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Genetic and clinical characteristics of Japanese patients with sporadic somatotropinoma

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Abstract. Most of acromegaly is caused by a sporadic somatotropinoma and a couple of novel gene mutations responsible for somatotropinoma have recently been reported. To determine the cause of sporadic somatotropinoma in Japanese patients, we analyzed 61 consecutive Japanese patients with somatotropinoma without apparent family history. Comprehensive genetic analysis revealed that 31 patients harbored guanine nucleotide-binding protein, alpha stimulating (*GNAS*) mutations (50.8%) and three patients harbored aryl hydrocarbon receptor interacting protein (*AIP*) mutations (4.9%). No patients had G protein-coupled receptor 101 (*GPR101*) mutations. The patients in this cohort study were categorized into three groups of *AIP*, *GNAS*, and others and compared the clinical characteristics. The *AIP* group exhibited significantly younger age at diagnosis, larger tumor, and higher nadir GH during oral glucose tolerance test. In all patients with *AIP* mutation, macro- and invasive tumor was detected and repetitive surgery or postoperative medical therapy was needed. One case showed a refractory response to postoperative somatostatin analogue (SSA) but after the addition of cabergoline as combined therapy, serum IGF-I levels were controlled. The other case showed a modest response to SSA and the switching to cabergoline monotherapy was also effective. These data suggest that although resistance to SSA has been reported in patients with *AIP* mutations, the response to dopamine agonist (DA) may be retained. In conclusion, the cause of sporadic somatotropinoma in Japanese patients was comparable with the previous reports in Caucasians, patients with *AIP* mutations showed unique clinical characteristics, and DA may be a therapeutic option for patients with *AIP* mutations.

Key words: Acromegaly, Gigantism, *GNAS*, *AIP*, *GPR101*

SOMATOTROPINOMA causes unregulated growth hormone (GH) hypersecretion, which results in acromegaly or gigantism [1]. Patients with acromegaly or gigantism without adequate treatment show a reduced life expectancy and impaired quality of life [2, 3]. Interestingly, endocrinological and clinical phenotypes of somatotropinoma greatly vary among patients; regarding serum GH and IGF-I levels, tumor size, tumor invasiveness, and response to medical therapy.

The underlying mechanism which yields this diversity has not been fully elucidated. Somatotropinoma is reportedly caused by unrestrained somatotroph proliferation by cell-cycle dysfunction as well as altered intracellular signaling regulating GH synthesis/secretion [4]. Although somatotropinoma tumorigenesis has not been fully understood, genetic analysis of somatic or germline mutations in patients with somatotropinoma revealed several pathogenetic mutations, which determine unique cellular and clinical characteristics. Therefore clarifying these mutations lead us to not only the understanding these tumorigenic processes but also to better management of the disease.

One of the most well-documented pathogenesis of somatotropinoma is activating guanine nucleotide-

binding protein, α stimulating (*GNAS*) mutations [5]. Approximately 40% of patients with sporadic acromegaly harbor somatic *GNAS* mutations, leading to constitutively activated cAMP pathway [6]. In general, somatotropinoma harboring *GNAS* mutations tended to have higher serum GH and IGF-I levels, smaller tumor, and good response to somatostatin analogues (SSA) and dopamine agonists (DA) [7, 8].

Analysis of familial pituitary adenoma revealed that germline mutations in the aryl hydrocarbon receptor interacting protein (*AIP*) gene cause pituitary adenoma, including somatotropinoma, prolactinoma, ACTHoma, or non-functioning pituitary adenoma [9]. Occurrence of germline mutations and loss of the normal allele in tumors suggest that *AIP* acts as a tumor suppression gene. Typically, patients with *AIP* mutations have a young age at disease onset, macro- and invasive tumor, and poor response to SSA. It is noteworthy that *AIP* mutations are occasionally detected in patients without family history of pituitary adenoma.

Recently, Trivellin *et al*. reported that microduplications on chromosome Xq26.3 cause somatotropinoma in children with gigantism, named X-linked acrogigantism (X-LAG) [10]. In addition, they also reported that among the duplicated genes on Xq26.3, the expression of G protein-coupled receptor 101 (*GPR101*) was highly upregulated in the pituitary tumors. Furthermore, *GPR101* sequence analysis revealed a missense mutation, c.924G>C (p.Glu308Asp), which resulted in an enhanced action of GPR101, in 11 of 248 (4.4%) patients with sporadic acromegaly.

The diagnosis of syndromic pituitary adenoma, such as McCune-Albright syndrome, Carney complex, or multiple endocrine neoplasia type 1 is not generally difficult because the patients exhibit several clinical characteristic manifestations. However, it is difficult to differentiate the acromegalic patients clinically caused by *GNAS*, *AIP*, or G*PR101*, but it would be useful to clarify these mutations because it may help to make the strategy for treatment. Here, we investigated the prevalence of pathogenetic somatic mutations of *GNAS*, *AIP*, and *GPR101* in Japanese patients with sporadic somatotropinoma and analyzed the clinical characteristics.

Materials and Methods

Patients

This study was approved by the Kobe University Hospital and Toranomon Hospital Ethics Committee.

We recruited 61 consecutive patients with sporadic acromegaly or gigantism without a family history of pituitary tumors, who underwent surgical treatment at Toranomon Hospital between May 2005 and May 2010. All patients provided written informed consent for genetic analysis. The diagnosis of somatotropinoma was histologically confirmed. Clinical data were retrospectively collected from patients' medical records. The diagnosis of acromegaly was based on clinical signs, lacking of serum GH suppression to <1.0 ng/mL during a 75 g oral glucose tolerance test (OGTT), elevated serum IGF-I levels corresponding to the normal range for age- and sex-matched individuals, and the presence of pituitary tumor according to the guideline [11]. The duration of acromegaly was assessed visually by comparison of photographs and by the onset of related symptoms as previously described [11]. Serum GH and IGF-I levels were measured by an immunoenzymometric assay using the ST AIA-PACK hGH kit (TOSOH Co., Tokyo, Japan) and an immunoradiometric assay using "Daiichi" IGF-I IRMA kit (FUJIFILM RI Pharma Co., Tokyo, Japan), respectively. Intra- and inter-assay coefficients of variation (C.V.) for the assay of GH and IGF-I were as follows: GH (intra-C.V. 1.3% and inter-C.V. 3.3%) and IGF-I (intra-C.V. 1.1% and inter-C.V. 2.2%), respectively. Acute octreotide test (SSA test) and bromocriptine test (BRC test) were performed as previously described [12]. In brief, following the subcutaneous injection of 50 μg octreotide or the oral administration of 2.5 mg bromocriptine, serum GH level was measured every two hours until eight hours after administration.

DNA extraction

Tumor DNA was extracted using the Gentra Puregene Tissue Kit (QIAGEN, Venlo, Netherlands). Genomic DNA was extracted from peripheral leukocytes using the Gentra Puregene Blood Kit (QIAGEN, Venlo, Netherlands).

Genetic analysis

Using tumor DNA, *GNAS* gene analysis was performed with amplifications of regions containing 2 sites of activating somatic mutations in codons 201 and 227 [5]. *AIP* gene analysis was performed in patients who revealed negative for *GNAS* mutations as previously described [9]. We also analyzed deletions/duplications in *AIP* gene in the patients without *AIP* mutation with younger age (< 40 years), invasive

tumor, or macroadenoma (> 10 mm). Deletions/duplications analysis was performed using SALSA MLPA kit P244 designed to detect deletions/duplications in *AIP*, *MEN1*, and *CDKN1B* genes (MRC-Holland, Amsterdam, Netherlands) according to the manufacturer's instructions. We further analyzed *AIP* mutations using genomic DNA in patients who were positive for the *AIP* mutations in the tumor. *GPR101* gene analysis was also performed with amplifications of region containing codon 308, which was previously reported as a recurrent somatic mutation in acromegaly [10]. Each primer sequence is available with request. All sequencing analysis was performed as follows; 35 cycle of PCR involved a denaturation step at 95 °C for 20 s, annealing step at 60 °C for 30 s, and extension step at 72 °C for 30 s. PCR products were sequenced using BigDye Terminator v3.1 Cycle Sequence Kit (Applied Biosystems, Tokyo, Japan), followed by analysis with ABI Prism 310 Genetic analyzer (Applied Biosystems, Tokyo, Japan).

Immunohistochemistry

Immunohistochemistry for paraffin-embedded tumor samples was performed by using avidin-biotin-peroxidase complex (ABC) as previously described [13]. The following primary antibody were used: AIP (HPA004063, SIGMA-ALDRICH, U.S.A.), E-cadherin, somatostatin receptor subtype 2 and 5 (SSTR2, 5) (SS-800 and SS-838, Gramsch Lab., Germany), and dopamine D2 receptor (D2R).

Statistical analysis

Data are expressed as means ± standard deviations or medians [interquartile range]. Continuous data in multiple groups were compared by a one-way analysis of variance or a Kruskal−Wallis test with a *post-hoc* test. Categorical data in multiple groups were compared by a χ^2 test followed by Tukey's honestly significant difference test. *P*-values of 0.05 or less were considered significant. Statistical analyses were performed using JMP Statistical Database Software version 8.0.1 (SAS Institute, Inc. Cary, NC, USA).
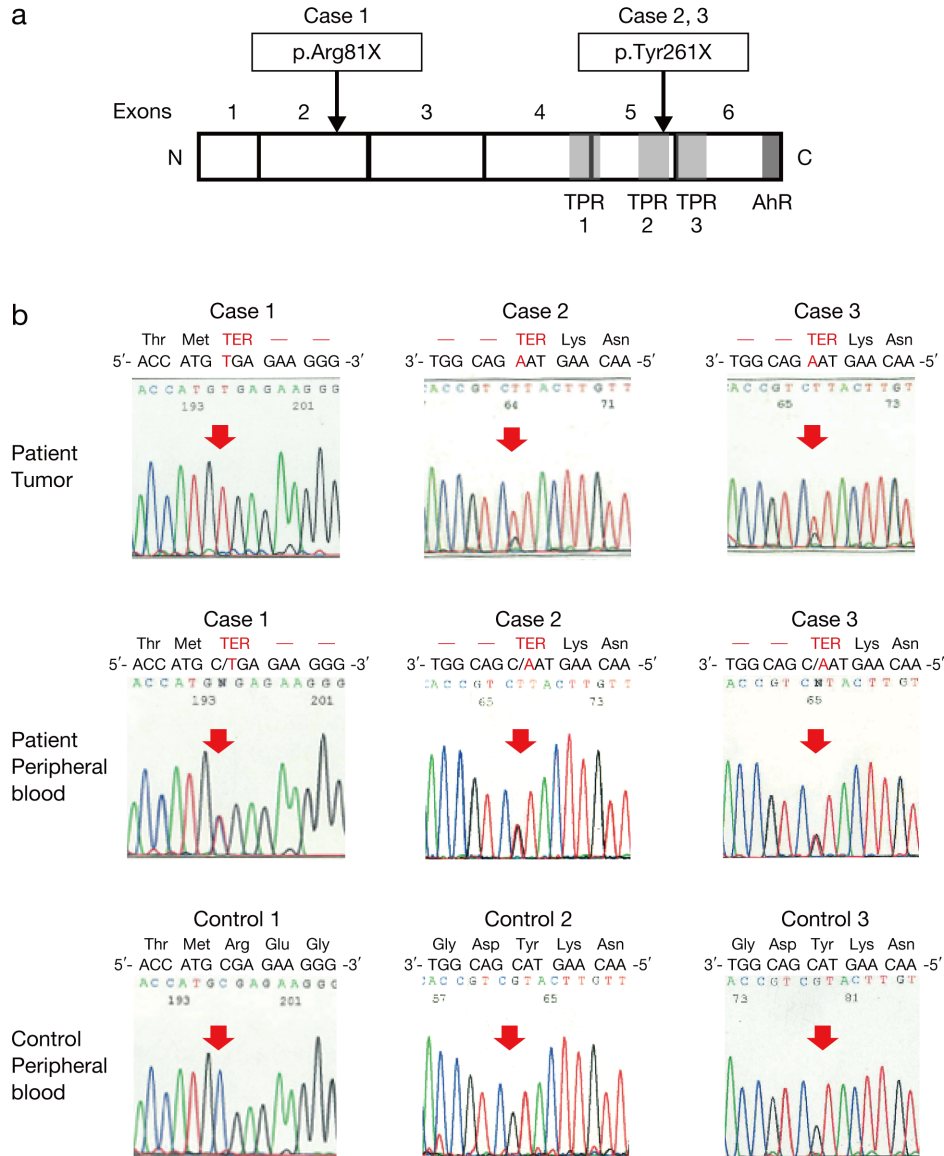
Results

Genetic analysis

Clinical characteristics of the patients are shown in Table 1. The patients cohort consisted of 28 male (46%) and 33 female (54%). Eighty five percent of the patients revealed a macroadenoma. The analysis of *GNAS* mutation in the tumor revealed 31 heterozygous mutations (50.8%); 22 case of c.601C>T (p.Arg201Cys), 4 case of c.680A>T (p.Gln227Leu), 3 case of c.602G>A (p.Arg201His), 1 case of c.601C>A (p.Arg201Ser), and 1 case of c.680A>G (p.Gln227Qrg), in which all mutations reportedly cause somatotropinoma. The analysis of *AIP* gene revealed that 3 patients exhibited a germline mutation with loss of heterozygosity (LOH) in the tumor (4.9%) (Fig. 1). A heterozygous c.241C>T (p.Arg81X) in exon 2 was detected in a 7-year-old male patient (Case 1) and heterozygous c.783C>A (p.Tyr261X) in exon 5 was detected in a 26-year-old male (Case 2) and 46-year-old female patient (Case 3) in the DNA from peripheral blood. Both mutations cause stop codon and result in a truncated AIP protein, which have been previously described [14] (Fig. 1a, b). We further performed a MLPA analysis to detect *AIP* deletions/duplications in 27 patients, who were in suspicion of AIP abnormality because of the clinical characteristics. However, no *AIP* gene deletions/duplications were detected. At last, somatic *GPR101* mutation in codon 308, which was detected in sporadic acromegaly [10] was examined in remaining 27 patients. No mutations in codon 308 of *GPR101* were detected. These results demonstrate that the prevalence of *GNAS* mutation was 50.8%, *AIP* mutation was 4.9%, and unknown causes were 44.3% in Japanese patients with somatotropinoma.

Table 1 Clinical characteristics of patients with somatotropinoma

Age (years)	46.2 ± 13.5
Sex (male/female)	28/33
Height (cm; male/female)	175.0 ± 9.3 / 158.1 ± 7.8
Body weight (kg; male/female)	82.6 ± 12.2 / 62.7 ± 10.4
BMI	25.9 ± 3.3
Disease duration (years)	3.0 [6.3]
Macroadenoma (%)	85
Tumor volume (cm^3)	1.1 [2.1]
Knosp grade (%; 0/1/2/3/4)	36/17/19/25/3
Random GH (ng/mL)	11.6 [14.5]
Nadir GH (ng/mL)	5.7 [9.9]
IGF-I (ng/mL)	640 [372]
IGF-I SDS	6.7 ± 2.6
PRL (ng/mL)	11.7 [22.0]
n	61

Fig. 1 (**a**) *AIP* gene structure and the location of *AIP* gene mutations. (**b**) Somatic and germline *AIP* mutations in each case. A heterozygous mutation in peripheral blood and LOH in the tumor were detected. All mutations were predicted to result in a production of truncated AIP protein. TPR, tetratricopeptide repeat domain; AhR, Aryl hydrocarbon receptor binding domain.

We next compared clinical indices of each group to clarify the clinical characteristics. The *AIP* group showed younger age at the disease onset, higher height, heavier body weight, larger tumors, and higher nadir GH among three groups (Table 2). The *GNAS* group showed smaller tumor size, higher serum GH levels corresponding to the tumor size, and better responses to SSA and BRC test as compared to the *AIP* and others group.

Patients with AIP mutation
Case 1.

A 7-year-old boy presented with tall statue (146.4 cm, +4.8 SD). His serum GH and IGF-I levels were markedly elevated (54.6 ng/mL and 1,108 ng/mL (+5.3 SDS), respectively). Nadir serum GH level during OGTT was 46.2 ng/mL. Pituitary magnetic resonance imaging (MRI) revealed a 38-mm-sized inva-

Table 2 Comparison of clinical characteristics of *AIP*, *GNAS*, and others groups

|	AIP	GNAS	Others	p
Age (years)	23.7 ± 20.1 **	48.5 ± 11.3	46.2 ± 13.3	<0.01 **
Sex (male/female)	2/1	15/16	11/16	0.64
Disease duration (years)	3.0 [3.0]	4.0 [4.0]	2.0 [8.8]	0.97
Height (cm; male/female)	146.4, 193.6 / 163.3	175.4 ± 6.0 / 157.3 ± 9.3	175.8 ± 3.5 / 158.5 ± 6.6	—
Height SDS	3.3 ± 1.9 **	0.47 ± 1.3	0.63 ± 0.9	<0.01 **
Body weight (kg; male/female)	47.9, 102.0 / 88.1	85.4 ± 9,7 / 63.7 ± 10.1	81.3 ± 6.3 / 59.3 ± 7.5	—
Body weight SDS	5.7 ± 1.4 **	1.9 ± 1.4	1.3 ± 1.1	<0.01 **
BMI	27.5 ± 1.9	26.5 ± 0.7	24.7 ± 0.7	0.14
Tumor volume (cm^3)	8.1 [11.4] **	1.0 [1.4]	1.4 [2.4]	<0.01 **
Knosp grade (%; 0/1/2/3/4)	0/0/0/100/0	37/26/26/11/0	43/7/14/29/7	0.06
Random GH (ng/mL)	23.3 [29.6]	11.8 [15.6]	7.2 [13.2]	0.09
Nadir GH (ng/mL)	12.2 [38.6] *	7.6 [8.8]	3.4 [7.1]	0.03 *
IGF-I (ng/mL)	927 [448]	654 [444]	566 [283]	0.12
IGF-I SDS	5.7 ± 3.4	7.3 ± 2.7	6.2 ± 2.2	0.21
PRL (ng/mL)	21.9 [27.4]	17.2 [59.5]	10.9 [7.7]	0.47
Hypertension (%)	66.7	40.7	30.4	0.49
Dyslipidemia (%)	33.3	7.4	0	0.08
Diabetes (%)	0	22.7	10.5	0.33
HbA1c (%)	5.9 ± 0.46	5.7 ± 0.88	5.5 ± 0.74	0.66
DGA/SGA (%)	33/67	73/27	57/43	0.36
MIB-1 index (%)	0.5 ± 0.9	0.8 ± 0.2	0.8 ± 0.3	0.88
SSA test (%change)	51.0 ± 15.8	90.2 ± 10.4 **	71.7 ± 22.7	<0.01 **
BRC test (%change)	25.6 ± 8.8	74.6 ± 21.2 *	56.8 ± 19.2	0.02 *
n	3	31	27	

* indicates *P* value < 0.05. ** indicates *P* value < 0.01.

sive pituitary adenoma (Fig. 2a). He was diagnosed with gigantism. Presurgical long-acting octreotide acetate (octreotide LAR) was started; however, apparent tumor shrinkage was not observed (16.3 cm^3 to 13.8 cm^3). After 3 months administration of octreotide LAR, transsphenoidal surgery was performed. After the surgery, his serum GH, nadir GH, and IGF-I levels were improved but slightly higher than normal range (0.46−2.4 ng/mL, 1.5 ng/mL, and 307.8−509.2 ng/mL, respectively) (Fig. 2b). However, seven months after the surgery, his residual tumor became enlarged without any symptoms. Then, he underwent the second surgery. The second surgery achieved complete tumor resection and his serum GH and IGF-I levels became within normal range (0.03 ng/mL and 119-208 ng/mL, respectively) (Fig. 2b). *AIP* sequence analysis revealed a germline heterozygous nonsense mutation c.241C>T (p.Arg81X) in exon 2 with LOH in the tumor (Fig. 1a, b).

Case 2.

An 18-year-old man presented with tall statue (193.6 cm, +4.0 SD) and acral enlargement. His random GH level was 23.3 ng/mL and serum IGF-I level was 660 ng/mL (+2.5 SDS). Nadir serum GH level during the OGTT was 12.2 ng/mL. Pituitary MRI revealed a 30-mm-sized invasive pituitary adenoma (Fig. 2c). He was diagnosed as gigantism. Presurgical octreotide LAR did not reduce tumor size (4.9 cm^3 to 4.7 cm^3). After 4 months administration of octreotide LAR, he underwent transsphenoidal surgery; however his serum GH and IGF-I levels remained higher than normal range, then he was treated with octreotide LAR. Although his IGF-I levels had been controlled by 40 mg/month of octreotide LAR for 5 years, his serum IGF-I levels escaped and elevated higher than normal range (484.7 ng/mL, +3.3 SDS) (Fig. 2d). Therefore, cabergoline with octreotide LAR was started as a combination therapy. Thereafter, his serum GH and IGF-I levels were well-controlled for at least 3 years. *AIP* gene analysis revealed a germline nonsense mutation c.783C>A (p.Tyr261X) in exon 5 with LOH in the tumor (Fig. 1a, b).
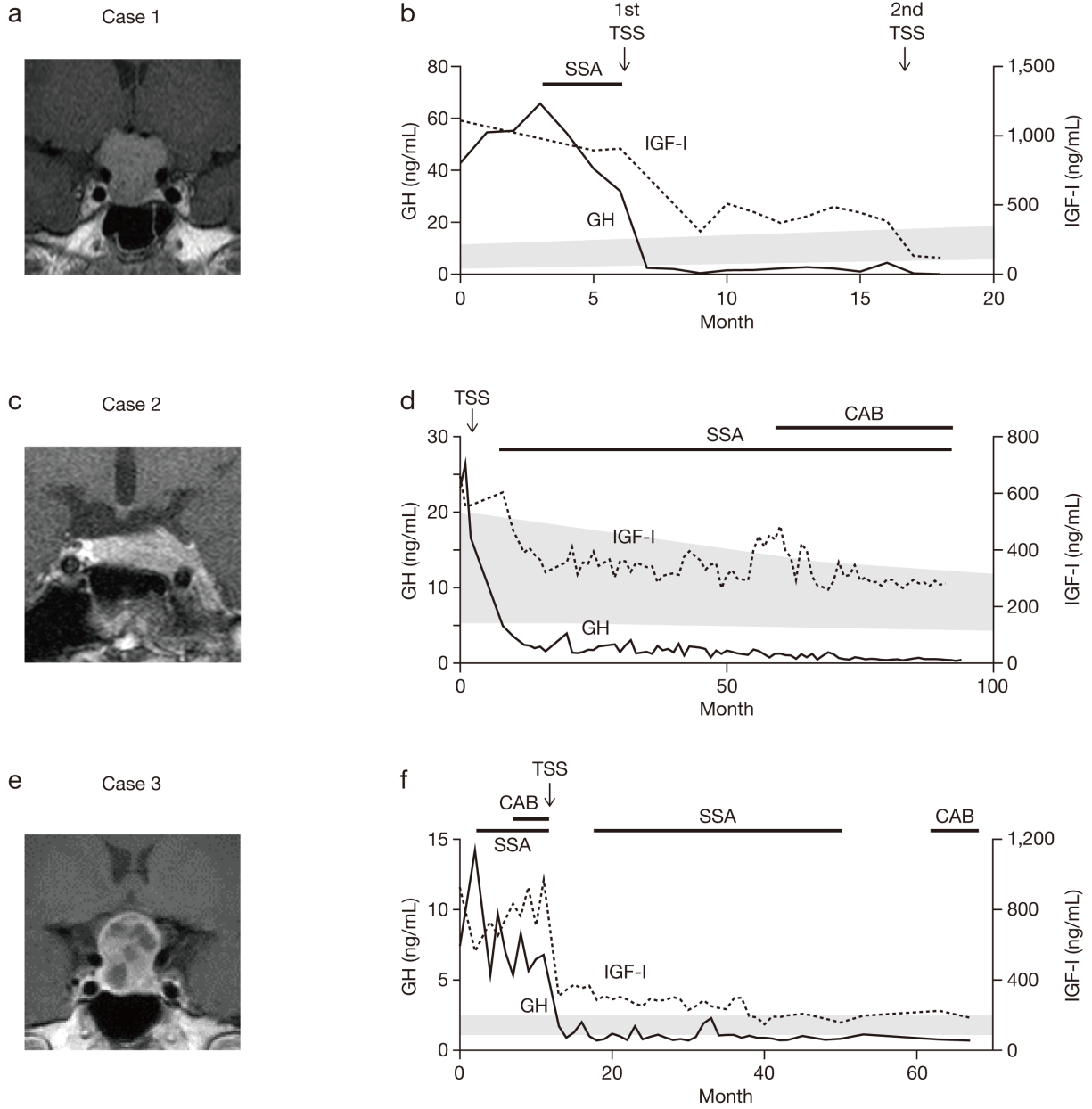
Fig. 2 MRI findings and clinical courses of patients with *AIP* mutations
Case 1, (**a**) and (**b**); Case 2, (**c**) and (**d**); Case 3, (**e**) and (**f**). Shaded areas indicate IGF-I normal range corresponding to age- and sex-matched Japanese population. TSS, transsphenoidal surgery; SSA, somatostatin analogue; CAB, cabergoline. (**g**) Results of acute octreotide test (SSA test) and bromocriptine test (BRC test).

Case 3.

A 46-year-old woman presented with a visual disturbance. Although her height was within normal limit (163.3 cm), she had acromegalic face. Her serum GH and IGF-I levels were elevated (13.2 ng/mL and 927 ng/mL (+9.6 SDS), respectively). Nadir serum GH level during the OGTT was 7.7 ng/mL. Pituitary MRI showed a 36 mm-sized invasive pituitary adenoma compressing optic chiasm (Fig. 2e). She was diagnosed with acromegaly. Because she first refused surgery, she was treated with octreotide LAR and then cabergoline was added as a combination therapy (Fig. 2f). Although visual disturbance improved and serum GH levels decreased after combination therapy, serum IGF-I levels and the size of the tumor assessed by MRI (8.1 cm^3 to 7.3 cm^3) did not show any apparent changes. She then underwent transsphenoidal surgery. Most of the tumor was removed and serum GH (1.2 ng/mL) and IGF-I (302 ng/mL, +3.9 SDS) levels improved. But serum GH and IGF-I levels remained higher than the normal range, octreotide LAR was started. *AIP* gene analysis revealed a germline nonsense mutation c.783C>A (p.Tyr261X) in exon 5 with LOH in the tumor (Fig. 1a, b). After 2 years of treatment, finally her serum IGF-I levels decreased to normal range; however, she eventually stopped visiting the hospital. After the cessation of the treatment for 1 year, she came to the hospital. Because her serum IGF-I level was slightly elevated (224 ng/mL, +2.1 SDS), we tried cabergoline monotherapy. Thereafter, serum GH and IGF-I levels were normalized.

Immunohistochemical analysis in cases with AIP mutation

Immunohistochemical analysis using primary antibody against AIP protein, which recognizes 21-134 amino acids of the protein, revealed a complete loss of AIP protein expression in all cases with *AIP* mutations (Fig. 3).

Interestingly, two patients with *AIP* mutation showed response to cabergoline therapy as a combined or monotherapy. Then we analyzed the expression of D2R in the tumor as well as that of SSTR2 and 5. It has been reported that somatotropinoma with *AIP* mutation is not associated with a reduced expression of SSTR2 and 5 and downstream signaling of SSTR2 is impaired [15, 16]. In line with this, SSTR2 and 5 were strongly expressed on cell membrane in all cases (Fig. 3). Intriguingly, D2R was also expressed in all cases,

supporting the efficacy of the D2 agonist.

Loss of membranous E-cadherin expression is reportedly correlated with the tumor size and resistance to SSA treatment [17]; however, E-cadherin expression level in somatotropinoma with *AIP* mutation has not been reported. Therefore, we analyzed E-cadherin expression in the tumor tissues. We found that E-cadherin expression was substantially diminished in all cases with the *AIP* mutations (Fig. 3).

Familial screening

Although these 3 patients with *AIP* mutation had no family history of endocrine disorders including pituitary adenoma, familial screening was performed in the relatives of case 1 and 2. Mutation carriers were found in both of the families. Father of case 1 had the same *AIP* mutation as the proband. However, he had neither pituitary hormone abnormality nor pituitary tumor. In case 2, father and sister revealed the same *AIP* mutation, but no pituitary hormone abnormality nor pituitary tumor was detected thus far.

Discussion

In the present study, we showed the prevalence of *GNAS*, *AIP*, and *GPR101* gene mutations in sporadic Japanese patients with somatotropinoma. The prevalence of *GNAS* mutations were comparable with the previous report of Japanese acromegalic patients [18]. On the other hand, although it has been reported that *AIP* mutations in sporadic Japanese patients with somatotropinoma may be extremely rare [19], our cohort study showed a comparable prevalence of *AIP* mutations (4.9%) to that of European patients [19-21]. In addition, our results suggest that the prevalence of *GPR101* mutation is rare in Japanese patients. These data suggest that the ratio of causal genes in Japanese patients is comparable with the previous report in Caucasians.

In this study, we detected no p.Glu308Asp mutation in the *GPR101* gene. Trivellin *et al*. reported that the mutation was detected in 11 of 248 (4.4%) patients with sporadic acromegaly. Functional study revealed that the mutant GPR101 exhibit a higher ability of GH secretion in GH3 cells, suggesting that it plays causal role in somatotropinoma. However, a population based exome-sequencing data set, the ExAC Browser showed that c.924G>C (p.Glu308Asp) in *GPR101* gene is observed in 0.37% of total cohort in 61,500 normal individuals, which is approximately 100 times

	Case 1	Case 2	Case 3	Control
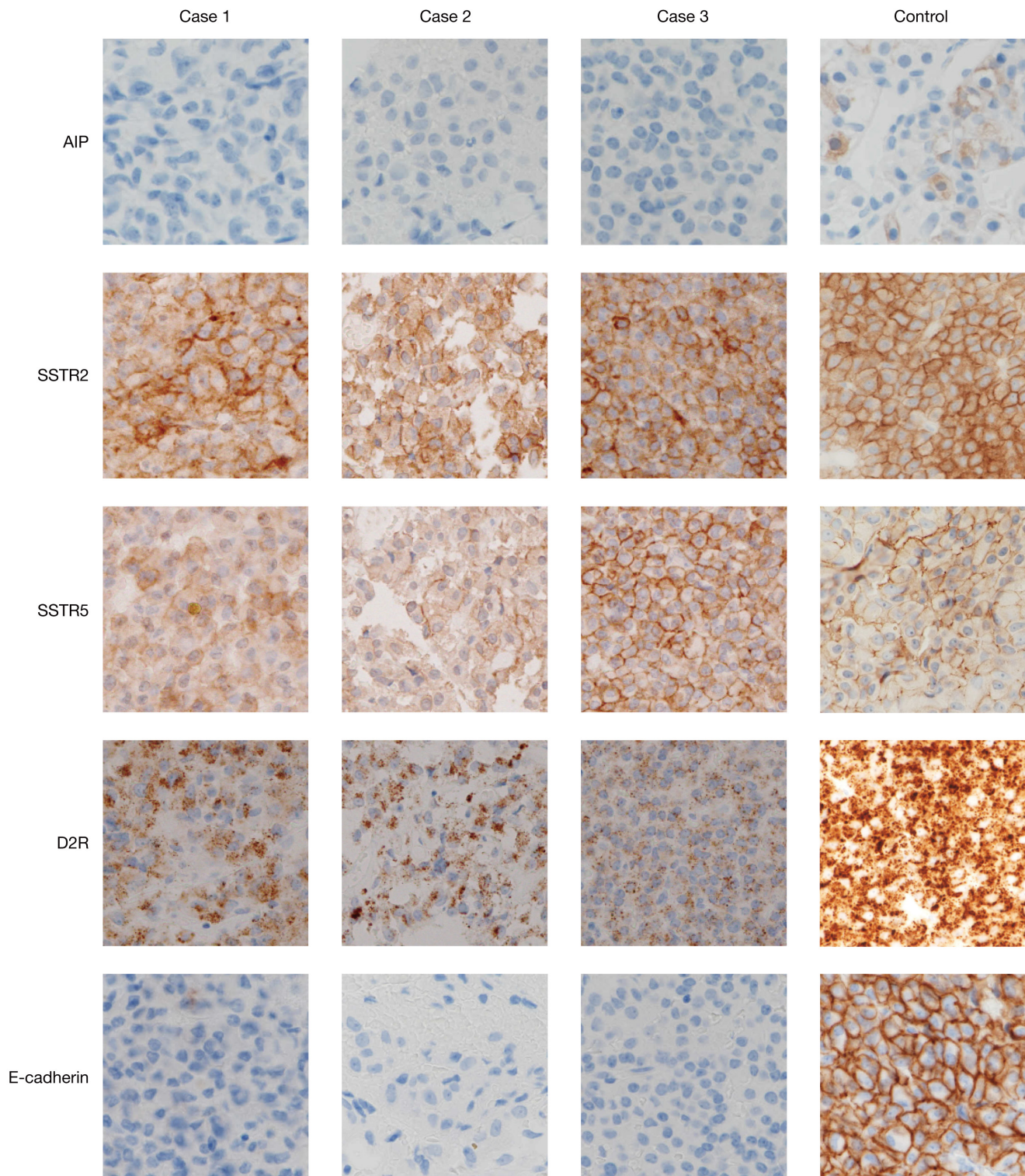
Fig. 3 Immunohistochemical analysis in somatotropinomas with the *AIP* mutations
AIP was not expressed and SSTR2, SSTR5, and D2R were strongly expressed in somatotropinomas with the *AIP* mutations.
E-cadherin expression was obviously decreased in somatotropinomas with the *AIP* mutations. The results of control samples
include AIP staining (normal anterior pituitary) and SSTR2, SSTR5, D2R, and E-cadherin staining of AIP mutation-negative
somatotropinoma. SSTR, somatostatin receptor; D2R, dopamine D2 receptor.

higher than population prevalence of acromegaly (40 cases per million; 0.004%) [22], suggesting that most individuals who harbor this *GPR101* mutation may not develop somatotropinomas. Thus, a caution has been urged in the interpretation of this mutation as a cause of the disease [23]. These data suggest that the role of *GPR101* mutation in the development of somatotropinoma still remains inconclusive.

In this study, 4.9% of somatotropinomas carried *AIP* mutations. Immunohistochemical analysis demonstrated a loss of AIP protein in the tumor in all 3 cases (Fig. 3). Case 2 and 3 possess p.Tyr261X mutation, which result in a truncation in the C-terminal portion of AIP protein, suggesting a presence of N-terminal epitope. However, immunohistochemical analysis showed an absence of AIP protein expression in these cases. These results suggested that this mutation may affect AIP protein expression, degradation, or intracellular localization as well as producing the truncated protein. These results are compatible with the LOH in the tumor. Generally, somatotropinoma with *AIP* mutation showed aggressive phenotype and resistance to SSA therapy [24, 25]. Interestingly, we found that E-cadherin expression was lost in tumor tissues of all cases with the *AIP* mutations, which may explain at least in part, the mechanism underlying the aggressive phenotype and resistance to SSA. As the molecular mechanisms underlying SSA resistance, it has been suggested that AIP protein exerts anti-proliferative effect *via* ZAC1 in the downstream of SSTR2 [15]. Intriguingly, two patients with *AIP* mutations in this study demonstrated the efficacy of D2 agonist cabergoline, suggesting that the inhibitory signals downstream of D2R were preserved even when function of AIP protein was impaired. This is important because somatotropinoma with *AIP* mutations generally show a large and invasive tumor, indicating that it is likely that the postoperative medical therapy is necessary and in addition, it generally exhibits resistance to SSA. Although further investigations with more number of patients with *AIP* mutations are necessary, these data suggest that cabergoline monotherapy or combination therapy with SSA is a potential option when SSA resistance is observed. Interestingly, Case 2 and 3, in whom cabergoline combined or monotherapy was effective, did not show a good response to BRC test (Fig. 2g), indicating that cabergoline therapy should be taken into consideration, even if a poor response in BRC test was observed.

We clearly demonstrated that clinical indices of age,

tumor diameter, and nadir GH discriminated *AIP* from *GNAS* and others group. *AIP* group showed younger age, larger tumor, and higher nadir GH levels and *GNAS* group showed a relatively smaller tumor and higher GH production corresponding to the tumor size. These characteristics may be reflected by the role of AIP and Gsα in cell proliferation and GH production. In comparison with the *AIP* and *GNAS* group, the others group showed relatively heterogeneous phenotype in terms of the hormonal activities and tumor sizes, suggesting that this group may consist of somatotropinoma caused by several different causes.

In the present study, 44.3% of patients revealed unknown cause. Recently, Valimaki *et al*. reported the result of whole-genome sequencing in 12 somatotropinomas [26]. They showed that somatotropinoma had a low number of somatic genetic alterations and only recurrent somatic mutations were the *GNAS* mutations (p.Arg201Cys), suggesting that there may be no major genetic alterations except for the *GNAS* mutations in somatotropinomas. Recently, epigenetic modifications such as DNA methylation or histone acetylation reportedly play a role in the development of pituitary adenomas [27]. Also, it has been reported that non-coding RNA, for example, MEG3 functions as a growth suppressor in pituitary tumor cells, indicating that non-coding RNA might have a tumorigenic role [28]. These mechanisms may explain the unknown pathogenesis of somatotropinomas.

This study has several limitations. First, we performed a sequential analysis of each gene, indicating that all genes were not analyzed in all patients. However, there have been no reports that multiple driver mutations were detected in the same patient as far as we have searched, justifying this strategy. Also gene deletion/duplication analysis of *AIP* was performed in a subgroup of patients. We can not exclude the possibility that we might miss some patients with deletion in atypical patients. Second, *GNAS* and *GPR101* gene analysis were restricted in their hot spots, codon 201/227 and codon 308, respectively. However, although numerous analysis of *GNAS* gene were performed in various tumors, constitutive active form of Gsα was observed only in codon 201/227 mutations, indicating that it is sufficient to analyze codon 201/227. Regarding *GPR101* mutation, p.Ala397Lys change has theoretically been reported to be activating mutation [10]. However, Trivellin *et al*. has not detected this mutation in their study. Finally the number of patients was lim-

ited so that more number of patients may be necessary to clarify an accurate ratio of causal mutations.

In conclusion, we demonstrated that the prevalence of *GNAS, AIP*, and *GPR101* gene mutations in Japanese patients with sporadic somatotropinomas was comparable with the previous studies. *AIP* mutation discriminates clinical phenotypes from *GNAS* or others. Cabergoline therapy may be efficacious in patients with the *AIP* mutations.

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Disclosure

The authors declare no conflict of interest associated with this research.

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