and

Table 1 Analytical response to medical treatment for acromegaly

	Mar/2014	**Sept/2014**	Dec/2014	Sept/2015	Feb/2016	Aug/2016	Oct/2016	Feb/2017	May/2017	Jul/2017
IGF-1 (RV)	1216 µg/L (71–284)	**Transphenoidal endoscopic surgery**	485 µg/L (71–284)	192 µg/L (71–284)	335 µg/L (81–238)	302 µg/L (81–238)	340 µg/L (81–238)	303 µg/L (71–284	349 µg/L (71–284)	114 µg/L (36–200)
GH (RV < 8 µg/L)	—		—	0.82 µg/L	0.73 µg/L	1.39 µg/L	2.06 µg/L	1.38 µg/L	0.59 µg/L	—
OGTT	0 min 8.54 µg/L Nadir 11.4 µg/L		0 min 4.18 µg/L Nadir 1.12 µg/L	—	—	—	—	—	—	—
Medical treatment	—		—	Lanreotide 120 mg/monthly				Octreotide LAR 40 mg/monthly		Pegvisomant

RV reference values, *OGTT* oral glucose tolerance test

electron transport chain [4, 5]. The genes encoding the four SDH subunits—*SDHA, SDHB, SDHC*, and *SDHD*—have been demonstrated to be tumour-supressor genes, as they predispose to the development of hereditary PGL/Pheo. The proposed tumorigenic mechanism behind *SDHx* mutations involves succinate accumulation in the tumour tissue (substrate accumulation), with consequent inhibition of prolyl hydroxylases (PHDs). PHDs are iron-dependent and oxygen-dependent enzymes, which hydroxylate and inhibit the hypoxia-inducible factor-α (HIF-1α). In the event of a *SDHx* mutation, succinate accumulation inhibits PHDs, which results in lack of HIF-1α hydroxylation and consequent stabilization. When stabilized, HIF-1α translocates to the nucleus, where it forms a complex with additional proteins and triggers the activation of the hypoxia pathway to promote tumour formation [5]. Another consequence of SDH inactivation is an increased production of reactive oxygen species, which also contributes to PHDs inhibition [2, 4]. Succinate accumulation is also able to inhibit other enzymes, including histone demethylases resulting in epigenetic modification [2, 5].

SDHx mutations are classically associated with familial PGL/Pheo, but they are also linked to other tumours, such as gastrointestinal stromal tumours (GISTs), renal-cell carcinomas, and more recently to PA [4, 5]. In the last 5 years, mutations in any of the four genes encoding SDH subunits and the assembly factor *SDHAF2* were identified in patients with PA and PGL/Pheo [2]. To our knowledge, only six patients were reported in literature as having both PA and PGL/Pheo, while also carrying a germline *SDHB* mutation [2].

Phenotypically, the PA of the previously described six patients were non-functioning adenomas (two patients), prolactinomas (three patients) and a somatotropinoma (one patient) [2]. This last case was a male patient who presented a unilateral vagal body PGL at the age of 70, a nonsecreting adrenal nodule at the age of 71, and a somatotropinoma at the age of 72. Genetic testing from peripheral blood lymphocytes showed a *SDHB* exon 7 pathogenic mutation (c.689 G > A, p.Arg230His), but analysis for LOH on PA was not performed [1]. Only two out of the six patients (one with a prolactinoma and the other patient presenting a non-functioning pituitary adenoma) had demonstrated LOH at *SDHB* locus in the PA.

In the presence of a germline *SDHB* gene mutation, LOH is the predominant mechanism of inactivation of the wild-type allele, nonetheless, combinations of germline mutations with somatic mutations and epigenetic silencing can also occur and may account for the clinical variability observed among carriers of an identical *SDHx* germline mutation [5].

In our patient, the germline *SDHB* mutation was known for many years before somatotropinoma diagnosis. Since a

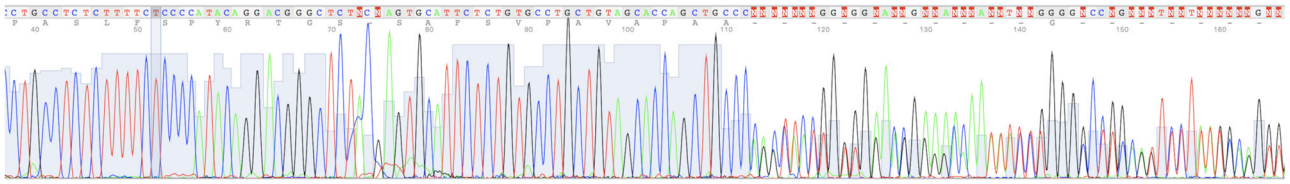

Fig. 4 Pituitary adenoma electropherogram (Sanger sequencing method)

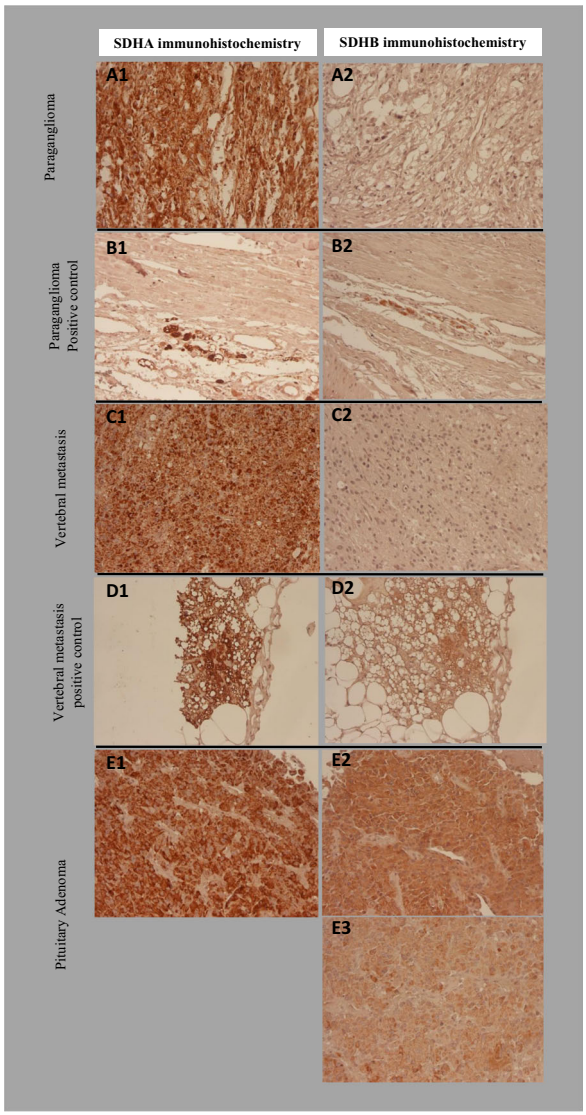

Fig. 5 Immunohistochemical analysis. The initial PGL and the vertebral metastasis were both positive for SDHA and negative for SDHB. (Fig. 5A1, A2, C1, C2). At the pituitary level we found positivity for SDHA (Fig. 5E1) and heterogeneous staining pattern for SDHB, with areas within the tumour where cells are markedly positive for SDHB staining (Fig. 5E2) and other areas where there are positive and negative cells (Fig. 5E3). Negative and positive controls were used simultaneously to ensure specificity and reliability of the staining process. For the negative controls we omitted the primary antibody from the immunohistochemistry. As positive internal control, we used normal cells in the PGL section, outside the tumour bed (pointed out in the Fig. 5B1, B2) and normal cells in the vertebral metastasis section, outside the metastasis area (pointed out in the Fig. 5 D1, D2)

positive SDHA staining, together with negative SDHB staining in the tumour tissue has been considered a surrogate marker for the absence of functional SDH [4, 5], we used immunohistochemical staining to demonstrate the involvement of this mutation in the development of the three lesions. Our analysis showed that both initial PGL and the vertebral metastasis were positive for SDHA and negative for SDHB, which is in agreement with their common origin. In the PA, however, we found positivity for SDHA and a partial preservation of SDHB (areas with only positive cells and areas where positive and negative cells coexist). Together with the apparent lack of LOH in *SDHB*, our results raise questions regarding the causative link between the *SDHB* mutation and the development of PA as initially hypothesized. Two situations might explain this finding:

1. The PA is a coincidental finding in this patient, not related with the *SDHB* mutation
2. The PA development does not require a complete loss of SDHB expression, rather a partial loss that would be enough to trigger PA.

Considering the already available reports in the literature, which argue for a causative link between *SDHB* mutations and PA development, the latter explanation might be the most plausible one. Noteworthy, complete SDHB loss was detected in the malignant lesion—PGL—and in the respective metastasis, as opposed to the benign nature of the PA where partial SDHB loss was found.

Although evidence is sparse regarding the clinical characteristics of PA harbouring *SDHx* mutations, an analysis of the literature suggests that *SDHx*-related PA can have different phenotypes (somatotropinomas, prolactinomas, and non-functioning adenomas), be associated with more aggressive features and be more resistant to somatostatin analogues (SSA) [1, 4]. Specific histological findings typical of PA, namely pseudonuclear inclusions [2, 6], were also found in this case (Fig. 3b, d).

We achieved disease control in our patient, using lanreotide during about 12 months, although she progressed to resistance to SSA. This temporary response to first generation SSA is in accordance with the literature, showing that these tumours display more aggressive features and are

more resistant to SSA [1]. The mechanism behind this resistance is still not known, but considering the initial response to SSA observed in our patient, one may hypothesize that a reduction in SSTR2 receptors expression might be involved.

An additional question is the possible contribution of GH excess to the malignant behaviour of PGL. Although GH excess has been associated with increased risk for some malignancies, namely risk for colorectal carcinoma, and the fact that acromegaly presentation is insidious and the diagnosis frequently performed some years later, the fact that our patient harboured a *SDHB* gene mutation, which in turns is typically associated with a more aggressive behaviour of PGL, seems a more plausible explanation.

In conclusion, our study delivers more information about these new genetic syndromes and particular features, which may differentiate them from sporadic cases.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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