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ORIGINAL

Acromegaly caused by a somatotroph adenoma in patient with neurofibromatosis type 1

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Abstract. Although acromegaly has been reported in patients with Neurofibromatosis type 1 (NF1), these cases have not been associated with growth hormone (GH)-producing somatotroph adenoma, but with optic pathway glioma. A 68 year-old Japanese woman, who had been clinically diagnosed with NF1, was referred to our hospital due to a thyroid tumor and hypercalcemia. Acromegaly was suspected due to her facial features, and subsequent examinations revealed the presence of GH excess with a pituitary tumor, leading to the diagnosis of acromegaly. Histological and immunohistochemical analysis demonstrated an eosinophilic pituitary adenoma with diffuse positivity for GH, indicating typical somatotroph adenoma. In addition, her thyroid tumor was diagnosed histologically as follicular thyroid carcinoma (FTC) with primary hyperparathyroidism (PHPT). To investigate the pathogenesis of this untypical multiple endocrine tumor case of NF1, genetic analysis was performed using peripheral leukocytes and tissue of resected tumors. A heterozygous novel germline nonsense mutation (p.Arg1534*) in exon 35 of the *NF1* gene was detected from peripheral leukocytes, which results in a truncated protein lacking the critical domain for GTPase activity, strongly suggesting its causal role in NF1. The loss of heterozygosity (LOH) in exon 35 of the *NF1* gene was not detected in the somatotroph adenoma, parathyroid adenoma, and FTC. Although any mutations of the following genes; *MEN1*, *CDKN1B*, and *PAX8-PPARγ* were not detected, a heterozygous *GNAS* R201C mutation was detected in the somatotroph adenoma. To our knowledge, this is the first rare MEN1-like case of genetically diagnosed NF1 complicated with acromegaly caused by a somatotroph adenoma.

Key words: Acromegaly, NF1, Somatotroph adenoma, Primary hyperparathyroidism, Thyroid follicular carcinoma

ACROMEGALY is characterized by excess GH secretion, mostly due to GH-producing somatotroph adenomas [1]. The majority of somatotroph adenomas are sporadic, with approximately 30–50% caused by activating mutations in the *GNAS* gene that encodes the G protein alpha-subunit [2]. A mosaic mutation in the *GNAS* gene has been described in McCune-Albright syndrome, which presents as sporadic acromegaly or gigantism [3]. A small proportion of somatotroph adenomas arise as familial pituitary adenomas, including multiple endocrine neoplasia type 1 (MEN1), MEN4, Carney complex, familial isolated pituitary adenomas (FIPA), X-linked acrogigantism (XLAG), and SDH-related syndrome [2]. These conditions are caused by mutations in the *MEN1*, cyclin-dependent kinase inhibitor 1B (*CDKN1B*), *PRKAR1A*, aryl hydrocarbon receptor-interacting protein (*AIP*), G-protein coupled receptor 101 (*GPR101*), and *SDHx* gene, respectively [2].

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder, caused by a heterozygous germline mutation in the *NF1* gene. Patients with NF1 develop multiple café'-au-lait macules and cutaneous neurofibromas. They

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also manifest skeletal abnormalities, vascular diseases, neurologic abnormalities, respiratory diseases, and multiple benign or malignant tumors [4]. Inactivation of the neurofibromin that activates RAS pathway signaling has been shown in NF1-related tumors [5].

Acromegaly has been described in some NF1 patients [6]. This autonomous GH hyper-secretion has only been observed in the presence of optic pathway gliomas (OPG) without somatotroph adenoma, which are located within or around the hypothalamus area. Thus, it has been considered that OPG play a pivotal role in the development of acromegaly in NF1; however, the pathophysiology remains largely unknown [6].

Here, we describe a case of acromegaly caused by a somatotroph adenoma in a patient with NF1 that was confirmed by genetic analysis.

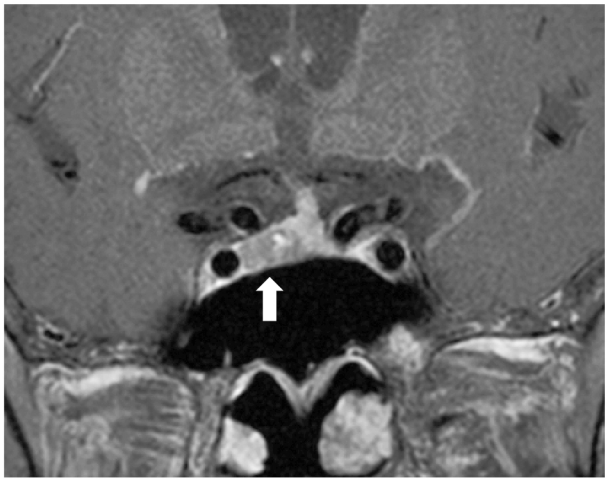

Fig. 1 MRI findings gadrinium enhanced T1WI showed the tumor as 5 mm less enhanced region.

Case Presentation

A 63-year-old Japanese woman who was clinically diagnosed with NF1 at 58 years of age was referred to the otolaryngology division at our hospital for evaluation of an enlarging thyroid tumor. It was noted that her serum Ca and intact PTH levels were elevated; she was referred to our division on suspicion of primary hyperparathyroidism (PHPT) with thyroid cancer. Her surgical history included uterine myoma at 37 years of age, bladder paraganglioma at 54 years of age, retroperitoneal fibrosis at 54 years of age, abdominal aneurysm at 61 years of age, and scoliosis at 62 years of age. Family history demonstrated that many relatives were clinically diagnosed with NF1 due to presence of neurofibromas. She showed acromegalic features of nose, lower lip, and frontal bone enlargement, as well as jaw and mandibular protrusion and enlarged extremities. These features have emerged from 48 years age of her photo.

Serum GH and IGF-I levels were elevated (28.7 ng/mL and 447 ng/mL (+5.7 SD), respectively). Serum nadir GH levels during 75 g-OGTT were 25.3 ng/mL, demonstrating autonomous hyper GH secretion. Magnetic resonance imaging (MRI) showed a 5 mm diameter pituitary adenoma at the right pituitary lobe (Fig. 1). She was diagnosed with acromegaly according to the diagnostic guideline [7]. She also had impaired glucose tolerance, sleep apnea syndrome, and a thyroid tumor, all considered associated with acromegaly. Octreotide test showed significant GH suppression (serum GH levels: 30.8 to 6.5 ng/mL, 79% decrease), while bromocriptine test showed no suppression (serum GH levels: 43.0 to 36.1 ng/mL, 16% decrease). Remarkably high PTH levels (362 pg/mL) with elevated Ca levels (11.1 mg/dL) and a parathyroid tumor with an accumulation of ^{99m}Tc-methoxy-isobutyl-isonitrile (MIBI) indicated a diagnosis and localization of primary hyperparathyroidism (PHPT). Thus, we performed a right lobectomy of the thyroid and parathyroidectomy. After the surgery, her serum Ca and intact PTH levels decreased (Ca 9.6 mg/dL, intact PTH 123 pg/mL). Pathological diagnoses were parathyroid adenoma and follicular thyroid carcinoma (FTC), respectively. Meanwhile, she was treated with a long-acting somatostatin analog; this started as octreotide-LAR 20 mg/M and increased to 30 mg/M. Subsequently, serum GH levels decreased from 28.7 to 8.59 ng/mL, although IGF-I levels remained unchanged (447 to 511 ng/mL). She then underwent trans-sphenoidal surgery and serum GH and IGF-I levels normalized (nadir GH: 0.2 ng/mL, IGF-I: 95 ng/mL (–0.6 SD)). Histological and immunohistochemical analysis demonstrated an eosinophilic adenoma with diffuse positivity for GH, indicating typical somatotroph adenoma (Fig. 2a–b). CAM5.2 staining showed both intracytoplasmic dot pattern and perinuclear pattern, indicating an intermediate mixed type of densely granulated and sparsely granulated adenoma (Fig. 2c). E-Cadherin staining was also heterogenous (Fig. 2d). Both SSTR2A and SSTR5 were highly expressed (Fig. 2e–f). Ki-67 positive cells were scant (less than 1%) (Fig. 2g).

To clarify the genetic cause of this complicated condition, with written informed consent, we analyzed the *MEN1* gene using peripheral blood samples; however, no mutations were found in any exons in the gene. Genetic analysis of the *NF1* revealed a heterozygous mutation c. 4600C>T, p.Arg1534* in exon 35 (Fig. 3a). Analysis of the tumor DNA demonstrated an absence of loss of heterozygosity (LOH) in exon 35 of the *NF1* gene in somatotroph adenoma, parathyroid adenoma, and FTC (Fig. 3b, data not shown). However, somatic *GNAS* R201C mutation was detected in the somatotroph adenoma (Fig. 3c).

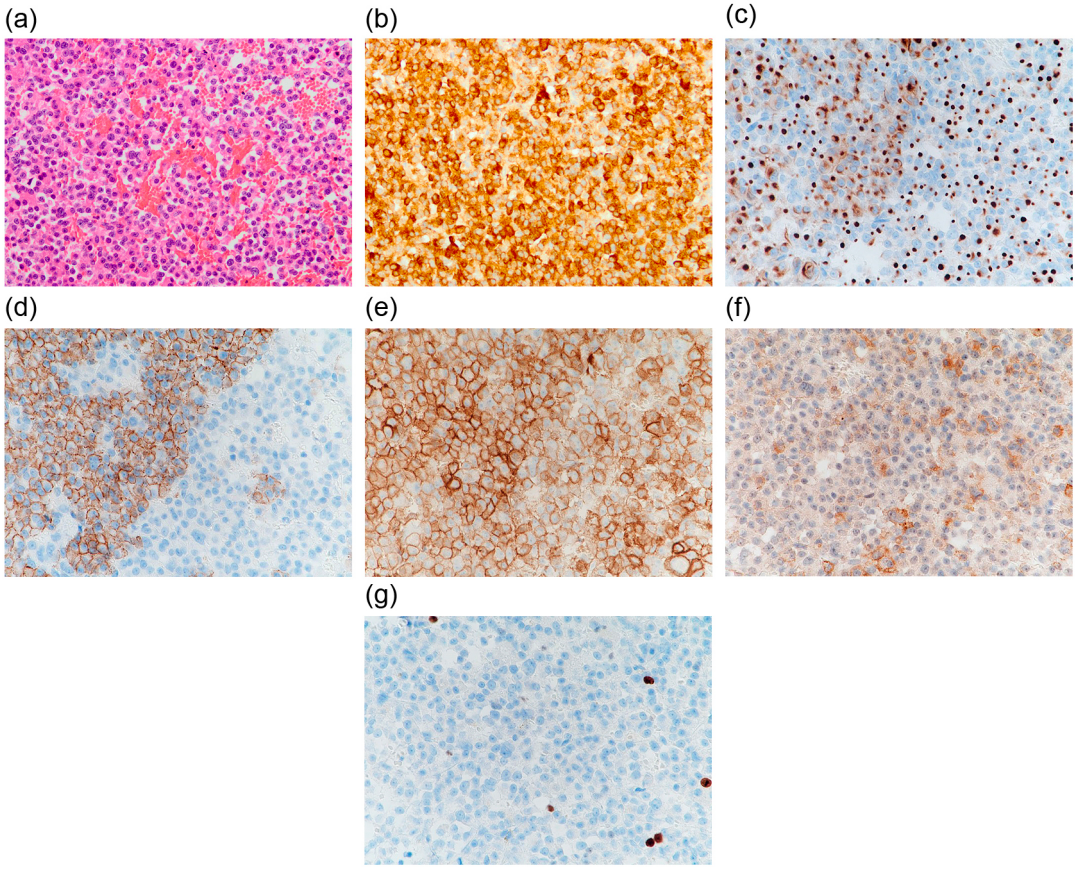

Fig. 2 Hematoxylin and eosin staining showed eosinophilic adenoma (a). Growth hormone immunoreactivity was diffusely stained in the cell body (b). CAM5.2 staining showed both intracytoplasmic and perinuclear patterns (c). E-Cadherin immunoreactivity was strongly positive in the cells with intracytoplasmic pattern of CAM5.2 staining (d). Both SSTR2A (e) and SSTR5 (f) are highly expressed. Ki-67 positive cells were scant and MIB-1 index was less than 1% (g).

We further analyzed *PAX8-PPARγ* gene rearrangements in FTC, and *CDKN1B* gene mutation status in parathyroid adenoma, did not show any mutations (data not shown). Genetic analysis was performed as previously described [8-12]. Briefly, genomic DNA was extracted using a QIAamp® DNA FFPE tissue kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. All the coding exons and the flanking introns of the exons in the *MEN1* gene were analyzed by direct sequencing [8]. *NF1* gene analysis were performed by a target sequencing panel with the next-generation sequencing technology [9]. *GNAS* mutation status (coding 201 and 227) were analyzed as previously described [10]. *CDKN1B* gene sequencing were performed using specific primers as shown before [11]. For *PAX8-PPARγ* rearrangements analysis, mRNA was isolated from tumor tissues by TRIzol reagent (Invitrogen, Tokyo, Japan). One μg of RNA was reverse transcribed into cDNA by reverse transcription with ReverTra Ace qPCR RT kit (TOYOBO Co., Ltd., Osaka, Japan). Combined RT-PCR with primers in exons 4–8 of *PAX8* and in exon 1 of *PPARγ* were performed as previously described [12].

Discussion

To our knowledge, this is the first case report of genetically determined NF1 with acromegaly caused by somatotroph adenoma. GH hypersecretion has been described in some patients with NF1, resulting in acromegaly. As these cases have exclusively considered the association with OPG and GH hypersecretion [2, 6]. On the other hand, the disruption of *NF1* gene in mice has been shown to suppress the hypothalamic-pituitary axis mediated by reduction of GHRH expression [13], suggesting the strong interaction between *NF1* gene and GH/IGF-I axis. The somatotroph adenoma in this case may be a coincidence. However, co-existent of pituitary adenoma, PHPT, and FTC is hard to explain only as a coincidence.

In the present case, germline heterozygous nonsense mutation c.4600C>T.p.Arg1534* in exon 35 of the *NF1* gene was detected. The *NF1* gene encodes the GTPase-activating protein (GAP) neurofibromin. Neurofibromin inactivates RAS, resulting in suppression of downstream signaling, including MAPK and PI3K. Therefore, loss of protein function causes RAS-related tumorigenesis,

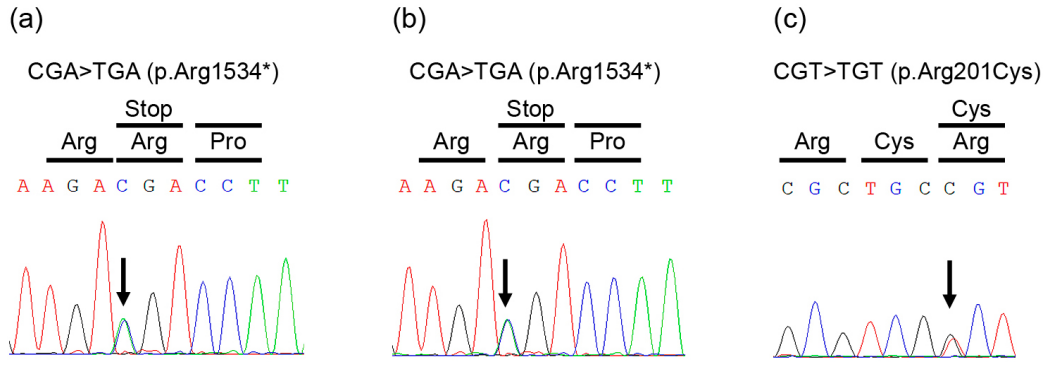

Fig. 3 Genetic analysis using DNA from patient's peripheral blood. A heterozygous non-sense mutation in exon35 of *NF1* gene; *NF1* c.4600C>T (p.Arg1534*) were detected (a). Genetic analysis using DNA from patient's somatotroph adenoma, LOH of *NF1* gene were not detected (b). Genetic analysis using DNA from patient's somatotroph adenoma, revealed a somatic heterozygous mutation in the *GNAS* gene; *GNAS* R201C (c).

called RASopathies [5]. This novel mutation (c.4600C>T.p.Arg1534*) results in a truncated protein lacking the critical domain for GTPase activity, strongly suggesting its causal role in NF1.

In the somatotroph adenoma, parathyroid adenoma, or FTC, LOH in exon 35 of the *NF1* gene was not detected. Although compound heterozygous *NF1* mutation including mutations or deletions other than exon 35 in these tumors were not excluded, these results suggest that a second hit might have occurred in these tumors to cause tumorigenesis. A heterozygous R201C mutation in the *GNAS* gene was detected in the somatotroph adenoma. However, *PAX8-PPARγ* gene rearrangements in FTC, and *CDKN1B* gene mutation were not detected. In NF1-related tumors, the frequency of somatic LOH in the *NF1* gene has been reported in both high [14-16] and low [17-19]. LOH in cell cycle-related genes, including *TP53*, *CDKN2A*, and *RB1* has been detected in some NF1-related tumors, suggesting DNA instability in NF1. This DNA instability might cause the *GNAS* gene mutation in the pituitary. However, a coincidence of somatotroph adenoma with the *GNAS* gene mutation cannot be ruled out.

In this case, primary hyperparathyroidism was complicated with pituitary tumor, indicating MEN1-resemble clinical manifestation. Thus, we analyzed the *MEN-1* gene but did not find any mutations. There are several reports of NF1 complicated with PHPT [20]. Although a causal relationship remains unknown, considering the tumor-prone condition in NF1, PHPT may be a rare but relevant complication. In terms of FTC, no reports have described the coexistence of FTC in NF1. However, a somatic mutation in the *NF1* gene was recently detected from FTC by whole genome analysis [21], suggesting that germline mutation in the *NF1* gene could play a role in FTC development.

In conclusion, we demonstrated the first case of NF1 complicated with acromegaly caused by somatotroph adenoma with somatic *GNAS* R201C mutation. This case also had co-existent with PHPT and FTC without *MEN1* mutations. Further pathogenic analysis is required for this quite rare case of NF1 why tumor prone in several atypical endocrine organ is occurred.

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Disclosure

None of the authors have any potential conflict of interest associated with this research.

Compliance with Ethical Standards

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Ethical Approval

All procedures performed in this case report were approved by the Kobe University Hospital ethical committee (IRB No. 1363). Written informed consent was obtained from the present patient.

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