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Case Report

A case of ^{123}I-MIBG scintigram-negative functioning pheochromocytoma: immunohistochemical and molecular analysis with review of literature

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Abstract: A 70-year-old Japanese woman was referred to our hospital due to hyperhidrosis and rapid weight loss of 10 kg in a month. A lump measuring 26 mm in diameter was detected in the left adrenal gland by computed tomography. Biochemical tests showed high levels of serum and urinary norepinephrine and epinephrine. However, a ^{123}I-MIBG scintigram failed to detect any accumulation in the left adrenal tumor. A left adrenalectomy was performed post clinical diagnosis of ^{123}I-MIBG negative pheochromocytoma. Microscopically, the tumor exhibited pheochromocytoma compatible features. Immunohistochemical analysis revealed low expression of VMAT1 in the tumor compared to the normal, surrounding tissue. To test for a possible genetic alteration of the monoamine transporter genes, we performed whole-exome sequencing of the *VMAT1*, *VMAT2*, and *NET* genes in the tumor. No significant base sequence substitution or deletion/insertion was found in any transporter. This suggests that MIBG negativity is caused by a change that is independent of the base sequence abnormalities, such as an epigenetic change. Furthermore, a retrospective literature review of ^{123}I-MIBG negative-scintigraphy cases indicates that a negative finding in the ^{123}I-MIBG scintigram is frequently associated with metastatic pheochromocytomas or *SDHB* mutations. However, a *SDHB/D* gene mutation has not been identified in the reported case. Although the patient needs careful monitoring following the surgery, to date she has been disease free for 12 months. This study could not find clear reasons for negative conversion, however, investigations of the negative conversion mechanism might reveal significant insights towards the improvement of patient survival.

Keywords: Pheochromocytoma, MIBG, monoamine transporter, VMAT1

Introduction

Pheochromocytomas (PHEOs)/extra-adrenal paragangliomas (PGLs) are rare tumors arising from chromaffin cells of adrenal medullas (PHEOs) or extra-adrenal paraganglionic tissues (PGLs). Chromaffin cells produce catecholamines, which are synthesized from the amino acid tyrosine in a multi-step process. First, L-tyrosine is oxidized to L-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase in the cytoplasm of the chromaffin cells. Subsequently, L-DOPA is decarboxylated to dopamine, which is transported to and stored in the secretary vesicles of the cells. Here, it is converted to norepinephrine (NE) by dopamine-β-hydroxylase. Subsequently, a part of the NEs are released from the vesicles to the extracellular environment and rapidly routed back inside the cells into the cytoplasm by a norepinephrine transporter (NET). In the adrenal gland, a part of the NEs are again transported to the cytoplasm of chromaffin cells where they are converted to epinephrine (EP) by phenylethanolamine-N-methyltransferase (PNMT). Furthermore, EP is transported by the vesicular monoamine transporter (VMAT) 1 or 2 to the intracellular vesicles and released outside the cells (**Figure 1**).

Metaiodobenzylguanidine (MIBG) is a guanidine analogue similar to NE, which can enter chromaffin cells through both, active uptake via NET or passive diffusion. It is stored in the neurosecretory granules through VMAT [1, 2]. On this basis, $^{123/131}$I-MIBG scintigraphy is used as a diagnostic tool for the detection of PHEOs/PGLs [3, 4] and treatment of unresectable or

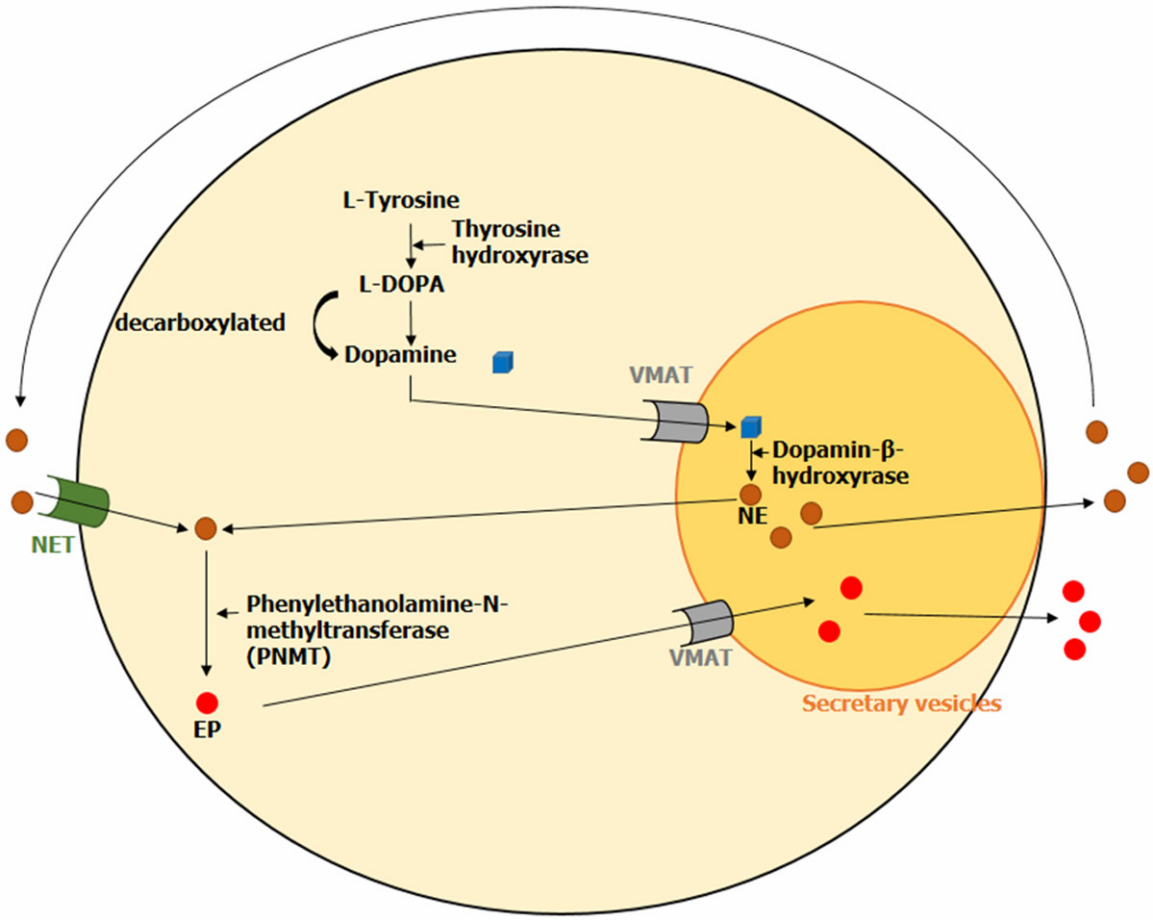

Figure 1. Illustration of the biochemical events occurring in a neuroendocrine cell of adrenal medulla.

multiple metastatic PHEOs/PGLs that demonstrate avid MIBG uptake [5].

Decreased sensitivity of $^{123/131}$I-MIBG scintigraphy may be caused by various reasons such as low affinity to NET, loss of NET, lack of storage granules, altered VMAT, or drug interferences [6, 7]. However, most of the reported $^{123/131}$I-MIBG -negative cases have no drug intervention, and the majority of them show high levels of serum or urinary NE. These findings suggest that in the false-negative cases the tumor cells do have neurosecretory vesicles thus producing NE in the vesicles but the conversion process of NE to EP in the cytoplasm by the uptake of NE to vesicles is altered.

Additionally, it has been reported that many patients with von Hippel-Lindau disease (vHLD) characterized by low NET expression, show MIBG negative-scintigraphy [7]. A recent report suggested that lack of the VMAT1 expression as probed by immunohistochemistry could be used to predict MIBG negative cases [8]. Those reports suggest that the majority of false-negative cases of $^{123/131}$I-MIBG scintigraphy could be attributed to abnormality of the monoamine transporters including VMAT.

Alternatively, it is known that PHEOs and PGLs in patients with negative $^{123/131}$I-MIBG single photon emission computed tomography (SPECT) follow a more aggressive course [9]. Generally, it is estimated that the rate of malignant PHEOs/PGLs is 15%. However, the rate of malignancy among patients with negative $^{123/131}$I-MIBG SPECT is higher (71%) [9]. Moreover, it was reported that $^{123/131}$I-MIBG negative cases are frequently linked to the presence of *SDHB* mutations [9, 10]. It is well established that PHEOs or PGLs caused by *SDHB* mutations are associated with shorter survival [11] and a higher incidence of malig-

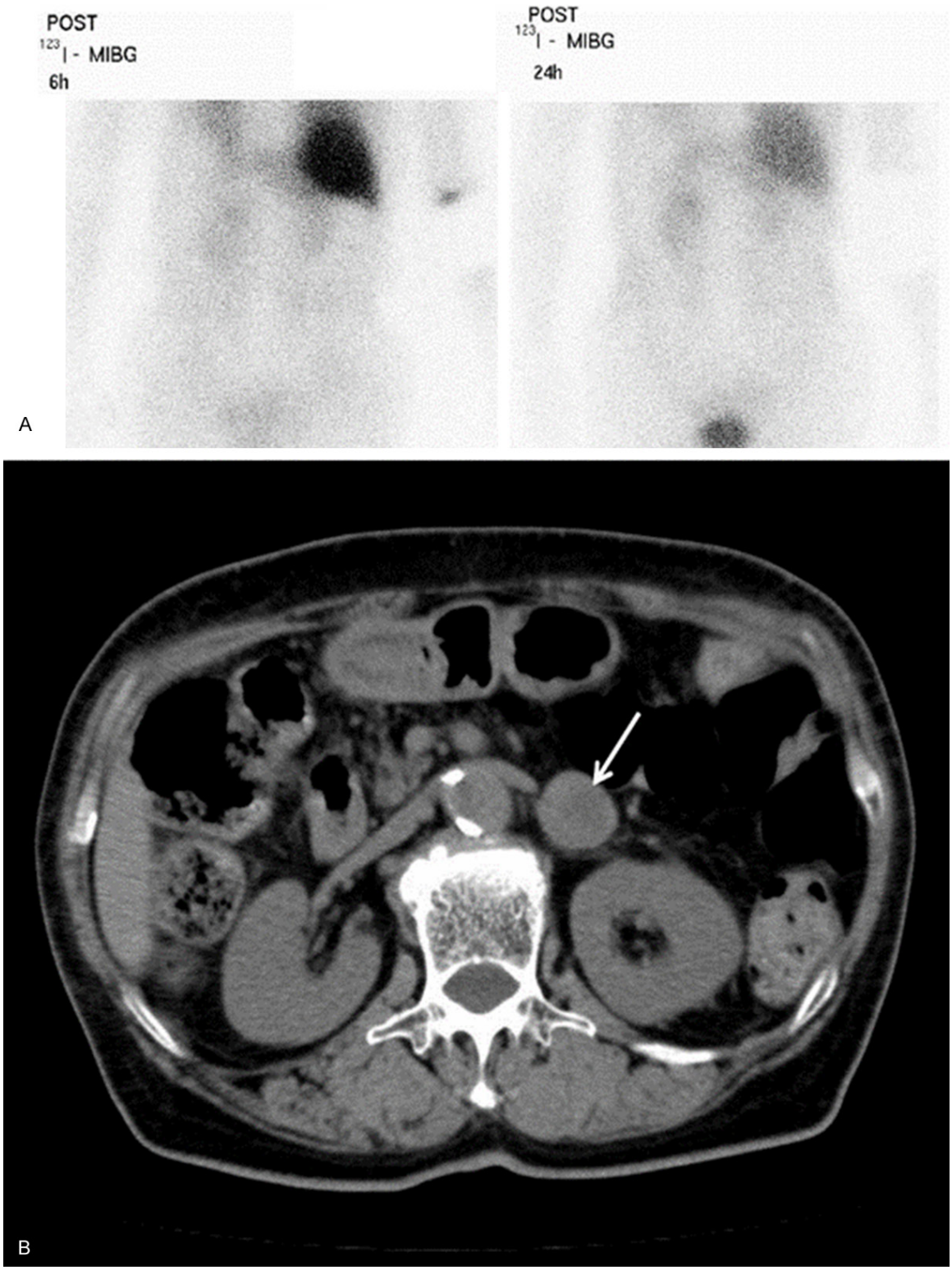

Figure 2. A: Functional imaging of a patient with MIBG-negative pheochromocytoma. The left adrenal tumor was not detected by ^{123}I MIBG scintigraphy. B: A computed tomography scan showing a tumor mass of 26 mm in diameter located in the left adrenal gland (arrow).

nancy [12]. Therefore, it is expected that the *SDHB* genetic alteration in MIBG negative cases will likely be strongly linked to the malignant phenotype.

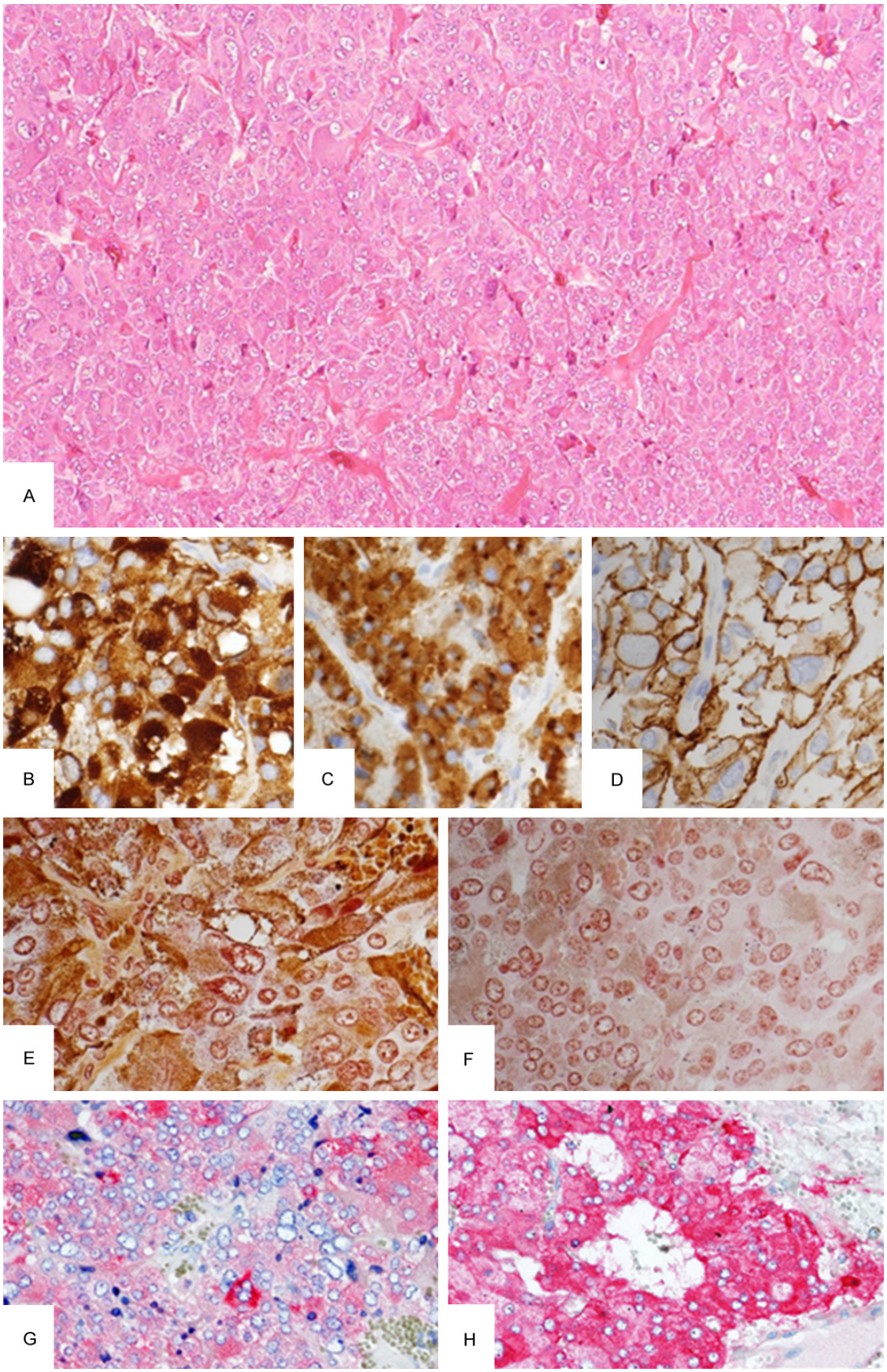
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Figure 3. (A) "Zellballen" architecture of the tumor as shown by hematoxylin and eosin staining. The tumor is composed of chief cells and sustentacular cells. (B-D) Immunohistochemical staining of chromogranin-A (B), synaptophysin (C), and CD56 (D) in MIBG negative-scintigraphy case. Tumor showed strong cytoplasmic expression of chromogranin-A, synaptophysin, and membranous expression of N-CAM. (E, F) Grimelius silver staining (E) and Masson-Fontana staining (F). Grimelius staining demonstrates numerous neurosecretory vesicles in the cytoplasm of the tumor cell but vesicles were close to negative for Masson-Fontana stain. (G, H) Immunohistochemical staining for VMAT-1. (G) In the ^{123}I-MIBG-negative case, approximately 15% of tumor cells showed positive cytoplasm staining (score 2+). (H) In the ^{123}I-MIBG-positive case, approximately 85% of the tumor cells showed positive staining (score 6+).

Table 1. MIBG-negative pheochromocytoma/paraganglioma reported in literatures

Ref	Age	Sex	Site	Size	Catecholamin type	Outcome	Genetic abnormality	Conversion case
[10]	56	M	LtAG		NA	Multiple meta→die (a few months later)		
[10]	51	M	RtAG		NA, A	Good	neurofibromatosis	
[10]	35	M	RtAG		NA, A	Good		
[19]	60	M	Liver	5 × 5 cm	NA	Spinal bone meta		
[18]	34	M	Aortic bifurcation	7.7 × 4.4 cm	NA	Multiple meta		
[18]	46	F	LtAG		?→not elevated	Multiple meta		MIBG+→ -
[18]	64	F	Aortic bifurcation		NA	Multiple meta		
[18]	25	F	Chest wall		NA	Multiple meta→die		
[18]	54	F	RtAG	5.5 × 5 × 2.2 cm	NA	Multiple meta		
[20]	39	F	RtAG	Φ3.1 cm	NA	Not available		
[21]	58	M	LtAG		NA	Multiple meta		
[21]	38	M	Urinary bladder	7.0 × 4.5 cm	NA	LN metastasis (15 month after OPE)		
[6]	54	F	BilateralAGs		NA	Multiple meta (6 years later)	vHLD	MIBG+→ -
[9]	13	F			NA, A	Not available	Sporadic	
[9]	47	F			NA, A	Not available	RET	
[9]	51	F			NA, A	Not available	Sporadic	
[9]	61	M			Not elevated	Not available	SDHB	
[9]	49	F			NA	Not available	Sporadic	
[9]	46	F			NA, A	Not available	Sporadic	
[9]	36	F			NA	Not available	SDHB	
[9]	40	F			NA	Recurrence	Sporadic	
[9]	55	M			NA	Meta	Neg for SDHX	
[9]	54	M			NA	Meta	SDHB	
[9]	42	F			NA	Meta	SDHB	
[9]	36	F			NA	Meta	SDHB	
[9]	32	M			NA, A	Meta	Sporadic	
[9]	47	F			Not elevated	Meta	SDHB	
[9]	43	F			NA	Meta	SDHB	
[9]	34	M			NA	Meta	SDHB	
[9]	37	M			NA	Meta	SDHB	
[9]	43	F			Not elevated	Meta	SDHB	
[9]	45	F			NA	Meta	Sporadic	
[9]	33	M			Not elevated	Meta	SDHB	
[9]	22	F			NA	Meta	Sporadic	
[22]	53	M	ltAG		NA	Not available		

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To our knowledge, genetic abnormalities of these transporters of MIBG-negative cases have not been reported to date. We herein report the first case of MIBG-negative pheochromocytoma in conjunction with immunohistochemical and genetic analysis.

Case report

Clinical history

Our patient was a 70-year-old Japanese woman referred for hyperhidrosis and a 10 kg per

Table 2. PCR and RT-PCR primer sequences for *VMAT1*, *VMAT2*, *NET*, *SDHB*, and *SDHD*

VMAT1	Forward	Reverse	PCR
Exon3 F1R1	tcatcctactacttgcaaccct	cccacagcaaattaaccctcagc	
F2R2	tcatcctactacttgcaaccct	aaccctcagcacaaagaccca	
Exon4 F1R1	tttcagtgccaattgtgccccacc	tcttcaacagccacggtgtt	
F2R2	aacaacaccgtggctgttgaa	gccacgaatgggttgaccag	
F3R3	tgcttcaaaggctgtgatgca	ccgaatagctgactcccctgagg	
Exon5	tcatactcatccacatgccct	accctgctatctacaccgca	
Exon6	gcaaaggcaatttgctaactgca	gccagtgttacatgcaacagat	
Exon7	aagggctctgagaggcggat	gtgccacttacccagcaacc	
Exon8	ctggaatctgtgtctcctatgc	atgcattcccaacctgacct	
Exon9	cagtagacacgctgaactgtc	cttcttatggtttggcaggca	
Exon10	actgtagatggtctctaagccca	cttacctgcagccaccaggat	
Exon11	caggtaagctggcgatgacctcc	cgaaaggactcatggcaccca	
Exon12	ggttacaggggtggacttcag	ctttcaaccgtatggaagctgtt	
Exon13	acaggtcagcatggcggtgat	tctgacagtccttgccaagc	
Exon14	caagatgggtcggtggctgtgtt	cagggaaaaccgatggcctt	RT-PCR
Exon15 F1R1	caagatgggtcggtggctgtgtt	aagccacatcagcgatggcgta	RT-PCR
Exon15 F2R2	tctcattggccccaatgcagg	cagggaaaaccgatggcctt	RT-PCR
Exon16	gtgtcctacgtggaggatgc	acagtcggtgctccttacaa	
Exon17	gttttccctggctcatggtc	aagaggtggtggtcactgaggca	RT-PCR
VMAT2	Forward	Reverse	PCR
Exon2 F1R1	ctccagattgggtccccgac	cgccaggaacacgatgaacag	
F2R2	ctccagattgggtccccgac	agtgagcagcatgttgtcca	
F3R3	tcgcggaagctcatcctgtt	ttgcaaagcgagttctccaa	
Exon3 F1R1	ctggagagggtttcagtgtg	gcggtctgatgaagtgtcagg	
F2R2	ctatggtcaccgggaatgct	acatgatgcagaatcccgca	RT-PCR
F3R3	ccttccgactgtcccagtgaa	aagcagaattccaggaaggac	
Exon4	gatcccaaagggtagccagg	taattcccggctcatgggcat	
Exon5	cactgatgtgcggtcttgga	atgagtccagctcactctgca	
Exon6	agggaaggcgagtcacgcat	gtcagtgcaaactgcgccag	
Exon7	gagagggcatgtgtcccagg	cagcacgatgccaaggtcat	
Exon8	acctgctttctgaagaggggct	ggttaggggtgtccccttctgac	
Exon9	catggtgctgcttctgacccgt	atcacctcgcattcccctgactc	
Exon10	ggaggcagaagccactacaa	caactcagcagggatgccct	
Exon11	ctgctctcatcaacattgtggt	ctccccattttgtgtgcaagta	
Exon12	acttgcacacaaaatggggaggt	ggtgattcttgcccaaagcgcaa	
Exon13	acacaagttcaagactttccgag	gaggaactacagtctaccagagg	
Exon14	tgactgtcttcaggaatggtgg	caccagcagaaggacctttcagg	
Exon15 F1R1	tgctataggtaaggacattggct	tctttggcaggtggacttcg	
F2R2	aaggcaattggatttccatggct	gctatcaggaaggccatgct	
Exon16	gcgattgctccaaatgactgg	ttctggatgagttgtgtcactgg	
NET	Forward	Reverse	PCR
Exon1 F1R1	ccctttatccaagcagagcctcg	gaagccgactacggacagca	
F2R2	tgctggtggtgaaggagcgcaa	gaagccgactacggacagca	
Exon2	acacggatccaagactggga	gggaagcctttatgaacaaccgt	
Exon3 F1R1	ggcctgggagactcctacct	ccaagcacggagccattgag	
F2R2	tcaacctgccctggaccgac	tatcctgacggcatgcgtctc	
Exon4	cgacatttaccctggtcccctc	caggccttgcacttccagct	

month weight loss. A CT scan showed in the left adrenal gland a tumor of 26 mm in diameter. Biochemical tests showed high levels of serum and urinary norepinephrine and epinephrine (serum noradrenaline 5.2 ng/mL, serum adrenaline 0.31 ng/mL, urinary normetanephrine 1.2 mg/mL, urinary metanephrine 0.42 mg /mL). However, ^{123}I-MIBG scintigraphy did not show any radioactivity in the left adrenal tumor. Left adrenalectomy was performed under the clinical diagnosis of ^{123}I-MIBG-negative pheochromocytoma (**Figure 2A** and **2B**). The patient was disease free at 12 months after the surgery.

Pathological findings

Grossly, the left adrenal tumor showed a clearly demarcated brownish, solid appearance and measured 23 × 20 × 20 mm^3. Microscopically, tumor cells displayed a "salt and pepper"-like chromatin pattern with eosinophilic cytoplasms and round nuclei. Tumor nests were surrounded by spindle-shaped supporting cells and organized in a "zellballen" architecture. No mitotic figures or nuclear pleomorphism were found. Marked sinusoidal neovascularization was observed within the tumor. Vascular or adrenal capsular invasion could not be visualized (**Figure 3A**).

Immunohistochemical study

Immunohistochemical staining was performed on a

Exon5	cagagcgaggctctcacctg	ggatcctgtccaaggctacac	
Exon6	gttccagtgttgtaggaagca	tctctgccacccaacaagag	
Exon7	cacagggttgagggtgtcaa	aataaggtgggcttctcagc	
Exon8	gctcagaccaatggtttcagcca	cagcactgtgtgttggggaa	
Exon9	gggagaccctaattcctgcacc	ttgaaatgcggcctcagagg	
Exon10	cacgtttgaccaaagagggcctc	cacacctccaggtgcaggat	
Exon11	tcatgggaggacctggccctg	agctccatcctggacacagc	
Exon12 F1R1	ccctttgctgtgatgctcac	accatggaggacagggcgat	
F2R2	cttcaagccactcacctacgacg	aggaatgagagtcgtcccgt	
Exon13	gtgtccctgggccaagctgag	aaaggcagctcacactgggt	
Exon14	cgggacaagggtgctgtgta	ccagctgagcggtaactcct	
SDHB	Forward	Reverse	PCR
Exon1	agagcgacctcggggttaag	tatgcttcctcagtctctcc	
Exon2	gccagcaaaatggaattatc	aagaactctccttcaatagctg	
Exon3	tgataaagtgtagggaggttg	agctgttttccagatgtctc	
Exon4	ggatatgggtgaggatgtg	cccccatgcaaataaaaac	
Exon5	tgaggtgatgatggaatctg	cacactcctggcaatcatc	
Exon6	gagtctctcccgtcacaag	cttctggatgcttgagtttc	
Exon7	ctgttgattggcagctcag	ttgtgagcacatgctacttc	
Exon8	gttttccctttcagtttcag	gctgtattcatggaaaaccaag	
SDHD	Forward	Reverse	PCR
Exon1	attgtcgcctaagtggttc	atgagtcctcacttccatcc	
Exon2	gtcctgttaaaggagaggttc	atggctagagcccagaaag	
Exon3	tgtgtgtttctcacatcaac	ctcacagcaaacaaactgag	
Exon4	gcagccaagttatctgtatagtc	aaaaaggtcagagcttccac	

semi-automated BENCHMARK XT system, (Ventana, Arizona, AZ) using the standard IHC protocol. Briefly, tissue sections serially cut from formalin-fixed paraffin specimens. For immunohistochemistry, tissue samples were stained with the following antibodies: Chromogranin-A A0430 (DAKO, Glostrup, Denmark) 1:200, rabbit polyclonal; Synaptophysin A0010 (DAKO, Glostrup, Denmark) 1:200, rabbit polyclonal; CD56 07-5603 (Invitrogen, Camarillo, CA) 1:50, mouse monoclonal; Glucagon A0565 (DAKO, Glostrup, Denmark) 1:100, rabbit polyclonal; Somatostatin A0566 (DAKO, Glostrup, Denmark) 1:100, rabbit polyclonal; Serotonin NCL-SEROTp (Novocastra, Newcastle, UK) 1:400; S-100 Z0311 (DAKO, Glostrup, Denmark) 1:800, rabbit polyclonal; SSTR-2A (Gramsch Laboratories, Schwabhausen, Germany) 1:800, rabbit polyclonal. Moreover, to confirm the previously reported role of VMAT1 expression in ^{123}I-MIBG negative tumors [8], we manually performed VMAT1 and VMAT2 immunohistochemical staining on a ^{123}I-MIBG negative pheochromocytoma and 7 ^{123}I-MIBG positive tumors randomly chosen from the pathology records of our hospital between 2009 and 2013. Slides were deparaffinized, hydrated and heat-induced epitope retrieval was conducted in pH 6.0 citrate buffer. The multi-step IHC protocol included peroxide blocking (15 min), overnight primary antibody incubation (VMAT1 HPA007210 (SIGMA-ALDRICH, Sweden) 1:50, rabbit polyclonal; VMAT2 HPA016856 (SIGMA-ALDRICH, Sweden) 1:100, rabbit polyclonal) and incubation with a rabbit anti-goat secondary antibody (Nichirei Bioscience, Tokyo, Japan) labeled with alkaline phosphatase. The reaction was developed with the Simplestain AP system (Nichirei Bioscience, Tokyo, Japan) and Fast-red was used to visualize the reaction. Tissues were counterstained with hematoxylin, allowed to dry in the air, perforated with xylene and covered by a glass coverslip.

The number of VMAT1, VMAT2 and SSTR-2A immunoreactive tumor cells was semi-quantitatively scored on a scale from – , absent; +, less than 10%; 2+, 10 to less than 20%; 3+, 20 to less than 40%; 4+, 40 to less than 60%; 5+, 60 to less than 80%; 6+, 80-100% as previously described [8].

Tumor cells were positively stained for chromogranin-A (**Figure 3B**), synaptophysin (**Figure 3C**) and CD56 (**Figure 3D**) and displayed focal positivity for S-100, serotonin, and somatostatin. Glucagon was negative, and SSTR-2A showed a +1 positive score. Ki-67 labeling index was approximately 1%. Grimelius stain showed numerous neurosecretory vesicles in the tumor cells (**Figure 3E**). However, the vesicles were close to negative for Masson-Fontana stain (**Figure 3F**).

Immunohistochemical staining for VMAT1 exhibited decreased positivity on the tumorous area of MIBG-negative case (2+). Conversely, all 7 MIBG positive cases were strongly positive for VMAT1 (5+, 1 case; 6+, 6 cases) (**Figure 3G** and **3H**). All cases were VMAT2 positive but the highest positivity was scored on the tumorous area of the MIBG-negative case.

Genetic testing

This case was tested for *VMAT1, VMAT2* and *NET* genetic alteration. DNA was extracted from the paraffin embedded tissues according to previously published procedures [13]. Informed consent and appropriate treatment of research subjects were obtained. DNA sequence was examined by polymerase chain reaction (PCR) following direct sequencing for the full open reading frames of these genes *VMAT1*, *VMAT2*, and *NET* using originally designed primer pairs (**Table 2**). For selected regions that could not be amplified on genome-based PCR, we also used RT-PCR based analysis. No significant abnormality in the nucleotide base sequence of any of the genes was identified.

Furthermore, we also screened for mutations in *SDHB* and *SDHD*. SDHB/SDHD is an enzymatic subunit of the mitochondrial complex II (succinate dehydrogenase, succinate: ubiquinone oxidoreductase) which participates in the aerobic electron transport and Krebs tricarboxylic acid cycle. It also plays an important part in tumor suppression [14]. Chromaffin tumors in patients with SDHB mutations are 6-fold more likely to be extra-adrenal than is expected for these tumors as a whole [15]. SDHD variants are most often associated with head and neck paragangliomas [16]. However, some adrenal sporadic cases of SDHB/SDHD mutations have been reported. We could not identify a SDHB/SDHD gene mutation for our case study.

Discussion

^{123}I-MIBG scintigraphy is used as a diagnostic tool for the detection of PHEOs/PGLs [3, 4]. The sensitivity of ^{123}I-MIBG scintigraphy in PHEOs is reported to be 95%, whereas it is only approximately 15% in PGLs occurring at extra-adrenal gland [8]. **Table 1** summarizes the reported cases of ^{123}I-MIBG negative scintigraphy of PHEOs/PGLs. This review clearly tells us that negative findings in ^{123}I-MIBG scintigraphy correlate with a malignant phenotype. In addition, we note the association with SDHB mutation given that the frequency of *SDHB* mutations in PHEOs and PGLs were reported to be approximately 4% [17].

In our case study, the tumor cells had neuroendocrine vesicles as revealed by Grimerius staining. However, they were negative for Masson-Fontana staining. This implies that tumor cells store NEs. The most significant increase of NE was observed in serum and urine. Therefore, we hypothesize that tumor cells may have neuroendocrine granules where dopamine is converted to NE but are not taken up via VMAT of EP, converted from NE in the cytoplasm (**Figure 1**). In reviewing the previously reported cases (**Table 1**), we note that the majority of MIBG negative cases (30 out of 35 cases) showed noradrenergic biochemical phenotypes, while 5 had normal levels and were thought to lack granules. Alternatively, the epinephrine reuptake to granules in the cytoplasm may have some functional disorder where norepinephrine is converted to epinephrine despite the tumor cells have neuroendocrine granules and could produce more norepinephrine. Our study showed decreased immunoreactivity of VMAT1 (2+) in a tumorous area of the MIBG-negative case, however, all of the MIBG-positive cases showed strong positivity for VMAT1 immunohistochemical staining (5+ to 6+). These findings are consistent with the aforementioned hypothesis.

Aiming to find possible abnormalities in the monoamine transporters in this case, we performed sequencing of exons 3 to 17 of *VMAT1,* 2 to 16 of *VMAT2*, 1 to 14 of *NET*, 1 to 8 of *SDHB*, and 1 to 4 of *SDHD* gene expanding over the entire coding region of each gene in the tumorous and non-tumorous tissues derived DNA of pheochromocytomas obtained by micro dissection. No genetic alteration was found in any of the tested transporters, despite the decreased expression of VMAT1 in the tumorous areas of the ^{123}I-MIBG negative case. This suggests that MIBG negativity is likely to arise through some change that does not involve base sequence abnormalities such as the epigenetic change. Two previously described cases of positive to negative transitions in the ^{123}I-MIBG up-take during recurrence of metastatic process of PHEOs or PGNs suggest the

possible presence of epigenetic regulation of these genes [6, 18].

Alternatively, ^{123}I-MIBG negative-scintigraphy is frequently related to metastatic pheochromocytomas and SDHB mutations as unfavorable prognostic factors. Our review of ^{123}I-MIBG negative-scintigraphy cases (**Table 1**) revealed a higher incidence of malignancy (92%; 24 out of 26 cases available for prognostic information) in ^{123}I-MIBG-negative cases than it is estimated for these tumors as a whole (15%).

We conclude that we have experienced a relatively rare case of ^{123}I-MIBG-negative pheochromocytoma. We speculate that genetic alterations in the monoamine transporters might cause dysfunction of these proteins, although the whole-exome sequencing failed to detect any significant abnormality. However, because VMAT1 expression has decreased only in the tumorous area of the MIBG-negative case, it is more likely that the negative result of the MIBG scintigraphy originates from a functional loss of this monoamine transporter. A more comprehensive study of the mechanism of negative conversion will be carried out on a large series of MIBG-negative PHEOs using a variety of methods such as methylation analysis for epigenetic research or proteomic analysis for identifying the difference in the protein expression between MIBG positive and negative cases.

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Disclosure of conflict of interest

None.

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References

[1] Bomani J, Levison DA, Flagman WD, Home T, Bollox PM, Ross G, Britton KE, Besser GM. Uptake of iodine-123 MIBG by pheochromocytomas, paragangliomas, and neuroblastomas: a histological comparison. J Nucl Med 1987; 28: 973-978.

[2] Havekes B, Lai EW, Corssmit EP, Romijn JA, Timmers HJ, Pacak K. Detection and treatment of pheochromocytomas and paragangliomas: current standing of MIBG scintigraphy and future role of PET imaging. Q J Nucl Med Mol Imaging 2008; 52: 419-429.

[3] Sisson JC, Frager MS, Valk TW, Gross MD, Swanson DP, Wieland DM, Tobes MC, Beierwaltes WH, Thompson NW. Scintigraphic localization of pheochromocytpma. N Engl J Med 1981; 305: 12-17.

[4] Castellani MR, Seghezzi S, Chiesa C, Aliberti GL, Maccauro M, Seregni E, Orunesu E, Luksch R, Bombardieri E. (131)I-MIBG treatment of pheochromocytoma: low versus intermediate activity regimens of therapy. Q J Nucl Med Mol Imaging 2010; 54: 100-113.

[5] Rose B, Matthay KK, Price D, Huberty J, Klencke B, Norton JA, Fitzgerald PA. High-dose 131I-metaiodobenzylguanidine therapy for 12 patients with malignant pheochromocytoma. Cancer 2003; 98: 239-248.

[6] Taketani A, Sibata T, Koide N, Toyama K, Yoshida T, Huse M, Otsuka Y, Noguchi Y, Takino I, Saito Y. A case of vHLD related extra-adrenal paraganglioma, MIBG negative but showed activity for FDG-PET. Jap J endocrinol 2004; 80: 109. In Japanese.

[7] Eisenhofer G. The role of neuronal and extraneuronal plasma membrane transporters in the activation of peripheral cathecholamines. Pharmacol Ther 2001; 91: 35-62.

[8] Fottner C, Helisch A, Anlauf M, Rossmann H, Musholt TJ, Kreft A, Schadmand-Fischer S, Bartenstein P, Lackner KJ, Kloppel G, Schreckenberger M, Weber MM. 6-18F-fluoro-L-dihydroxyphenylalanine positron emission tomography is superior to 123I-metaiodobenzylguanidine scintigraphy in the detection of extraadrenal and hereditary pheochromocytomas and paragangliomas: correlation with vesicular monoamine transporter expression. J Clin Endocrinol Metab 2010; 95: 2800-2810.

[9] Fonte JS, Robles JF, Chen CC, Reynolds J, Whatley M, Ling A, Mercado-Asis LB, Adams KT, Martucci V, Fojo T, Pacak K. False-negative 123I-MIBG SPECT is most commonly found in SDHB-related pheochromocytoma or paraganglioma with high frequency to develop metastatic disease. Endocr Relat Cancer 2012; 19: 83-93.

[10] Mackenzie IS, Gurnell M, Balan KK, Simpson H, Chatterjee K, Brown MJ. The use of 18-fluoro-dihydroxyphenylalanine and 18-fluorodeoxyglucose positron emission tomography scanning in the assessment of metaiodobenzylgu-

anidine-negative phaeochromocytoma. Eur J Endocrinol 2007; 157: 533-537.
[11] Amar L, Baudin E, Burnichon N, Peyrard S, Silvera S, Bertherat J, Bertagna X, Schlumberger M, Jeunemaitre X, Gimenez-Roqueplo AP, Plouin PF. Succinate dehydrogenase B gene mutations predict survival in patients with malignant pheochromocytomas or paragangliomas. J Clin Endocrinol Metab 2007; 92: 3822-3828.
[12] Brouwers FM, Eisenhofer G, Tao JJ, Kant JA, Adams KT, Linehan WM, Pacak K. High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing. J Clin Endocrinol Metab 2006; 91: 4505-4509.
[13] Qian X, Jin L, Shearer BM, Ketterling RP, Jalal SM, Lloyd RV. Molecular diagnosis of Ewing's sarcoma/primitive neuroectodermal tumor in formalin-fixed paraffin-embedded tissues by RT-PCR and fluorescence in situ hybridization. Diagn Mol Pathol 2005; 4: 23-28.
[14] Klein RD, Jin L, Rumilla K, Young WF Jr, Lloyd RV. Germline SDHB mutations are common in patients with apparently sporadic sympathetic paragangliomas. Diagn Mol Pathol 2008; 17: 94-100.
[15] Van Nederveen FH, Dinjens WN, Korpershoek E, De Krijger RR. The occurrence of SDHB gene mutations in pheochromocytoma. Ann N Y Acad Sci 2006; 1073: 177-182.
[16] Neumann HP, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M, Buchta M, Franke G, Klisch J, Bley TA, Hoegerle S, Boedeker CC, Opocher G, Schipper J, Januszewicz A, Eng C. European-American Paraganglioma Study Group. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA 2004; 292: 943-951.
[17] Kim J, Seong MW, Lee K, Choi H, Ku E, Bae J, Park S, Choi S, Kim S, Shin C, Kim S. Germline mutations and genotype-phenotype correlations in patients with apparently sporadic pheochromocytoma/paraganglioma in Korea. Clin Genet 2013; [Epub ahead of print].
[18] Mamede M, Carrasquillo JA, Chen CC, Del Corral P, Whatley M, Ilias I, Ayala A, Pacak K. Discordant localization of 2-[18F]-fluoro-2-deoxy-D-glucose in 6-[18F]-fluorodopamine- and [(123)I]-metaiodobenzylguanidine-negative metastatic pheochromocytoma sites. Nucl Med Commun 2006; 27: 31-36.
[19] Homma K, Hayashi K, Wakino S, Irie R, Mukai M, Kumagai H, Shibata H, Saruta T. Primary malignant hepatic pheochromocytoma with negative adrenal scintigraphy. Hypertens Res 2006; 29: 551-554.
[20] Otsuki M, Imafuku T, Morihiro T, Iigaya K, Tatematsu S, Sasaki S. A case of pheochromocytoma, negative for both 123/131I-MIBG and FDG-PET. Jap J Endocrinol 2012; 88: 1030. In Japanese.
[21] Iino K, Yamashita M, Goto S, Torizuka T, Onai Y, Oishi Y, Oki T, Nakamura H. The utility of PET for MIBG negative malignant pheochromocytoma. Jap J Endocrinol 2002; 78: 145. In Japanese.
[22] Jotoku M, Okura T, Nagao T, Enomoto D, Irita J, Miyoshi K, Kurata M, Fukuoka T, Higaki J. Case of 131I-meta-iodobenzylguanidine (MIBG) scintigraphy-negative and 2-[18F]-fluoro-2-deoxy-D-gulucose positron emission tomography (FDG-PET)-positive pheochromocytoma. Nihon Jinzo Gakkai Shi 2009; 51: 563-568. In Japanese.