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CASE REPORT

A non-catecholamine-producing sympathetic paraganglioma of the spermatic cord: the importance of performing candidate gene mutation analysis

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Abstract
Background Catecholamine-producing tumours are called pheochromocytomas when they are located in the adrenal gland and sympathetic paragangliomas when they are located elsewhere in the abdomen. Rarely these tumours do not produce catecholamines and even more rarely they arise in the spermatic cord. Over the past decade, systematic mutation analysis of apparently sporadic cases of pheochromocytomas and paragangliomas has elucidated the frequent presence of germ line mutations in one of five candidate genes, including *RET*, *VHL*, *SDHB*, *SDHC*, and *SDHD*.
Clinical history and methods We describe a 45-year-old man with a non catecholamine-producing paraganglioma of the spermatic cord. We performed SDHB immunohistochemistry and performed mutation analysis of the *SDHB*, *SDHC*, and *SDHD* genes.
Results There was no staining of tumour cells with SDHB immunohistochemistry, indicative of an *SDH* mutation. Mutation analysis demonstrated a germ line *SDHD* mutation (p.Val147Met).
Conclusions Systematic mutation analysis is required in paraganglioma patients for the detection of germ line mutations. This should be preceded by SDHB immunohistochemistry to limit the number of genes to be tested.

Keywords Sympathetic paraganglioma · Spermatic cord · SDHD · Immunohistochemistry

Precis Candidate gene mutation analysis on a paraganglioma of the spermatic cord

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Introduction

Extra-adrenal tumours originating from chromaffin cells are called sympathetic paragangliomas and arise from paraganglia that are distributed along the pre- and paravertebral sympathetic chains and the sympathetic nerve fibres, which innervate the pelvic and retroperitoneal organs. In contrast, tumours originating from the adrenal medulla are called pheochromocytomas [1]. Between 25% and 79% of sympathetic paragangliomas and about 90% of pheochromocytomas are associated with clinical signs of excess catecholamine secretion, while the remaining cases represent clinically non-functional tumours [2]. Most of these tumours produce but do not secrete catecholamines and may therefore evade detection for many years.

The majority of sympathetic paragangliomas occur in various abdominal sites, mostly in a paravertebral location, or in the organ of Zuckerkandl, a sympathetic paraganglion that plays an important role early in life. More rarely, sympathetic paragangliomas have been described in the bladder wall, which may elicit micturition-related complaints of excess catecholamine secretion [3, 4]. An even less frequent location is in the spermatic cord, where until

now eight cases had been described in the international literature [5–12]; however, none of these have been investigated for mutations in sympathetic paraganglioma-related genes. An overview of the clinical data of these previous eight patients is presented in Table 1.

Between 12% and 24% of apparently sporadic paragangliomas have been shown over the past decade to have a hereditary basis, involving mutations in one of five different genes: the REarranged during Transfection (*RET*) proto-oncogene, the von Hippel–Lindau (*VHL*) gene, and the succinate dehydrogenase subunits B (*SDHB*), C (*SDHC*), and D (*SDHD*) genes [13], [14].

In the present study, we report the first case of a spermatic cord sympathetic paraganglioma in which the tumour tissue was investigated by SDHB immunohistochemistry. This was shown to be a useful tool in diagnosing paraganglioma patients with *SDHx* mutations; negative immunostaining was seen in paragangliomas with *SDHx* mutations, whereas paragangliomas without mutations are positive with SDHB immunohistochemistry [15]. In this spermatic cord sympathetic paraganglioma, the negative immunostaining gave an important clue for the presence of an *SDHx* mutation, which was subsequently shown to be a previously unknown germ line *SDHD* mutation.

Clinical history

A 45-year-old man presented with a painless lump in his left hemiscrotum of months' duration. In his past medical history, an episode of acute left epididymitis, which subsided with antibiotics, was recorded 20 years earlier. On physical examination, a palpable, painless, firm mass was revealed in the upper pole of this left testicle. With a provisional diagnosis of a testicular neoplasm, a left inguinal orchiectomy was recommended and subsequently performed. His blood pressure was stable during and after surgery. The orchiectomy specimen displayed a tumour mass confined to the spermatic cord measuring 4.8×3.3×2.5 cm, weighing 71 g, surrounded by a capsule. The cut surface showed homogeneously reddish-white tumour tissue with an elastic consistency, while both the testis and epididymis were of normal colour, shape, and consistency (Fig. 1a).

Microscopically, there was a solid-looking tumour, with well-defined nests or trabeculae of tumour cells, separated by highly vascularized septa, focally thickened and hyalinized (Fig. 1b). Immunohistochemically, the tumour cells displayed strong and diffuse reactivity for vimentin, chromogranin A (Fig. 1c), synaptophysin, and neuron-specific enolase. Moreover, sustentacular cells were immunoreactive for S-100 protein. Taken together, a diagnosis of an abdominal, presumably sympathetic, paraganglioma was proffered. Following this histological diagnosis, the urologists performed an additional 24-h urinary analysis of catecholamines and their metabolites, which was shown to be normal. Three years after surgery, the patient is healthy, free of disease, and without tumours at other anatomic sites. There were no other family members known to the patient that had one or more pheochromocytomas or paragangliomas.

Material and methods

DNA was isolated from formalin fixed paraffin-embedded (FFPE) material. A region of at least 80% tumour cells was micro-dissected, and DNA was isolated using the Puregene DNA isolation kit (Gentra, Minneapolis, USA) according to manufacturer's protocol. An SDHB immunohistochemistry was performed using the rabbit polyclonal antibody HPA002868 (Sigma-Aldrich Corp, St. Louis, MO; 1:500) according to the method described by van Nederveen et al. [15]. Subsequently, mutation analysis was performed by direct sequencing of tumour tissue.

Results

The SDHB immunohistochemistry did not show any reactivity of the neoplastic cells (Fig. 1d). The mutation

Table 1 Clinical data of the previous eight patients with spermatic cord PGL

	Age	Symptoms	Hormonally active	Additional tumours	Reference
1	37	Painless mass right scrotal sac for 10 years	No	No	Eusebi et al. [5]
2	52	Painless mass left scrotal sac for 10 years/elevated blood pressure at operation	Yes	No	Soejima et al. [6]
3	18	Painless mass in right scrotal sac for 2 years	No	No	Bacchi at al. [7]
4	37	Painful mass in right scrotal sac	No	No	Mashat et al. [8]
5	40	Painless mass in left scrotal sac	No	No	Attaran et al. [9]
6	52	Lump within the right spermatic cord	No	No	Young et al. [10]
7	55	Painless left scrotal mass	Yes	Bilateral carotid body paragangliomas and bilateral pheochromocytomas	Abe et al. [11]
8	69	Weight loss, malaise, and mass in right testicle	No	No	Garaffa et al. [12]

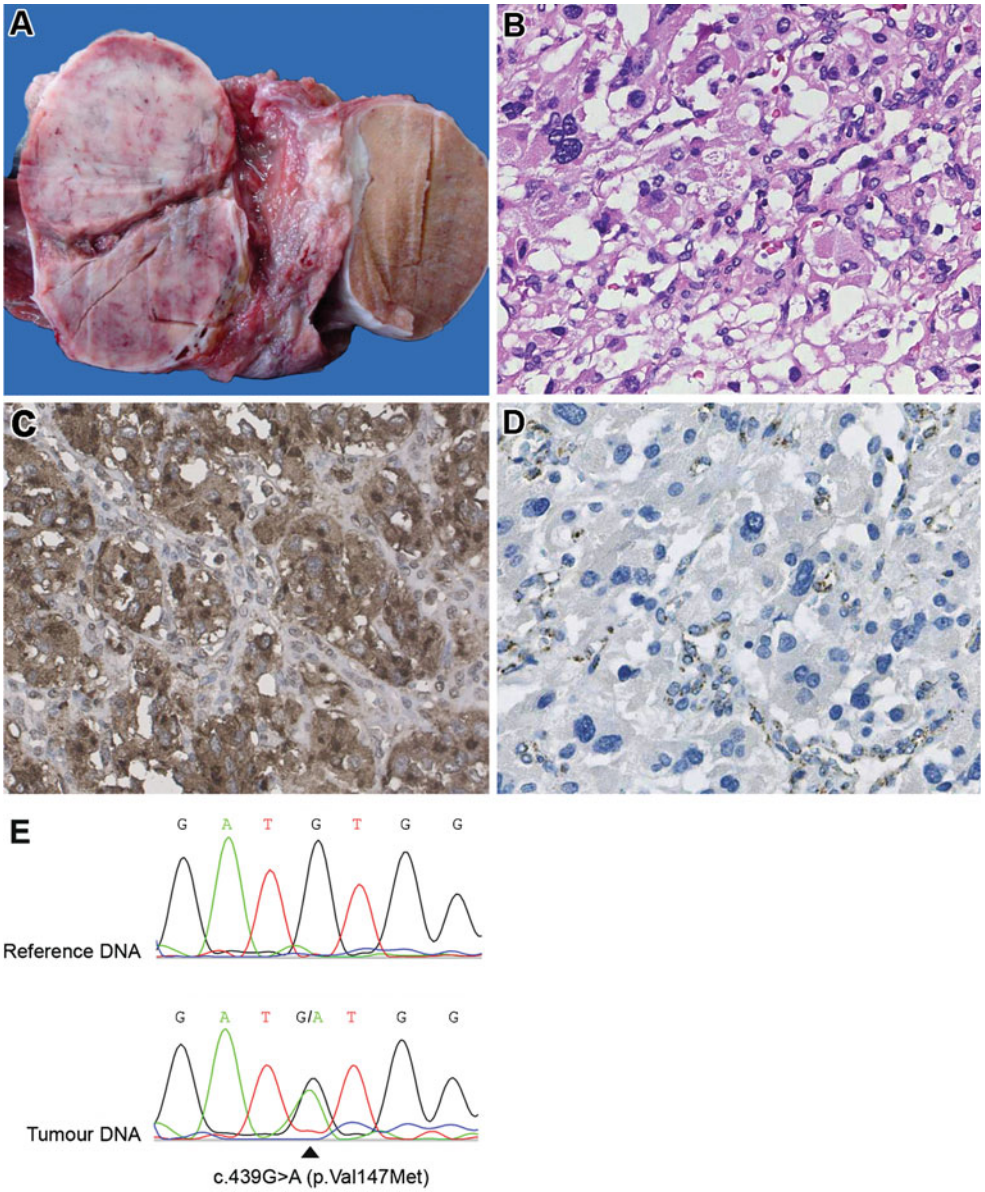

Fig. 1 **a** Gross aspect of spermatic cord paraganglioma. **b** Typical histology of the tumour showing large polygonal cells with ample amphophilic cytoplasm and moderate nuclear pleomorphism. **c** Chromogranin A immunohistochemistry showing positive staining in the tumour cells. **d** SDHB immunohistochemistry showing negative staining in tumour cells, while endothelial cells are immunoreactive. **e** Mutation analysis showing a p.Val147Met mutation in the tumour DNA compared with normal reference DNA

analysis that was performed by direct sequencing, on tumour tissue of this patient, showed an *SDHD* mutation in exon 4 (c.439 G→T, p.Val147Met) (Fig. 1e).

Discussion

In the present case report, we have described, for the first time in an individual case the use of SDHB immunohistochemistry for the guidance of subsequent DNA mutation analysis. Because of negative immunostaining, an *SDHx* mutation was predicted and eventually shown to be a p.Val147Met *SDHD* mutation.

This patient represents the ninth patient in the English literature with a spermatic cord paraganglioma, which was detected by its local mass effect, as it did not appear to produce catecholamines. The latter has not been formally proven, as no biochemical analyses had been carried out prior to surgery; however, the patient did not report any complaints that could be related to high blood pressure and/or catecholamine excess. The unusual location for a paraganglioma in the spermatic cord has been attributed to migration of neural crest progenitor cells, which are known to be present in the paraganglia throughout the abdomen, as is reflected by the various locations of abdominal sympathetic paraganglioma [16]. It is entirely conceivable that these progenitor cells migrate along with the developing male gonad and give rise to paragangliomas at low frequency.

Thus far, these paragangliomas have been described in middle-aged men, none of whom has had genetic testing, although one patient clearly had evidence of multiple tumours, for which we would strongly recommend systematic candi-

date gene mutation analysis [11]. Until about 10 years ago, pheochromocytomas and paragangliomas were known to occur in the context of various tumour syndromes, including multiple endocrine neoplasia type 2 (MEN 2), VHL disease, and NF1. Since the beginning of this decade, four additional genes (*SDHB*, *SDHC*, *SDHD, and SDHAF2*) have been added, causing the pheochromocytoma–paraganglioma syndrome, characterized by the occurrence of multiple pheochromocytoma and/or paraganglioma in the same patient and his or her family members. Whereas originally the frequency of germ line mutations in pheochromocytomas and paragangliomas was estimated at 10%, based upon patients from clearly recognizable familial tumour syndromes, systematic analysis of all genes (with the exception of *NF1*) has shown that an additional 15–25% of pheochromocytoma and paraganglioma patients are carriers of germ line mutations in each of these genes [13, 14]. Although somatic mutations in some of these genes have been described, they are quantitatively insignificant [4].

The finding of this mutation has important implications for further patient management, as it is known that patients with *SDHD* germ line mutations are at increased risk to develop further paragangliomas, either abdominal or head and neck, or even pheochromocytomas. In addition, other family members may also be affected, so they should be screened too.

Taken together, we show here that the spermatic cord can be a rare location for the occurrence of paragangliomas in male patients and that a stepwise immunohistochemical and genetic approach can be employed for the diagnosis of inherited paraganglioma, even in the absence of a positive family history.

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Conflict of interest statement We declare that we have no conflict of interest.

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