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CASE REPORT

L157X nonsense mutation of the succinate dehydrogenase subunit B gene in a Japanese patient with right paraaortic paraganglioma

Haruhiro Sato · Genta Kanai · Kenichi Hirabayshi · Hiroshi Kajiwara · Johbu Itoh · Robert Yoshiyuki Osamura

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Abstract Nuclear genes *succinate dehydrogenase B subunit* and *succinate dehydrogenase D subunit,* which encode two mitochondrial complex II subunits, are associated with the development of familial paraganglioma (PGL). *Succinate dehydrogenase B subunit gene* mutation is highly associated with extraadrenal PGL and subsequent distant metastasis. We describe the case of a 29-year-old Japanese man with a 3-year history of hypertension, headache, and palpitation. Endocrinological examinations showed that the patient had elevated levels of catecholamines, and imaging studies revealed a right paraaortic PGL without distant metastases. The PGL was surgically removed. Genetic analysis of the patient showed a heterozygous thymine deletion at position 470 (c.470delT) in exon 5 of the *succinate dehydrogenase B subunit gene* complementary DNA. This thymine deletion changed TTG (leucine) to TGA (stop codon) at codon 157 (L157X). It remains unclear whether this mutation was associated with PGL malignancy because the patient has had no metastases for the past 3 years. It has been recently reported that L157X is associated with malignant paraaortic PGL. Thus, strict follow-up is required because this *succinate dehydrogenase B subunit gene's* nonsense mutation (L157X) may be related to the malignancy.

Keywords Paraganglioma · *SDHB* · L157X · Malignancy

H. Sato (✉) · G. Kanai
Department of Medicine, Tokai University School of Medicine, Shimokasuya 143, Isehara, Kanagawa 259-1193, Japan
e-mail: hrhrsato@is.icc.u-tokai.ac.jp

K. Hirabayshi · H. Kajiwara · J. Itoh · R. Y. Osamura
Department of Pathology, Tokai University School of Medicine, Shimokasuya 143, Isehara, Kanagawa 259-1193, Japan

Introduction

A paraganglioma (PGL) is a tumor that derives from either sympathetic tissue in intraadrenal and extraadrenal locations or parasympathetic tissue of the head and neck. Intraadrenal PGL is also referred to as pheochromocytoma. The majority of sympathetic tissue-derived PGLs produce catecholamines, whereas parasympathetic PGLs usually do not [1, 2].

The mitochondrial complex II enzyme succinate dehydrogenase is a heterotetrameric protein consisting of A, B, C, and D subunits located on the inner mitochondrial membrane. Succinate dehydrogenase has a critical role in cellular energy metabolism through its dual role in the Krebs citric acid cycle and as part of the respiratory chain [3].

Germline mutations of the succinate dehydrogenase B subunit gene (*SDHB*) have been associated with susceptibility to intraadrenal PGL, extraadrenal PGL, and head and neck PGL (HNPGL) [4]. Typically, *SDHB*-related PGLs arise in extraadrenal locations and have a strong tendency for metastasis [5]. Among 84 patients with an apparently sporadic PGL, 8 patients had an *SDHB* mutation, and 7 of the 8 patients were related with extraadrenal site, and recurrence or malignancy [6]. Succinate dehydrogenase D subunit gene (*SDHD*) mutations were initially associated with multifocal HNPGL and benign PGL. Among 34 patients with *SDHD*-associated PGLs in a German/Polish cohort, 74% had multifocal PGL [7]. Succinate dehydrogenase C subunit gene (*SDHC*) mutations may also cause inherited HNPGL, and rarely, intraadrenal PGL [8, 9], although these are much less frequent than *SDHB* and *SDHD* mutations [10]. Interestingly, the succinate dehydrogenase A subunit (SDHA) has not been associated with neoplasia but can cause autosomal recessive juvenile encephalopathy [11].

In this study, we describe the case of a Japanese man with a germline *SDHB* nonsense mutation (L157X) and right paraaortic PGL; we also note the clinical course and management issues related to genetic testing.

Case report

A 29-year-old Japanese man with a 3-year history of hypertension, headache, and palpitation was referred to the hospital of Tokai University School of Medicine. During the previous 3 years, the patient had had headache and palpitation. He was diagnosed with hypertension at another hospital and received antihypertensive medication for 1 year. However, he was unwilling to continue antihypertensive therapy. On presentation, the patient was 178 cm in height and weighed 74 kg. His blood pressure was 182/116 mmHg and his heart rate was 138 beats/minute and regular. He had lost 8 kg during the previous 2 months and had a medical history of kidney stones. His father had normal blood pressure and heart rate and no medical history, whereas his mother was in remission from breast carcinoma and had no hypertension. His sister, aged 31, was healthy and had normal blood pressure. There was no family history of PGL.

Results from the endocrinological examinations are shown in Table 1. Levels of plasma and urine norepinephrine and its metabolites were unequivocally elevated, suggesting a norepinephrine producing tumor. Ultrasonographic examination of the neck showed no abnormality. Magnetic resonance imaging (MRI) of the neck, thorax, abdomen, and pelvis revealed only a right paraaortic tumor. ^{131}I-metaiodobenzylguanidine (MIBG) scintigraphy revealed accumulation at a right paraaortic site (Fig. 1) and no other accumulation was observed. Whole-body single-photon emission computed tomography (SPECT), i.e., ^{123}I-MIBG scintigraphy and computed tomography, demonstrated a tumor in the right paraaortic site (Fig. 2). These features were compatible with a right paraaortic PGL producing catecholamine. The whole-body SPECT also showed no accumulation suggesting no distant metastases. Oral administration of 16 mg/day doxazosin mesylate, 10 mg/day carvedilol, and 5 mg/day amlodipine besylate was initiated. Systolic blood pressure decreased to around 120 mmHg.

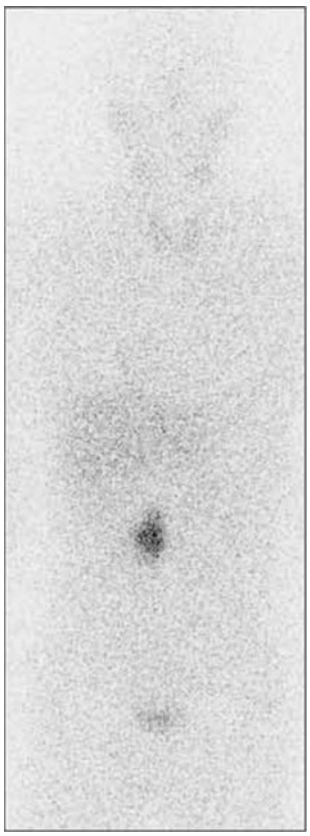

Fig. 1 ^{131}I-metaiodobenzylguanidine (MIBG) scintigraphy showed accumulation in the *right* renal hilum. No other accumulation was observed

Subsequently, the PGL was surgically removed. The blood pressure of the patient dropped to around 120 mmHg postoperatively without the administration of any antihypertensive medications. The patient has been doing well for

Table 1 Clinical course of catecholamine levels

Endocrinological tests	Initial	1 yr	2 yr	3 yr	Normal range
Serum epinephrine (pg/ml)	61	84	73	56	<100
Serum norepinephrine (pg/ml)	4395	428	448	324	100–450
Serum dopamine (pg/ml)	34	15	19	11	<20
Urinary epinephrine (μg/day)	14.2	N.D.	N.D.	N.D.	3.4–26.9
Urinary norepinephrine (μg/day)	1524.0	N.D.	N.D.	N.D.	48.6–168.0
Urinary dopamine (μg/day)	508.4	N.D.	N.D.	N.D.	365.0–961.0
Urinary metanephrine (mg/day)	0.11	N.D.	N.D.	N.D.	0.05–0.23
Urinary normetanephrine (mg/day)	2.01	N.D.	N.D.	N.D.	0.07–0.26

Initial at the first visit, *1 yr* 1 year after operation, *2 yr* 2 years after operation, *3 yr* 3 years after operation, *N.D.* not determined

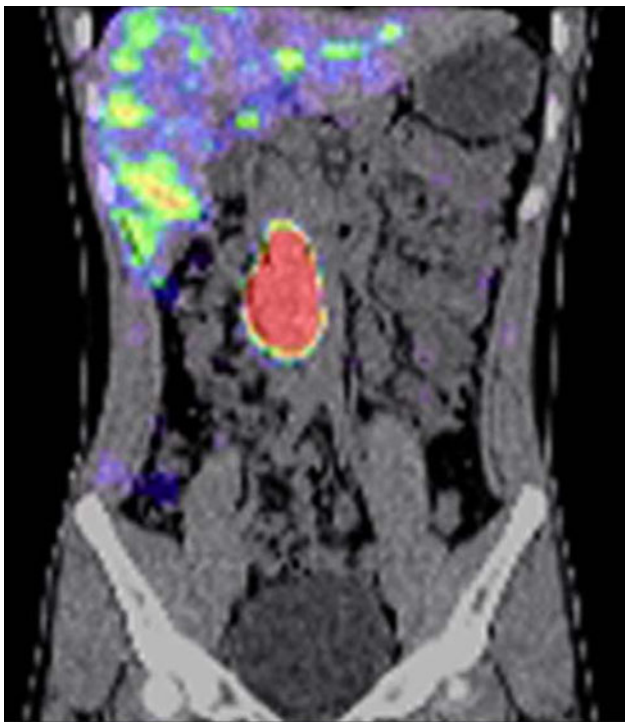

Fig. 2 Single photon emission computed tomography (SPECT) (^{123}I-MIBG scintigraphy and computed tomography) revealed a tumor at the *right* renal hilum

3 years and has normal blood pressure without any control of any antihypertensive medication. Annual endocrinological examinations have shown normal ranges for plasma catecholamines during the past 3 years (Table 1). Annual ultrasonographic examination and MRIs of the neck, abdomen, and pelvis have shown no abnormalities. These findings suggest that the PGL was completely removed and that metastasis and recurrence have not occurred during the past 3 years.

Gross examination revealed that the surgical specimen consisted of an encapsulated mass measuring 64 × 35 × 30 mm (Fig. 3a). The tumor cells were large and polygonal to spindle-shaped with granular basophilic or amphophilic cytoplasm; they were arranged in several architectural patterns, including trabeculae, solid sheets, and alveolar nests, surrounded by a capillary network imparting a Zellballen configuration (Fig. 3b). Vascular invasion of the tumor cells was observed (Fig. 3c). Immunohistochemical staining showed tumor cell positivity for chromogranin A (Fig. 4a) and synaptophysin (Fig. 4b). The Ki67 index was low (<1%) (Fig. 4c).

Materials and methods

Genetic analysis

The patient gave his informed consent for genetic testing and publication of his case history. The investigation was performed in accordance with the principles of the declaration of Helsinki. This study was approved by the institutional review board of the Tokai University School of Medicine. Blood samples were obtained from the patient and genomic DNA was isolated from the leukocytes using a standard protocol. Direct sequencing of amplified *SDHB* and *SDHD* was performed using the standard cycle on an automated sequencer, as described elsewhere [4, 12]. The patient's parents and sister also agreed to a medical interview. However, they were unwilling to undergo DNA testing of *SDHB* and *SDHD* and refused any genetic counseling or surveillance related to PGLs because they had no history of hypertension or PGL.

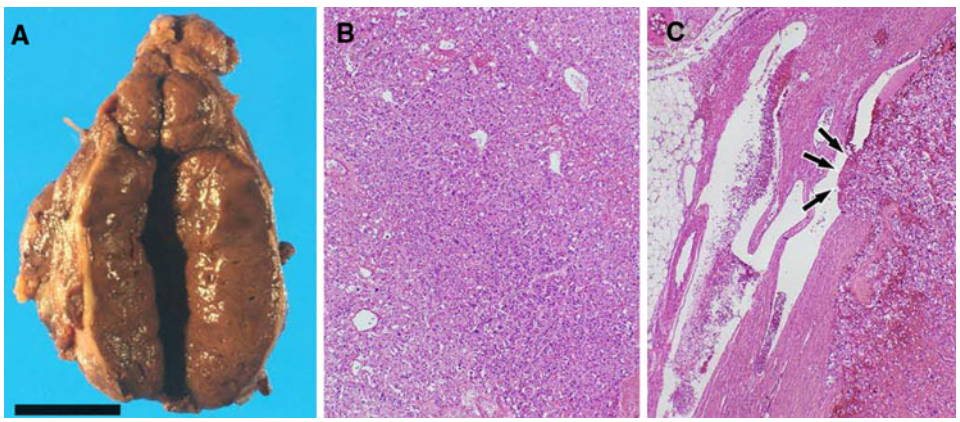

Fig. 3 **a** On gross examination, the surgical specimen consisted of an encapsulated mass measuring 64 × 35 × 30 mm. On cross-section, the tumor was *gray* and firm; areas of hemorrhage were not observed. *Black bar* indicates 2 cm. **b** The tumor had a thick fibrotic capsule without capsular invasion. Tumor cells were arranged in trabecular, solid, and Zellballen patterns in a vascular stroma. They were large, polygonal to oval in shape and had abundant finely granulated cytoplasm. **c** Vascular invasion of the tumor cells was observed (*arrows*)

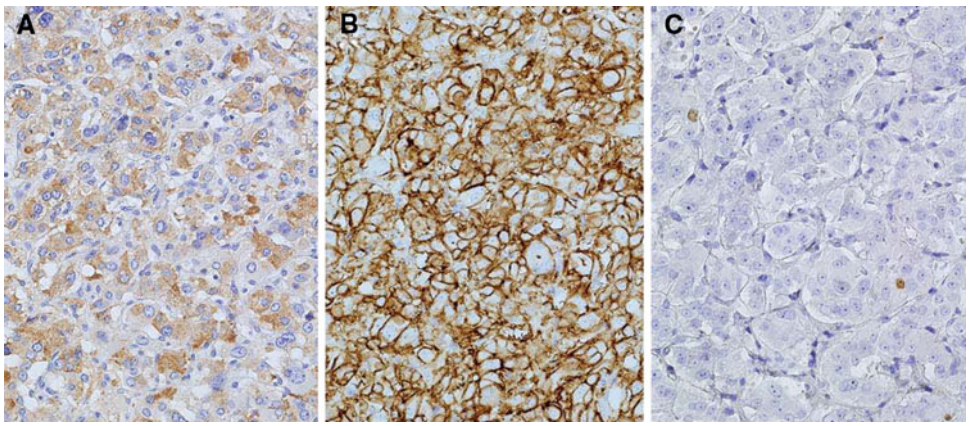

Fig. 4 Immunohistochemically, tumor cells stained positively for chromogranin A (**a**) and CD56 (**b**). The Ki67 index was low (<1%) (**c**)

Results

Genetic analysis

Genetic analysis revealed a heterozygous thymine (T) deletion of the complementary DNA at position 470 (c.470delT) in exon 5 of *SDHB*. This T deletion changed TTG (leucine) to TGA (stop codon) at codon 157 (L157X), predicting a truncated protein (Fig. 5). The patient had a wild type *SDHD* (data not shown).

Discussion

We identified a germline heterozygous nonsense *SDHB* mutation (L157X) in a patient with right paraaortic PGL without metastases. Metastasis is significant for diagnosing malignancy in PGL or for predicting the clinical course of PGL. Some metastatic PGLs show very little histological differences from their nonmetastatic counterparts. We identified vascular invasion of the tumor cells in this patient but malignant PGLs are defined by the presence of metastatic lesions at sites in which chromaffin tissue is normally absent [1]. A case of a *SDHB* nonsense mutation (L157X) in a 56-year-old Japanese patient with paraaortic PGL following malignant lung metastasis 7 years after the first operation was recently reported [13]. Thus, it is suggested that the *SDHB* nonsense mutation (L157X) may be related to PGL malignancy in our patient.

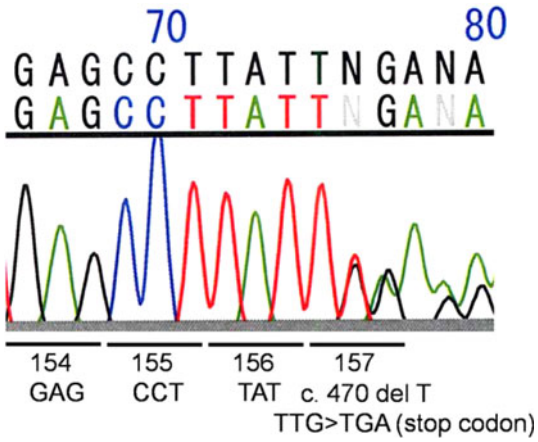

Fig. 5 Direct sequencing of *SDHB* genetic analysis of the patient revealed a heterozygous thymine (T) deletion at position 470 (c.470delT) in exon 5 of the *SDHB* complementary DNA. This T deletion changed TTG (leucine) to TGA (stop codon) at codon 157 (L157X)

Some reports have described the clinical course of *SDHB*-related PGL [14, 15]. Eight of 29 patients with *SDHB*-related PGLs presented with metastases and all but one eventually developed metastasis after 2.7 ± 4.1 years in a referral-based study [2]. It is suggested that in patients with *SDHB*-related PGLs, the rates of malignancy are high: 11 of 32 (34%) [7], 15 of 21 (71%) [16], and 18 of 48 (37.5%) patients [5]. Considering the high risk of developing additional *SDHB*-related tumors and/or metastatic lesions, it has been recommended that indefinite postoperative follow-up with bi-annual measurement of blood pressure and annual plasma or urine metanephrines and annual MRIs of the neck, thorax, abdomen, and pelvis should be performed [17]. Our patient may develop PGL-related metastasis and thus requires close monitoring.

The SDHB subunit contains three iron–sulfur clusters, is part of the hydrophilic catalytic domain, and binds to the SDHA subunit, which contains a covalently attached flavin adenine dinucleotide cofactor and the substrate binding site. The SDHB subunit also binds to the two hydrophobic membrane anchor subunits, the SDHC and SDHD subunits. SDHC and SDHD attach the complex to the mitochondrial inner membrane and also contain the ubiquinone binding site group to which the electrons are transferred from the iron–sulfur clusters within the SDHB subunit. The catalytic

domain of SDHB is necessary for the enzymatic activity of complex II [3]. It has been suggested that the *SDHB* mutation results in a complete loss of electron transport chain complex II activity in mitochondria, resulting in *SDHB*-related PGLs [18]. It has also been suggested that activation of hypoxic and angiogenic pathways may be related to the induction of PGLs [19].

Because the parents and sister of our patient were unwilling to undergo *SDHB* analysis, we were unable to determine if this mutation was inherited or sporadic. Twelve of 271 patients with apparently sporadic PGLs were shown to carry *SDHB* germline mutations in another study [20], and *SDHB* mutations in three families were reported to show variable penetration [21]. In these three families, the *SDHB* mutation-positive parents of each proband had no disease symptoms, yet the probands manifested the disease at an early age (12, 15, and 7 years). This pattern of disease presentation suggests that *SDHB* may be a variably penetrant gene with its expression influenced by genetic or environmental modifiers.

The patient's parents and sister were unwilling to undergo DNA analyses or receive any genetic counseling or surveillance related to PGLs because they had no history of hypertension or PGL. Thus, we were unable to perform further DNA analyses or undertake any genetic counseling and surveillance related to PGL for ethical reasons. It has been reported that approximately 1–5% of carriers of *SDHB* or *SDHD* mutations had been found to have renal cell carcinoma or papillary thyroid carcinoma [5, 22]. Recently, breast carcinoma was also identified in 6 out of 9 female carriers of *SDHB* or *SDHD* mutations [23]. The mother of our patient had a medical history of breast carcinoma, currently in remission. However, it is unclear whether the mother is a *SDHB* mutation carrier and whether her breast carcinoma was related to a *SDHB* mutation (L157X).

In conclusion, we described here a 29-year-old Japanese man with right paraaortic PGL. The patient had no distant metastases at initial presentation but carried the *SDHB* nonsense mutation (L157X). Strict follow-up is required because it is suggested that this mutation may be related to the malignancy.

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