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【 ORIGINAL ARTICLE 】

Paraganglioma with High Levels of Dopamine, Dopa Decarboxylase Suppression, Dopamine β-hydroxylase Upregulation and Intra-tumoral Melanin Accumulation: A Case Report with a Literature Review

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Abstract:

Object Exclusively dopamine-producing pheochromocytoma/paraganglioma (PPGL) is an extremely rare subtype. In this condition, intratumoral dopamine β-hydroxylase (DBH), which controls the conversion of norepinephrine from dopamine, is impaired, resulting in suppressed norepinephrine and epinephrine production. However, the rarity of this type of PPGL hampers the understanding of its pathophysiology. We therefore conducted genetic and immunohistological analyses of a patient with an exclusively dopamine-producing paraganglioma.

Methods Paraganglioma samples from a 52-year-old woman who presented with a 29.6- and 41.5-fold increase in plasma and 24-h urinary dopamine, respectively, but only a minor elevation in the plasma norepinephrine level was subjected to immunohistological and gene expression analyses of catecholamine synthases. Three tumors carrying known somatic PPGL-related gene variants (*HRAS*, *EPAS1*) were used as controls. Whole-exome sequencing (WES) was also performed using the patient's blood and tumor tissue.

Results Surprisingly, the protein expression of DBH was not suppressed, and its mRNA expression was clearly higher in the patient than in the controls. Furthermore, dopa decarboxylase (DDC), which governs the conversion of 3,4-dihydroxyphenyl-L-alanine (L-DOPA) to dopamine, was downregulated at the protein and gene levels. In addition, melanin, which is synthesized by L-DOPA, accumulated in the tumor. WES revealed no PPGL-associated pathogenic germline variants, but a missense somatic variant (c.1798G>T) in *CSDE1* was identified.

Conclusion Although pre-operative plasma L-DOPA was not measured, our histological and gene expression analyses suggest that L-DOPA, rather than dopamine, might have been overproduced in the tumor. This raises the possibility of pathophysiological heterogeneity in exclusively dopamine-producing PPGL.

Key words: Pheochromocytoma and paraganglioma, dopamine, 3,4-dihydroxyphenyl-L-alanine (L-DOPA), catecholamine synthase, dopamine decarboxylase

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Introduction

Pheochromocytoma/paraganglioma (PPGL) is a catecholamine-producing tumor that develops from chromaffin cells in the adrenal medulla or paraganglia (1). Tumors arising from the adrenal medulla or paraganglia are termed pheochromocytoma (PCC) and paraganglioma (PGL), respectively (1). PPGL is generally characterized by the overproduction of epinephrine and norepinephrine. However, some tumors overproduce dopamine (2). Although dopamine-producing PPGL is extremely rare (3, 4), most are poorly differentiated with metastatic potential (2).

Catecholamines are synthesized from tyrosine by the following catecholamine synthases: tyrosine hydroxylase (TH), dopa decarboxylase (DDC), dopamine β-hydroxylase (DBH), and phenylethanolamine-N-methyl transferase (PNMT) (5). TH, DDC, DBH, and PNMT control the conversion of tyrosine to 3,4-dihydroxyphenyl-L-alanine (L-DOPA), L-DOPA to dopamine, dopamine to norepinephrine, and norepinephrine to epinephrine, respectively (5). Norepinephrine and epinephrine are synthesized from dopamine. The synthesis of norepinephrine from dopamine is therefore suppressed by an impaired DBH function in exclusively dopamine-producing PPGL (2, 6, 7). However, because this tumor is rare, the understanding of the intratumoral enzymatic dynamics of catecholamine synthases is limited.

In the present study, we conducted immunohistological and genetic analyses of a patient with PGL who presented with high plasma and urinary dopamine levels, normal epinephrine levels, and minimal elevation of plasma norepinephrine levels. Surprisingly, the intratumoral *DBH* expression was markedly enhanced in our case, whereas the intratumoral DDC expression was suppressed at both the gene and protein levels. Although there is at times a discrepancy between clinical data and immunostaining results, in our case, PGL might have involved an intratumoral enzymatic pathway that overproduces L-DOPA rather than dopamine.

Materials and Methods

Patient details

A 52-year-old woman was referred to our department because of an expanding left retroperitoneal tumor. Further investigations revealed a PGL with high levels of dopamine (see Results). Given the metastatic potential of the lesion, genetic testing was indicated. Peripheral blood and tumor specimens were obtained from the patient. To analyze the genomic profile of the tumor, we compared its data with those of tumor samples from three other patients harboring PPGL-associated variants considered oncogenic or likely oncogenic by a web database (OncoKB, https://www.oncokb.org), including two *HRAS* variants (p.Q61K and p.Q61R) and one *EPAS1* variant (p.P531S) that had been reported in PPGL previously (8).

Written informed consent was obtained from all patients. Genetic analyses, including somatic and germline variant analyses, were approved by the institutional review board of the Clinical Research and Genome Research Committee at Yamanashi Central Hospital (G-2019-6), and the protocol complied with Declaration of Helsinki principles.

Tumor preparation and immunohistochemistry

Tumor tissues were fixed using 10% buffered formalin. Serial 10-μm-thick sections were prepared from formalin-fixed, paraffin-embedded (FFPE) tissues. Sections were stained with Hematoxylin and Eosin and reviewed by a pathologist to determine the tumor location. Immunohistochemistry of chromogranin-A (CgA), CD56, neuron-specific enolase (NSE), synaptophysin (SYN), neurofilament, and S-100 was used for the routine diagnosis of PPGL. In addition, Fontana-Masson stain was used to detect the accumulation of melanin. To detect TH, DDC, DBH, PNMT, and succinate dehydrogenase complex, subunit B (SDHB) protein expression, the sections were incubated with anti-TH, DDC, DBH, PNMT, and SDHB antibody, respectively, at 4°C overnight after blocking with Protein Block Serum-Free (DAKO, Tokyo, Japan) with partial reference to previous articles (9). The details of each antibody are described in Supplementary material 1. Subsequently, the Ventana BenchiMark ULTRA fully automated immunostaining system (Roche Diagnostics, Basel, Switzerland) was used with the I-VIEWDAB universal kit (Roche Diagnostics) containing secondary antibodies (biotinylated mouse anti-goat IgG antibody, biotinylated mouse anti-goat IgM antibody, and biotinylated rabbit anti-goat IgG antibody), avidin-horseradish peroxidase, and 3,3'-diaminobenzidine.

Gene expression analyses

Total RNA samples were prepared using an Ion Torrent Dx FFPE Sample Preparation Kit (Thermo Fisher Scientific, Waltham, USA) and reverse-transcribed using random hexamers and Superscript IV VILO Master Mix (Thermo Fisher Scientific). Quantiative reverse transcription polymerase chain reaction (RT-qPCR) was performed using a ViiA 7 Real-Time PCR System (Thermo Fisher Scientific) with Power Track™ SYBR™ Green Master Mix (Thermo Fisher Scientific) or TaqMan™ Gene Expression Assays (Thermo Fisher Scientific). Some primer sequences were generated with reference to previous articles (9), as listed in Supplementary material 2. The relative gene expression was calculated according to the threshold cycle values (Ct) using each primer set and standardized to Ct values for beta-actin (10).

Whole-exome sequencing (WES)

Exome sequencing was performed as previously described (11). Multiplex PCR was performed using genomic DNA with buffy coats and tumor FFPE DNA with a premixed primer pool using the Ion AmpliSeq™ Exome RDY (Thermo Fisher Scientific). PCR products were pooled and treated with FuPa reagent to partially digest primer se-

Table 1. Endocrinology Investigations Pre- and Post-surgery.

Blood test		Before surgery	6 months after surgery	Reference range
EPI	(pg/mL)	49	59	(<100)
NE	(pg/mL)	466	537	(100-450)
DA	(pg/mL)	592	14	(<20)
PRA	(ng/mL/h)	3.0		(0.3-2.9)
ALD	(pg/mL)	83.5		(29.9-158.8)
ACTH	(pg/mL)	18.5		(7.2-63.3)
Cortisol	(μg/dL)	7.26		(6.2-18.0)
DHEAS	(μg/dL)	151		(19-231)
Whole PTH	(pg/mL)	27.7		(8.3-38.7)

24-h urine collection		Before surgery	POD4	POD5	Reference range
EPI	(μg/day)	9.4	6.9	6.2	(3.4-26.9)
NE	(μg/day)	112.9	88.6	49.5	(48.6-168.4)
DA	(μg/day)	39,978.9	1,745.6	557.3	(365.0-961.5)
VMA	(mg/day)	5.3	3.4	3.1	(1.5-4.3)
HVA	(mg/day)	29.4	3.8	3.3	(2.1-4.3)
MN	(mg/day)	0.24	0.18	0.16	(0.0-0.2)
NMN	(mg/day)	0.48	0.16	0.14	(0.1-0.3)
Free cortisol	(μg/day)	51.5			(11.2-80.3)
ALD	(μg/day)	11			(<10.0)

EPI: epinephrine, NE: norepinephrine, DA: dopamine, PRA: plasma renin activity, ALD: aldosterone, ACTH: adrenocorticotrophic hormone, DHEAS: dehydroepiandrosterone sulfate, PTH: parathyroid hormone, VMA: vanillylmandelic acid, HVA: homovanillic acid, MN: metanephrine, NMN: normetanephrine, POD: post-operative day

quences. The amplicons were ligated to adapters with the diluted barcodes of the Ion Xpress Barcode Adapters Kit (Thermo Fisher Scientific). Purification was performed using Agencourt AMPure XP reagents (Beckman Coulter, Brea, USA). Library concentrations were determined using the Ion Library Quantitation Kit (Thermo Fisher Scientific). Emulsion PCR and chip loading were performed on the Ion Chef using the Ion PI Hi-Q Chef Kit. Sequencing was performed using the Ion PI Hi-Q Sequencing Kit on the Ion Proton Sequencer (Thermo Fisher Scientific).

Results

Patient's information and clinical course

The patient was a 52-year-old woman with a 48×48×39-mm^3 left retroperitoneal tumor that had been diagnosed 3 years previously as a non-functional tumor because her plasma levels of norepinephrine, epinephrine, and other adrenal steroid hormones were not elevated (Table 1). Because of the large tumor size, the patient was recommended to undergo surgery; however, she declined surgery and received annual imaging follow-up. Tumor growth was identified in the third year of follow-up (Fig. 1A), so iodine-123 metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy was conducted. Because a strong uptake in the left retroperitoneal tumor was confirmed (Fig. 1B), she was suspected of having a paraganglioma and was referred to our department.

Her history was significant for carcinoma of the right breast with surgery 4 months before presentation followed by treatment with tamoxifen 20 mg daily. She had also been diagnosed with uterine fibroids three years before presentation but had no family history of PPGL. She had no clinical symptoms, including headache, palpitation, cold sweats, or weight loss, and her blood pressure was normal at 96/65 mmHg. Laboratory investigations revealed a 29.6- and 41.5-fold increase in plasma and 24-h urinary dopamine levels, respectively and a 7-fold increase in 24-h urinary homovanillic acid (HVA) (Table 1). The plasma 3-methoxytyramine level, useful in the diagnosis of dopamine-producing PPGL (5), was not measured, as the test was not available at the time, and no sample was stored for a future analysis. Other relevant endocrinological data are also presented in Table 1. Contrast-enhanced computed tomography revealed a 56×49×39-mm^3 left retroperitoneal tumor in the hilum of a kidney that was in close contact with the left renal arteries and veins and the left ovarian vein (Fig. 1A), but no findings suggestive of multiple lesions or metastases were recorded. The patient was clinically diagnosed with a left retroperitoneal paraganglioma.

She received doxazosin and bisoprolol before laparoscopic surgery, which was uneventful. On days 4 and 5 after the operation, a marked decrease in 24-h urinary dopamine and HVA levels was observed, and the plasma dopamine level was normal at 6 months post-surgery (Table 1). To date, more than five years have passed since the tumor was first

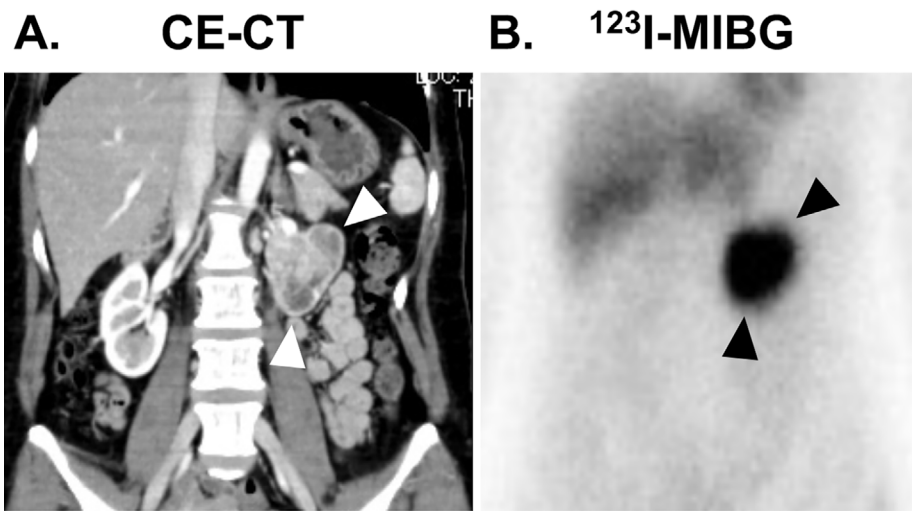


Figure 1. Images displaying a left retroperitoneal tumor. (A) Contrast-enhanced computed tomography (CE-CT) revealing a >50-mm tumor (white arrowheads). (B) Iodine-123 metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy demonstrated an intense uptake consistent with the tumor (black arrowheads).

detected, and the patient has remained well with no tumor recurrence or metastasis.

Histopathological analyses

Macroscopically, the tumor was 65×40×30 mm^3 in size with a distinct margin and no infiltration into the surrounding area (Fig. 2A, B). Pathologically, the tumor cells were less polymorphic, and no necrosis was observed. Immunohistologically, the mean Ki67 index was less than 1%. The cells were diffusely positive for CgA, CD56, NSE, and SYN, and some cells were positive for neurofilament and S-100. In addition, melanin was abundantly observed in the tumor (Fig. 2C, D) but was not seen in control cases. The grading system for adrenal pheochromocytoma and paraganglioma (GAPP) score (12) was 1 point, and the lesion was classified as a well-differentiated tumor with low metastatic potential. Furthermore, SDHB was diffusely positive (Fig. 2E).

Catecholamine synthetic enzyme analyses

In the immunohistological analysis of intratumoral catecholamine synthases, the patient's tumor was positive for TH, DBH, and PNMT, but it was not stained with DDC antibody (Fig. 2F). As controls, two tumors carrying *HRAS* variants (p.Q61K and p.Q61R) and one tumor carrying a known *EPAS1* variant (p.P531S), which had been reported previously (8), were also analyzed, and the tumors were positive for all examined catecholamine synthases, including DDC (Fig. 2G, H). Similar to the results of immunostaining, a reduced *DDC* expression and significantly increased *DBH* expression were confirmed in our case but not in the control tumors (Fig. 3).

Literature review

Because a literature review of 33 cases with dopamine-producing PPGL was reported recently (13), we elected to focus our review on the cases in which catecholamine synthases had been immunohistologically analyzed.

Using Pubmed, we identified three such cases, all from Japan (13-15). As shown in Table 2, only the tumor from case 1, which showed a decreased DBH expression and positive immunoreactivity for DDC, was consistent with the conventional theory of exclusive dopamine production (14). Similar to our case, the tumors from cases 2 and 3 were positive for DBH. In case 3, blood and urinary epinephrine and norepineprhine levels were normal, but urinary metaneprhine and normetaneprhine levels were elevated, which is consistent with positive DBH immunostaining. In comparison, similar to our case, metanephrine and normetanephrine levels were normal in case 2 despite DBH positivity, suggesting an exclusively dopamine-producing PGL. However, DDC expression was not assessed in case 2 and 3 (13, 15), and case 3 showed a decreased expression of TH (13). Taken together, these four cases demonstrated the heterogeneity of dopamine-producing PPGL. In contrast to our case, as detailed below, genetic tests (e.g., WES and mRNA expression analyses) were not performed in these three cases.

We further searched for cases of pigmented PPGL with melanin and melanin-like substance deposition, and 31 additional cases were found (16-23). Ten cases, including ours, with endocrine investigations and/or genetic testing were summarized in Table 3. The cases ranged in age from 18 to 78 years old, all were in women, and most had tumors over 40 mm in size; however, none had metastatic lesions at the diagnosis. Six patients had hypertension, seven had PCC, and three (including ours) had PGL. Some of the cases had a genetic background, such as an *SDHD* gene germline mutation (case D), neurofibromatosis type 1 (NF1) (case G), and multiple endocrine neoplasia type 2A (MEN2A) (case

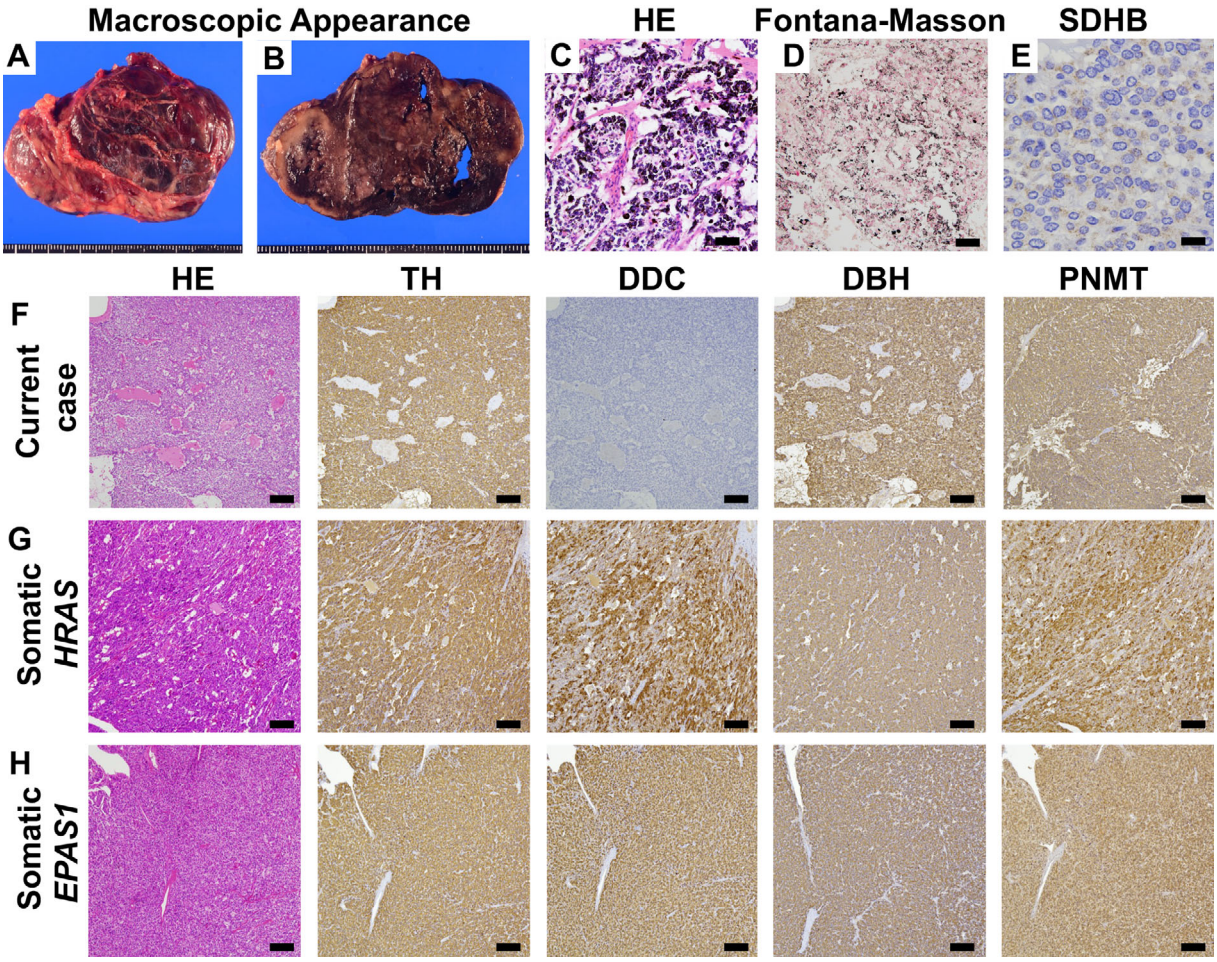


Figure 2. **Histopathological images. (A, B) Macroscopic appearance of the resected tumor. (C-H) Microscopic appearance of the tumor. The abundant pigment diffusely detected in the tumor (C) was confirmed to be melanin via Fontana-Masson staining (D). Scale bars=50 μm. (E) An immunohistological analysis of SDHB. Scale bars=20 μm. (F-H) An immunohistological analysis of catecholamine synthesis enzymes. The tumor tissues stained with Hematoxylin and Eosin staining were positive for TH, DDC, DBH, and PNMT. Our current case is presented in F, and control tumors harboring known PPGL-associated genes (*HRAS* and *EPAS1*) are presented in G and H. Scale bars=200 μm. SDHB: succinate dehydrogenase complex, subunit B, TH: tyrosine hydroxylase, DDC: dopa decarboxylase, DBH: dopamine β-hydroxylase, PNMT: phenylethanolamine-N-methyl transferase, PPGL: pheochromocytoma/paraganglioma**

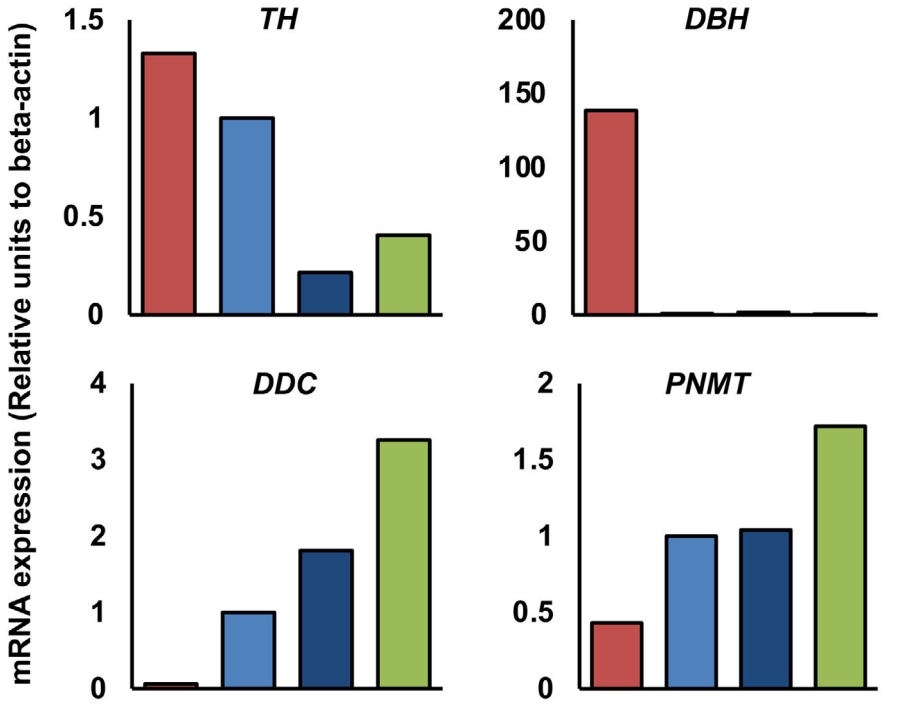
I). Endocrinological data were obtained in nine cases, and seven of them showed high levels of epinephrine, norepinephrine, or their metabolites (cases A-C, F-I). Case E and the present case showed minor elevations of plasma norepinephrine, urinary metaneprhine, normetanephrine, and vanillylmandelic acid levels. However, blood and urinary dopamine and urinary HVA levels were measured in none of the cases other than our own.

Gene variant analyses

Because patients with dopamine-producing tumors sometimes have germline variants (24, 25), we conducted WES using leukocytes along with tumor specimens. A missense somatic variant (c.1798G>T) in exon 15 of *CSDE1* (allele frequency, 36.88%) causing the substitution of aspartic acid with tyrosine at position 600 (p.D600T) was detected, but no other germline or somatic variants with genes associated with PPGL (11) nor catecholamine synthetic enzyme were identified (Supplementary material 3). We also searched for candidate tumoral somatic variants associated with impaired catecholamine synthesis among 18 somatic variants, including *CSDE1* variants, from our WES data (Supplementary material 4). Among these, we focused on a splice site variant (c.2610-2A>G) in *ATP13A2* (allele frequency, 12.73%). The estimated function of the variant based on our WES data was "unknown".

Discussion

In exclusively dopamine-producing PPGL, dopamine is not converted to norepinephrine because the intratumoral DBH expression is suppressed (2); however, as shown in our



Case	Mutation	Tumor	Hormone
Current case	None	PGL	Dopaminergic
Somatic *HRAS*	p.Q61K	Uni-PCC	Adrenergic
Somatic *HRAS*	p.Q61R	Uni-PCC	Adrenergic
Somatic *EPAS1*	p.P531S	Uni-PCC	Noradrenergic

Figure 3. An mRNA expression analysis of catecholamine synthesis enzymes. The expression of genes encoding TH, DDC, DBH, and PNMT was analyzed by RT-PCR. Our current case is presented in red bars, and control tumors harboring known PPGL-associated genes (*HRAS* and *EPAS1*) are presented in blue and green bars, respectively. TH: tyrosine hydroxylase, DDC: dopa decarboxylase, DBH: dopamine β-hydroxylase, PNMT: phenylethanolamine-N-methyl transferase, RT-PCR: reverse transcription polymerase chain reaction, PPGL: pheochromocytoma/paraganglioma

literature review, immunostaining of catecholamine synthases has only been conducted in three cases of exclusively dopamine-producing PPGL (Table 2) (14). One case showed a decreased expression of DBH, consistent with the conventional mechanism of dopamine producing PPGL (Fig. 4) (2), similar to our own case; however, while the DBH expression was detected in the other two cases, immunostaining for other catecholamine synthases, including DDC, was not performed (13, 15). In case 3, the DBH expression was considered to be due to the immature secretory vesicles or DBH gene mutation (13) based on abnormal vesicles demonstrated by electron microscopy; however, a genetic analysis was not conducted (13).

The presence of gene variants of catecholamine synthase in PPGL with abnormal catecholamine production is only speculative at present, as this has not been the focus of previous studies. However, it would be worthwhile to investigate the existence of such variants in PPGL, particularly in cases where certain catecholamine synthases, especially TH, DDC, and DBH, are immunohistochemically negative. Our case complemented the existing literature, as WES and immunohistochemical and gene expression analyses of all four catecholamine synthases were investigated. Interestingly, in our patient, enzymatic suppression was only observed in DDC, which converts L-DOPA to dopamine (5). However, the expression of DBH was sufficient at the gene and protein levels. Therefore, in our case, intratumoral catecholamine synthesis differed from that assumed in conventional exclusively dopamine-producing PPGL. We discussed the potential mechanisms of intratumoral dopamine synthesis in our case based on the results of our analyses.

Dopamine converted from L-DOPA in the cytoplasm is transported to secretory granules via the vesicular monoamine transporter (VMAT) (26) and hydroxylated by DBH (27, 28). Therefore, VMAT abnormalities may hamper the transport of dopamine to secretory granules and the sub-

Table 2. Literature Review of Dopamine-producing PPGL That Catecholamine Synthases Had Been Imunohistologically Analyzed.

Case No. Reference	Case 1 (14)	Case 2 (15)	Case 3 (13)	Current case
Age/Gender	65/M	70/F	64/F	52/F
Type	Uni-PCC	PGL	PGL	PGL
Size (mm^3)	NA	50×30×15	56×51×106	48×48×39
Metastasis at Dx	No	No	No	No
GAPP score	NA	1	6	1
Endocrinology tests [Reference range]				
P-DA (pg/mL)	280-880*	NA	870 [<30]	592 [<20]
U-DA (μg/day)	8,000-13,000*	7,933.2 [<1,100]	148,212.4 [<1,100]	39,978.9 [<961.5]
U-HVA (mg/day)	11.1*	NA	61.2 [<6.3]	29.4 [<4.3]
P-EPI (pg/mL)	20*	NA	60 [<111]	49 [<100]
P-NE (pg/mL)	460*	NA	60 [<750]	466 [<450]
U-EPI (μg/day)	5.1*	8.1 [<41]	24.3 [<41]	9.4 [<26.9]
U-NE (μg/day)	83.7*	102.2 [<160]	122.9 [<160]	112.9 [<168.4]
U-MN (mg/day)	0.1*	0.16 [<0.18]	1.3 [<0.19])	0.24 [<0.2]
U-NMN (mg/day)	0.13*	0.17 [<0.28]	1.3 [<0.33]	0.48 [<0.2]
U-VMA	1.9*	3.33 [4.90]	NA	5.3 [<4.3]
Immunohistological analysis				
TH	Positive	Positive	Decreased	Positive
DDC	Positive	NA	NA	Negative
DBH	Decreased	Positive	Positive	Positive
PNMT	Positive	NA	Decreased	Positive
SDHB	NA	Positive	Positive	Positive

PPGL: pheochromocytoma/paraganglioma, M: male, F: female, Uni: unilateral, PCC: pheochromocytoma, PGL: paraganglioma, Dx: diagnosis, P-DA: plasma dopamine, U-DA: 24-h urine dopamine, U-HVA: 24-h urine homovanillic acid, P-EPI: plasma epinephrine, P-NE: plasma norepinephrine, U-EPI: 24-h urine epinephrine, U-NE: 24-h urine norepinephrine, U-MN: 24-h urine metanephrine, U-NMN: 24-h urine normetanephrine, U-VMA: 24-h urine vanillylmandelic acid, TH: tyrosine hydroxylase, DDC: dopa decarboxylase, DBH: dopamine β-hydroxylase, PNMT: phenylethanolamine-N-methyl transferase, SDHB: succinate dehydrogenase complex, subunit B, NA: not available
*In case 1, individual reference range was not available.

sequent biosynthesis of norepinephrine in the granules, resulting in excess dopamine levels in the tumor cell cytoplasm. ^{123}I-MIBG is used to assess the localization and functional diagnosis of PPGL via its uptake into intratumoral secretory granules through VMAT (29). The observation of the accumulation of ^{123}I-MIBG in our patient was consistent with the normal tumoral VMAT function.

Because discrepancies between clinical data and immunohistochemistry results are occasionally observed, our case may simply represent a predominately dopamine-overproducing PGL based on blood and urinary endocrine test findings. However, our finding of reduced protein and transcriptional levels of intratumoral *DDC* without inhibition of both DBH and VMAT is of interest. One possibility is that this tumor may have been an L-DOPA-secreting tumor in which catecholamines downstream from dopamine were not overproduced. In the body, DDC is abundantly expressed in chromaffin cells, as well as in peripheral blood vessels and kidneys (30), and it converts L-DOPA to dopamine rapidly in the blood stream (31). Therefore, even if L-DOPA is only oversecreted from the tumor, dopamine would be converted from systemic L-DOPA via DDC derived from systemic blood vessels and kidneys. In fact, L-DOPA is used as a therapeutic drug for Parkinson's disease, and peripheral DDC inhibitors are used in combination to prevent L-DOPA from being converted by systemic DDC to dopamine, which less readily accumulates in the brain. In support of this is the finding of the upregulation of tumoral *DBH* in comparison with the control samples.

One prior study demonstrated that patients with dopamine-producing PPGL and oversecretion of norepinephrine and epinephrine also exhibited oversecretion of plasma and urinary L-DOPA (5). Thus, endocrinological tests using plasma and urinary samples are not sufficient to differentiate whether elevated plasma and urinary dopamine levels are attributable to the tumoral oversecretion of dopamine or oversecretion of L-DOPA and subsequent conversion to dopamine through systemic DDC. In addition, because urinary dopamine is mainly derived via the extraction of circulating L-DOPA in the kidneys and decarboxylation by DDC (5), urinary dopamine has a low sensitivity and specificity for validating dopamine overproduction. In dopamine-producing tumors with norepinephrine and epinephrine oversecretion, plasma and urinary dopamine levels are 56- and

Table 3. Literature Review of Pigmented PPGL.

Reference	CaseA (16)	Case B (17)	Case C (17)	Case D (18) (19)	Case E (20)	Case F (21)	Case G (22)	Case H (23)	Case I (23)	Our Case
Age/Gender	77/F	28/F	18/F	69/F	38/F	70/F	39/F	60/F	24/F	52/F
Type	Uni-PCC	Uni-PCC	Bi-PCC	PGL	Uni-PCC	PGL	Uni-PCC	Rt-PCC	Uni-PCC	PGL
Size (mm^3)	35×32×22	60×50×40	Rt: 44×40×40 Lt: 25×25×20	66×37×35	110×75×20	65	55×40×35	50×40×38	40	65×40×30
Hypertension	Yes	Yes	Yes	Yes	No	Yes	No	Yes	NA	No
MIBG	NA	Positive	Positive	NA	Positive	NA	NA	NA	NA	Positive
Metastasis at Dx	No	No	No	No	No	NA	No	No	NA	No
Hereditary PPGL	No	NA	NA	*SDHD* germline mutation*	NA	NA	NF1**	NA	MEN 2A***	No
P-DA	NA	NA	NA	NA	NA	NA	NA	NA	NA	High
U-DA	NA	NA	NA	NA	NA	NA	NA	NA	NA	High
U-HVA	NA	NA	NA	NA	NA	NA	NA	NA	NA	High
P-EPI	NA	NA	NA	NA	NA	High	NA	NA	NA	Normal
P-NE	NA	NA	NA	NA	NA	High	NA	NA	NA	Minimal↑#
P-MN	High	NA	NA	NA	NA	NA	NA	NA	NA	NA
P-NMN	High	NA	NA	NA	NA	NA	NA	NA	NA	NA
U-EPI	NA	Normal	NA	NA	NA	NA	NA	NA	NA	Normal
U-NE	NA	Normal	NA	NA	NA	NA	High	NA	NA	Normal
U-MN	High	NA	NA	NA	NA	NA	NA	NA	NA	Minimal↑#
U-NMN	High	NA	NA	NA	NA	NA	NA	NA	NA	Minimal↑#
U-VMA	NA	High	High	NA	Normal	NA	NA	High	High	Minimal↑#

*Diagnosed by genetic testing. **Diagnosed by clinical features. ***Diagnosed by family history. #Minimal high level that didn't meet the criteria of oversecretion. PPGL: pheochromocytoma/paraganglioma, F: female, Uni: unilateral, Bi: bilateral, Rt: right, Lt: left, PCC: pheochromocytoma, PGL: paraganglioma, MIBG: metaiodobenzylguanidine scintigraphy, Dx: diagnosis, NF1: neurofibromatosis type 1, MEN: multiple endocrine neoplasia, P-DA: plasma dopamine, U-DA: 24-h urine dopamine, U-HVA: 24-h urine homovanillic acid, P-EPI: plasma epinephrine, P-NE: plasma norepinephrine, P-MN: plasma metanephrine, P-NMN: plasma normetanephrine, U-EPI: 24-h urine epinephrine, U-NE: 24-h urine norepinephrine, U-MN: 24-h urine metanephrine, U-NMN: 24-h urine normetanephrine, U-VMA: 24-h urine vanillylmandelic acid, NA: not available

3-fold higher than the upper limit of normal in plasma and urine, respectively. Consequently, plasma dopamine levels are regarded as a more sensitive indicator of dopamine oversecretion than urinary dopamine (5). The finding of a remarkably elevated urinary dopamine level (41.5-fold above normal) in our patient relative to a 29.6-fold increase in the plasma level suggests intratumoral oversecretion of L-DOPA and its subsequent conversion to dopamine by DCC derived from the blood vessels and kidneys (Fig. 4). However, this conclusion remains speculative, as the plasma and urinary L-DOPA levels were not measured at the time, and no additional samples were collected for further analyses.

Interestingly, abundant melanin was observed in the patient's tumor. Melanin is generally produced from L-DOPA via various intermediate metabolites, including dopaquinone (32). Thus, under DDC suppression, the excess L-DOPA in the PGL can be used for melanin production instead of dopamine synthesis, and our pathological finding of abundant intratumoral melanin is consistent with our notion that the tumor might be an exclusively L-DOPA-producing PGL. Although melanin is mainly produced by melanocytes in the skin, in animal studies, tyrosinase, which is responsible for the conversion of tyrosine to L-DOPA and L-DOPA to dopaquinone, has been shown to be responsible for catecholamine synthesis in the adrenal medulla under TH deficiency (33). Furthermore, when the TH inhibitor metyrosine is used for malignant pheochromocytoma to suppress the production of norepinephrine and epinephrine, plasma L-DOPA and dopamine levels are significantly increased. As depicted in Fig. 4, the abundant melanin and suppressed DDC observed in our case support a predominantly L-DOPA tumor, given the conversion of tyrosine into L-DOPA followed by melanin synthesis from L-DOPA via intratumoral tyrosinase, which has been proposed as an alternative pathway (34).

The proposed mechanisms of pigmented PPGL include abnormal cell differentiation of adrenal medullary cells derived from the same neural crest as melanocytes, or the accumulation of degradation products of intra-tumoral catecholamines (17). To identify characteristics leading to certain pathological mechanisms, we summarized 10 total cases (including our own) of pigmented PPGL with catecholamine measurements and/or genetic backgrounds in Table 3. The clinical features in our case (female gender, large tumor, no metastatic lesions) were consistent with those previous cases. However, while eight of the nine previous cases had elevated levels of epinephrine, norepinephrine, or their metabolites, the levels of dopamine and its metabolites were not assessed in any case except our own. Hereditary PPGL were also identified in three cases, including the cases with

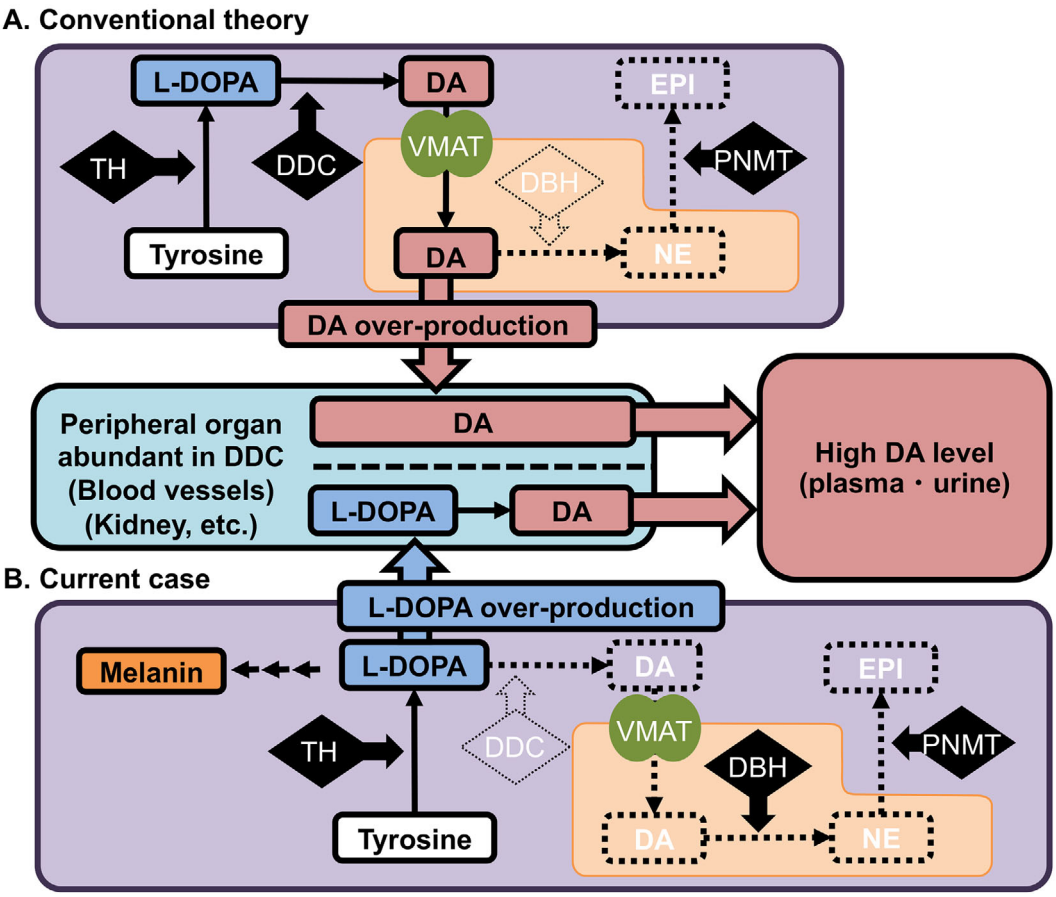


Figure 4. **Summary of this study. (A) The conventional theory of catecholamine synthesis in exclusively DA-producing PPGL. L-DOPA synthesized from tyrosine by TH is converted to DA by DDC and transported to secretory vesicles via VMAT. Because the function of DBH is suppressed in secretory vesicles, DA cannot be converted to NE and subsequently into EPI. As a result, only DA is oversecreted by the tumor, and laboratory tests revealed high levels of blood and urinary DA. (B) Possibility of exclusively L-DOPA-overproducing PGL. In our current case, suppressed DDC hampers the conversion of L-DOPA to dopamine. As a result, L-DOPA, but not DA, is overproduced. Some of the excess L-DOPA contributes to melanin synthesis through an alternative pathway. Excess L-DOPA secreted into blood is converted to dopamine in DDC-abundant organs such as the kidneys and blood vessels. Therefore, laboratory tests also revealed high blood and urinary DA levels, similar to the conventional exclusive dopamine-producing PPGL tumor in (A). PPGL: pheochromocytoma/paraganglioma, DA: dopamine, L-DOPA: 3,4-dihydroxyphenyl-L-alanine, TH: tyrosine hydroxylase, DDC: dopa decarboxylase, VMAT: vesicular monoamine transporter, DBH: dopamine β-hydroxylase, NE: norepinephrine, EPI: epinephrine, PNMT: phenylethanolamine-N-methyl transferase, PGL: paraganglioma**

an *SDHD* germline mutation, NF1, and MEN2A. These findings indicate that pigmented PPGL also has diverse clinical features and backgrounds. Therefore, the mechanisms described above may not explain our case of a dopamine-secreting pigmented DDC-negative PPGL, with the alternative pathway of melanin synthesis from L-DOPA that we proposed remaining a possibility. Elucidation of the pathogenesis of this rare type of PPGL will likely require detailed biochemical and histochemical analyes of catecholamine synthesis complemented by genetic studies.

Interestingly, a splice site variant of *ATP13A2* was detected in the tumor in our case. Splice site variants occasionally result in altered protein coding sequences. ATP13A2 is a lysosomal P5-type transport ATPase (35), and its gene is located adjacent to *SDHB* on chromosome 1p36. Germline variants in *ATP13A2* represent a cause of familial Parkinson's disease (Kufor-Rakeb syndrome, PARK9) or early- or late-onset parkinsonism via dopaminergic neurodegeneration attributable to lysosomal dysfunction (36-38). The pathogenic *ATP13A2* variant causing neurodegenerative diseases has been detected in various exons (37), including exon 24, which has a splice site variant site upstream, as observed in our case. It would be intriguing if the *ATP13A2* variant were found to be associated with the pathogenesis of predominately dopamine-producing PPGL.

In general, dopamine-producing tumors similar to our

case are associated with a high risk of metastasis because of DDC suppression on immunostaining, impaired catecholamine production, the large size, and extra-adrenal tumor development (39), particularly those with germline variants of *SDHB*. A molecular analysis of the current case revealed no known germline variants of *SDHB* according to WES, and only a somatic missense variant (c.1798G>T) in *CSDE1* was identified. Because frameshift and splice site somatic variants of *CSDE1* were reported in only PCCs and not PGLs (8), as in our case, the significance of the observed missense variant in the pathogenesis of PGL is unclear.

In a mini-review of 33 cases of exclusively dopamine-producing PPGL, 9 of the 25 cases (36%) whose prognosis could be followed showed recurrence or metastasis (13). We therefore initially thought that our patient with exclusive dopamine hypersecretion and a large tumor size had an increased metastatic potential. Interestingly, however, no recurrence or metastasis has been noted more than five years since the surgery in our case, and the low GAPP score of her tumor indicates a highly differentiated tumor (12). A review of the literature on dopamine-producing PPGLs using Pubmed that showed histological differentiation grading was performed in only three cases other than our own, including one PCC case with a pheochromocytoma of the adrenal gland scaled score (PASS) (40) of 4 points (malignant) (41) and the two cases shown in Table 2 (case 2 with 1 point, a highly differentiated tumor; and case 3 with 6 points, a moderately differentiated tumor). As pathological grading was only reported in four cases, the further accumulation of additional cases is necessary to clarify the relationship between tumor differentiation and clinical features in dopamine-producing PPGLs.

Increased plasma methoxytyramine, a metabolite of dopamine, is not only associated with an *SDHB* variant and extra-adrenal disease but is also seen in patients with metastases without *SDHB* variants (42). Therefore, it is possible that certain molecular backgrounds other than *SDHB* variants may have influenced the clinical presentation in patients without *SDHB* variants and metastasis, as with our patient. One limitation of the present study was that the control samples had obvious genetic backgrounds and were PCCs, whereas the current case had a PGL. Therefore, we could not exclude the possibility that the difference observed in our case was specific for PGL, with the differences in the two controls related to disease type and potentially the location of the disease. However, our case suggests that exclusively dopamine-producing PPGLs, which have been thought to be the result of a single pathological mechanism of DBH suppression, may represent a spectrum of heterogeneous tumors with diverse molecular mechanisms. Comprehensive analyses, including immunohistological and genetic analyses of multiple cases, are warranted to better understand the pathophysiology of dopamine-producing PPGL.

In conclusion, we reported a case of PGL with predominately dopamine production and the accumulation of melanin. Immunohistological and gene expression analyses revealed DDC downregulation and DBH upregulation in the tumor. Although we suggested that our case might be a PGL with L-DOPA production, no sample was available to measure the intratumor, plasma, or urinary L-DOPA levels. As the disease concept of exclusively L-DOPA-producing PPGL has yet to be established, it is possible that classical dopamine-producing PPGL should be classified into multiple pathological types according to the expression patterns of catecholamine synthesis enzymes.

The authors state that they have no Conflict of Interest (COI).

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