Hypokalemic rhabdomyolysis due to WDHA syndrome caused by VIP-producing composite pheochromocytoma in a case of neurofibromatosis type 1

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ABSTRACT

A 47-year-old woman with neurofibromatosis type 1 suffered from general muscle weakness and watery diarrhea. Results of laboratory tests showed elevated musclar enzymes, severe hypokalemia and excessive production of catecholamines and vasoactive intestinal polypeptide (VIP). CT scan showed a 10-cm left adrenal mass, and ^{131}I-metaiodobenzylguanidine scintigraphy showed uptake on the mass. After she underwent surgical removal of the tumor, all the symptoms and signs subsided. Histological study revealed that the mass consisted of pheochromocytoma and ganglioneuroma respectively producing catecholamines and VIP. In immunohistochemical staining of neurofibromin, pheochromocytoma and ganglion cells showed positive staining, whereas nerve bundles and Schwann cells showed negative staining. We concluded that the patient had hypokalemic rhabdomyolysis due to watery diarrhea, hypokalemia, and achlorhydria (WDHA) syndrome, which was induced by VIP-producing composite pheochromocytoma. Composite pheochromocytoma is neuroendocrine tumor that is composed of pheochromocytoma and ganglioneuroma, both of which is neural crest derivatives. Deficiency of neurofibromin in Schwann cells might have played an important role in the development and the growth of the composite pheochromocytoma in this patient.

Key words: composite pheochromocytoma, vasoactive intestinal polypeptide, hypokalemic rhabdomyolysis, watery diarrhea hypokalemia and achlorhydria syndrome, neurofibromin

Hypokalemic rhabdomyolysis is relatively rare presentation of hypokelemia. Gross et al first described hypokelemic myophathy caused by licorice ingestion in 1966 (1). Since then, various causes such as usage of laxative, diuretics, anorexia, or chronic alcoholism, infectious enterocolitis, aldosteronism, renal tubular acidosis were reported to be possible causes of hypokalemic rhabdomyolysis. Here, we present the first case with hypokalemic rhabdomyolysis due to watery diarrhea, hypokalemia, and achlorhydria (WDHA) syndrome. The unusual presentation of this case was caused by unusual tumor, which was revealed to be vasoactive intestinal polypeptide (VIP)-producing composite pheochromocytoma. In this case, the genetic background of neurofibromatosis type 1 (NF1) is considered to play an important role in multidirectional differentiation and proliferation of neuroendocrine cells, resulting in the development of VIP-producing composite pheochromocytoma.

REPORT OF A CASE

A 47-year-old woman was admitted to the Orthopedic Department of Asahikawa City Hospital in February 2001 with general muscular weakness and myalgia. She could not even stand up or walk for a few days before admission. She had suffered from watery diarrhea and weight loss for one month before admission. She had been treated for hypertension for seven years. She was diagnosed as having neurofibromatosis type 1 (NF1) when she had a cervical skin neurofibroma removed 14 years ago. Her mother and daughter were also diagnosed as having NF1. Her height was 150 cm, and physical examination on admission showed weight of 54.7 kg, blood pressure of 164/84 mmHg and regular heart rate of 76 beats/min. Multiple cafe-au-lait macules and neurofibromas were present on her hands and hip. Neurological examination was

unremarkable except for general muscle weakness.

Results of laboratory tests showed marked hypokalemia of 1.8 mEq/l and elevated muscular enzymes: AST, 164 IU/l; LDH, 629 IU/l; CPK, 12920 IU/l. White blood cell count (11.77 x 10^9 /l) and CRP (1.0 mg/dl) were slightly elevated. Results of other biochemical tests were within normal ranges. She was diagnosed as having rhabdomyolysis due to severe hypokalemia, and she was referred to our department for additional systemic examination. Although treatment with intravenous infusion of potassium resulted in steady clinical improvement of symptoms and signs of rhabdomyolysis, watery diarrhea persisted despite treatment with several antidiarrhetics. Repeated stool cultures were negative for bacterial infection.

An abdominal CT scan revealed a mass of 10 cm in diameter in the left adrenal gland (Fig.1). ^{131}I-labeled metaiodobenzylguanidine (MIBG) scintigraphy showed uptake in the left adrenal gland. An endocrinological study was then performed. Plasma levels and 24-hour urinary secretions of catecholamines were greatly increased. Plasma level of VIP was also elevated to 645 pg/ml (Table 1). Plasma levels of other adrenal hormones (cortisol, aldosterone and deoxycorticosterone) and other gastrointestinal hormones (gastrin, somatostatin, glucagons) were within normal ranges.

We concluded that she had hypokalemic rhabdomyolysis due to watery diarrhea, hypokalemia, and achlorhydria (WDHA) syndrome, which was assumed to be induced by VIP-producing pheochromocytoma. She underwent surgical removal of the tumor. Catecholamine and VIP levels returned to normal ranges (Table 1) and the diarrhea subsided soon after removal of the tumor. The patient was discharged and has not received any medication since discharge. She has been well without any sign of recurrence.

The tumor measured 11 x 13 x 7 cm and weighed 460 g. It was soft and brownish,

and it had a thin capsule. Multiple cystic degeneration and necrosis were seen on a section view (Fig.2). In the low power observation, the tumor was well defined from normal tissue. The adjacent adrenal grand was intact. Two different components were recognized in the tumor. The first component showed the typical zellballen pattern with high cellularity, and the second component showed relatively loose wavy pattern with fibrous stroma. The two components were separated but partially merged each other (Fig.3A). In the high power observation, pleomorphic small cells with abundant granules were arranged in nests (pheochromocytoma) (Fig.3B). Large cells with abundant cytoplasmic processes (ganglion cells) were scattered or aggregated within the proliferating nerve bundles and Schwann cells (ganglioneuroma) (Fig.3C). Cells of each component were well-differentiated, and no mitosis was observed. A diagnosis of composite pheochromocytoma was made on the basis of these findings.

Immunohistochemical staining of formalin-fixed, paraffin-embedded tissue was performed using EnVision System (DakoCytomation, Glostrup, Denmark), which is based on peroxidase-labelled polymer conjugated with secondary antibodies. The antibodies used for staining included chromogranin A (DakoCytomation, Glostrup, Denmark), synaptophysin (DakoCytomation, Glostrup, Denmark), neuron-specific enolase (NSE) (DakoCytomation, Glostrup, Denmark), VIP (Biomeda, CA, USA), vimentin (DakoCytomation, Glostrup, Denmark), S-100 protein (DakoCytomation, Glostrup, Denmark), neurofilament (DakoCytomation, Glostrup, Denmark). The immunohistochemical staining of neurofibromin was kindly performed by Dr. N. Kimura (Department of Pathology and Laboratory Medicine, Tohoku Rosai Hospital, Sendai) as described previously (2). Pheochromocytoma cells and ganglion cells were stained positively for chromogranin A, synaptophysin and NSE. The ganglion cells were strongly positive for VIP stain (Fig.4A). Nerve bundles and Schwann cells were

immunoreactive for vimentin, S-100 protein and neurofilament. Pheochromocytoma and ganglion cells showed positive staining for neurofibromin, whereas nerve bundles and Schwann cells showed negative staining (Fig.4B). Tumor cells showed negative immunoreactivity to pancreatic polypeptide, calcitonin, glucagon, serotonin, somatostatin and gastrin.

In electron microscopic analysis, high electron density core granules with wide halo (nor-epinephrine granule) and relatively low electron density core granules without halo (epinephrine granules) were observed in pheochromocytoma cells.

DISCUSSION

WDHA syndrome is caused by VIP-producing tumors (VIPomas). Although most VIPomas arise in the pancreas, as many as 20% of these occur in extra-pancreatic sites (3). Adrenal pheochromocytoma could be one of the extra-pancreatic VIPomas. Previously, sixteen cases of VIP-producing adrenal pheochromocytoma have been reported (4-19). Thirteen of those cases had clinical symptoms of watery diarrhea. Muscle weakness (4,6,10,12,17) was commonly observed in these cases possibly related to associate hypokalemia. All cases became free from such symptoms after resection of the tumor. Many cases did not show typical symptoms of pheochromocytoma such as hypertension (6,8,10,12,13,15,16,18). It is suggested in some reports that excessive VIP acts as a vasodilator and masks the vasospastic symptoms of catecholamines. Eleven cases were histologically diagnosed as pheochromocytoma (5-10,12,15,17-19) and only 5 cases were diagnosed as composite pheochromocytoma as our case (4,11,13,14,16). In cases of composite pheochromocytoma, it has been reported that the pheochromocytoma component and the ganglioneuroma component produced catecholamines and VIP, respectively, as in our case (4,11,13,14,16). Only one of

these 16 cases of WDHA syndrome due to VIP-producing pheochromocytoma was reported to have a genetic background of NF1 (18).

NF1 or von Recklinghausen's disease is characterized by proliferation and malignant transformation of neural-crest derivatives. NF1 is an autosomal dominant disorder, which is caused by single loss-of-function allele of the gene designated *NF1* (20). Neurofibromin, the product of *NF1*, contains a region homologous to mammalian RasGTPase-activating proteins that function as negative regulators of Ras by accelerating the conversion of Ras-GTP to Ras-GDP (21). The *NF1* gene appears to act as a tumor suppressor gene. Thus, it is conceivable that patients with NF1 have a higher incidence of malignancy. It has been reported that the incidence of pheochromocytoma in patients with NF1 is 10-times higher than that in the general population (22). Composite pheochromocytoma, known as mixed neuroendocrine and neural tumor, has been reported to be associated with NF1 (2). The role of neurofibromin in neurofibromas in NF1 patients has been extensively studied. Neurofibroma is a mixed tumor that consists all neural crest derivatives. It has been reported that only Schwann cells lose neurofibromin expression, whereas other components of neurofibroma retain neurofibromin expression (23). Zhu *et al.* demonstrated that loss of neurofibromin in Schwann cells (*NF1*-/-) is sufficient to generate a neurofibroma in a heterozygous (*NF1*+/-) mouse, which is considered to be counterpart model of human NF1 (24). Composite pheochromocytoma is also mixed neuroendocrine tumor that is composed of pheochromocytoma and ganglioneuroma, both of which is neural crest derivatives. Similar to neurofibroma, Schwann cells of composite pheochromocytoma in NF1 patients have been reported to lose neurofibromin expression as in our case (2). In our case, loss of neurofibromin in Schwann cells might also have played an important role in multidirectional

differentiation and proliferation of neuroendocrine cells, resulting in the development of VIP-producing composite pheochromocytoma.

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Figure Legends

Figure 1. Computed tomography revealed a cystic adrenal tumor on the left side.

Figure 2. Cut section of the tumor showed multiple cystic degeneration and necrosis.

Figure 3. Histophathological features of the tumor

A. Pheochromocytoma component (left) and ganglioneuroma component (right) were merged each other. (hematoxylin and eosin, original magnification x100)

B. Pleomorphic small cells with abundant granules were arranged in nests (pheochromocytoma) (hematoxylin and eosin, original magnification x400)

C. Large cells with abundant cytoplasmic processes (ganglion cells) were scattered or aggregated within the proliferating nerve bundles and Schwann cells (ganglioneuroma) (hematoxylin and eosin, original magnification x400)

Figure 4. Immunohistochemical features of the tumor

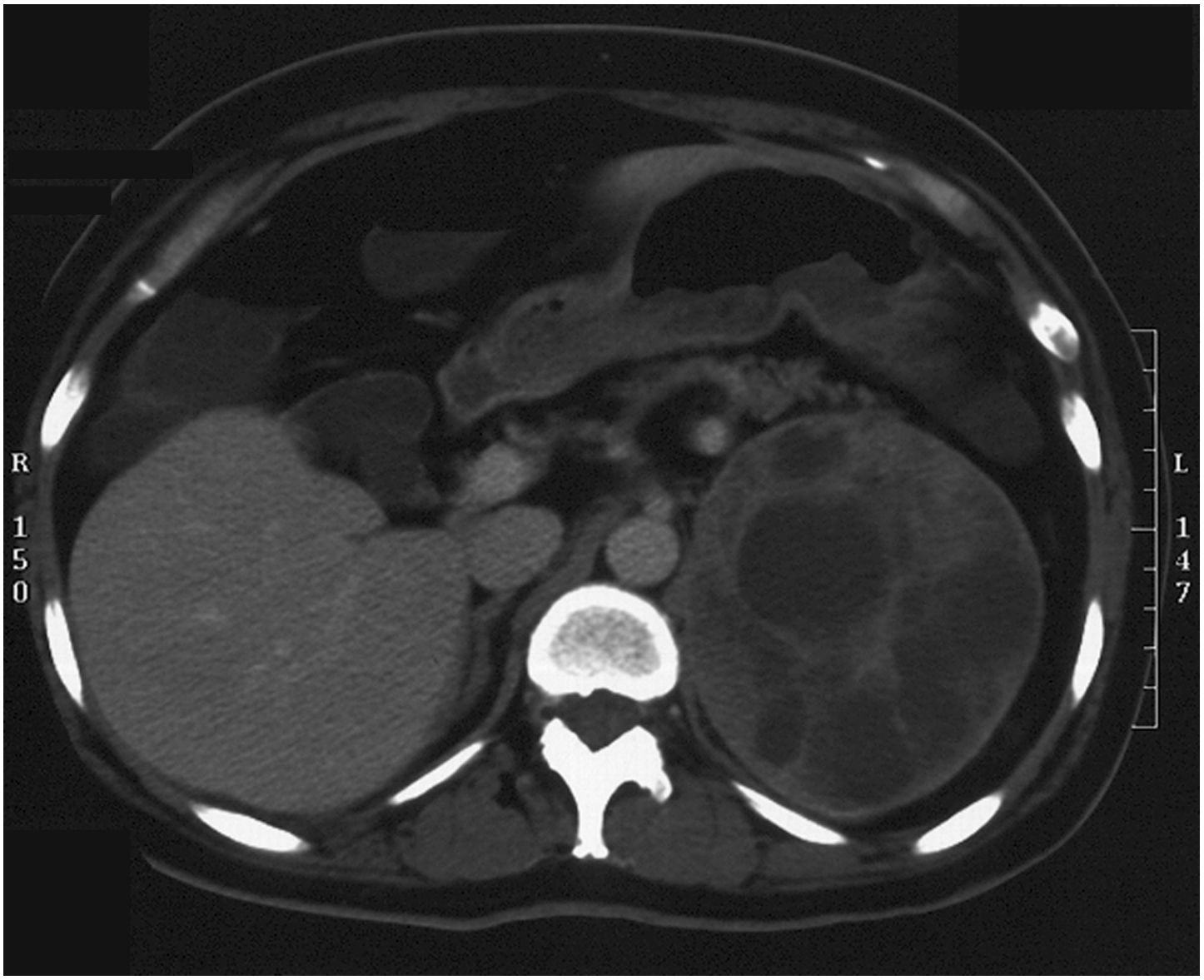
A. The ganglion cells were strongly positive for VIP stain. (VIP, original magnification x400)

B. Pheochromocytoma and ganglion cells were positive for neurofibromin stain, whereas nerve bundles and Schwann cells showed negative staining. (neurofibromin, original magnification x400)

Table 1. Pre- and post-operation hormone levels.

Hormone	Pre-operation	Post-operation	Normal range
Serum catecholamines			
Adrenaline	**0.36**	**<0.01**	**(~0.10 ng/ml)**
Noradrenaline	**6.72**	**0.11**	**(0.10~0.50 ng/ml)**
Dopamine	**0.71**	**<0.01**	**(~0.03 ng/ml)**
Serum VIP*	**645**	**16**	**(~100 pg/ml)**
24-hour urinary catecholamines			
Adrenaline	**119.1**	**3.8**	**(3.0~41.0 μg/day)**
Noradrenaline	**1266.4**	**81.7**	**(31.0~160.0 μg/day)**
Dopamine	**4473.2**	**491**	**(280.0~1100.0 μg/day)**
24-hour urinary metabolic products			
VMA*	**65.82**	**5.17**	**(1.9~5.9 mg/day)**
HVA*	**17.74**	**4.52**	**(2.40~6.00 mg/day)**
Metanefurine	**3.56**	**0.05**	**(0.04~0.18 mg/day)**

***VIP: vasoactive intestinal polypeptide**
***VMA: vanillylmandelic acid**
***HVA: homovanillic acid**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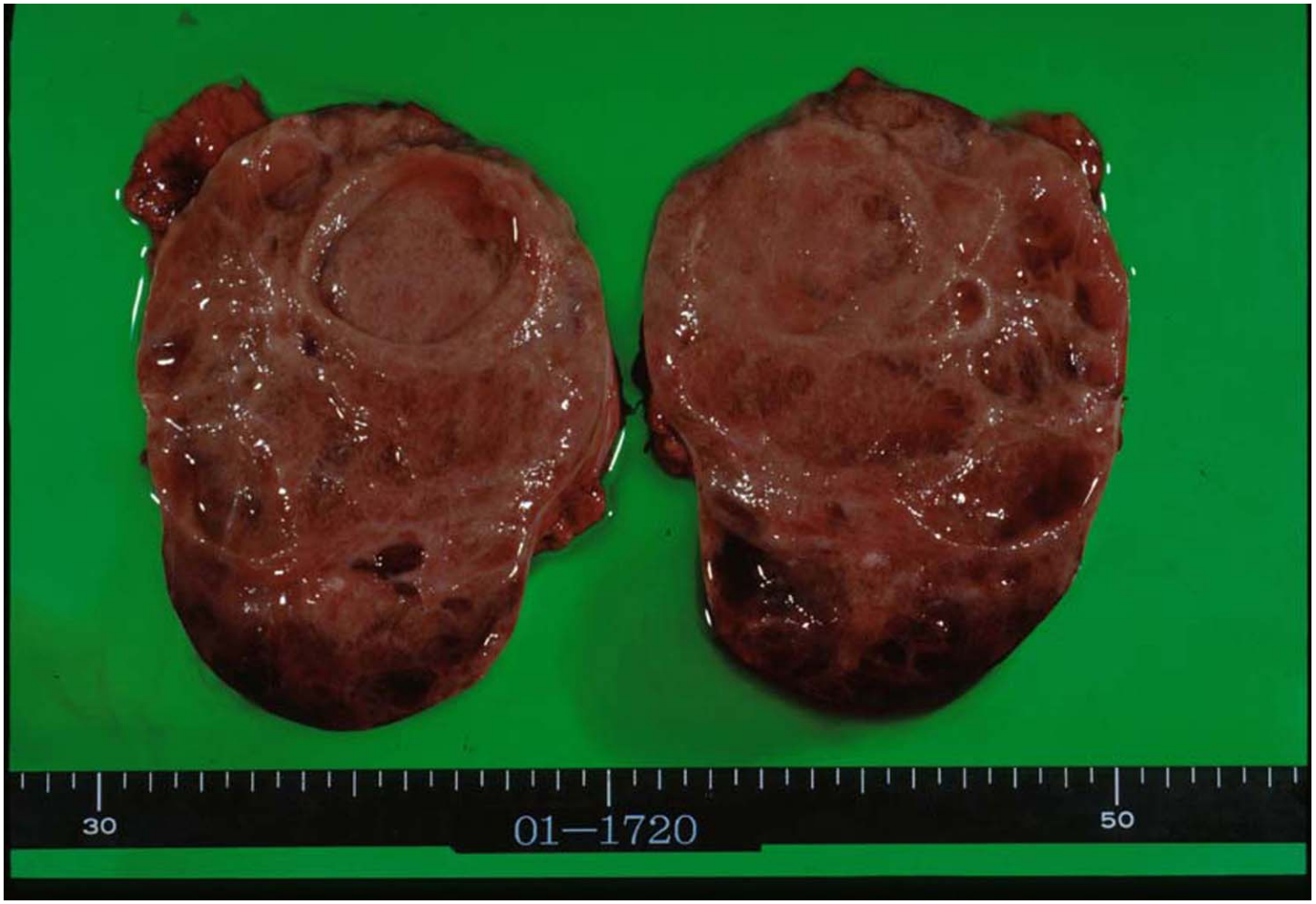
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