

[CASE REPORT]

Pasireotide-resistant Refractory Cushing's Disease without Somatostatin Receptor 5 Expression

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Abstract:

Pasireotide, which has a high affinity for somatostatin receptor (SSTR) 5, has attracted attention as a new treatment for refractory Cushing's disease. The patient was a 28-year-old man. He had refractory Cushing's disease and underwent multiple surgeries, radiotherapy, and medication therapy. An examination of the adenoma by immunohistochemistry revealed a low SSTR5 expression. An *USP8* mutation was not detected by reverse transcription polymerase chain reaction. Although we administered pasireotide, it was ineffective. While a further investigation is necessary, the analysis of SSTR5 expression may support the prediction of the efficiency of pasireotide for Cushing's disease. We report this case as a useful reference when considering whether or not to use pasireotide for refractory corticotroph adenomas.

Key words: pasireotide, Cushing's disease, corticotroph adenoma, somatostatin receptor 5, *USP8* mutation

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Introduction

Cushing's disease is caused by the excessive secretion of adrenocorticotrophic hormone (ACTH) from an ACTH-producing adenoma in the pituitary gland (1). Overabundant ACTH secretion causes excessive glucocorticoid expression. Consequently, excess ACTH causes infection and cardiovascular complications, leading to a high mortality rate (2). This disease can be fatal if not treated; however, medication therapy is often ineffective. The most reliable treatment is surgical resection.

However, pasireotide is currently attracting attention as a new drug for Cushing's disease. In the clinical field, the indications of pasireotide for the treatment of Cushing's disease have been expanded. The first-generation somatostatin analogs octreotide and lanreotide have been conventionally used for the treatment of neuroendocrine tumors. These drugs have a high affinity for somatostatin receptor (SSTR) 2A. However, many patients with Cushing's disease show a greater expression of SSTR5 than SSTR2 (3). Pasireotide is

a second-generation somatostatin analog that has a high affinity for SSTR5 (4). The use of pasireotide, which can be expected to have a direct effect on the tumor, has been approved for use in patients with Cushing's disease who have a residual tumor or recurrence.

For somatotroph tumors, an octreotide loading test is usually performed, and the reactivity is used as a reference to determine the adaption of first-generation somatostatin analogs (5). However, in Japan, there is no short-acting formulation of pasireotide, and it is not possible to determine the adaption by referring to the reactivity demonstrated by the loading test.

We herein report a case of a corticotroph adenoma with repeated recurrence that was not effectively treated with pasireotide. A low SSTR5 expression and no *USP8* mutations were observed in this case. As an alternative to the loading test, the examination of pathological specimens for SSTR5 expression and *USP8* mutations may be useful for predicting the effect of pasireotide.

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Table 1. Circadian Rhythm of Plasma Adrenocorticotrophic Hormone (ACTH) and Serum Cortisol before the First Transsphenoidal Surgery (TSS). the Circadian Rhythm of Serum Cortisol was Lost.

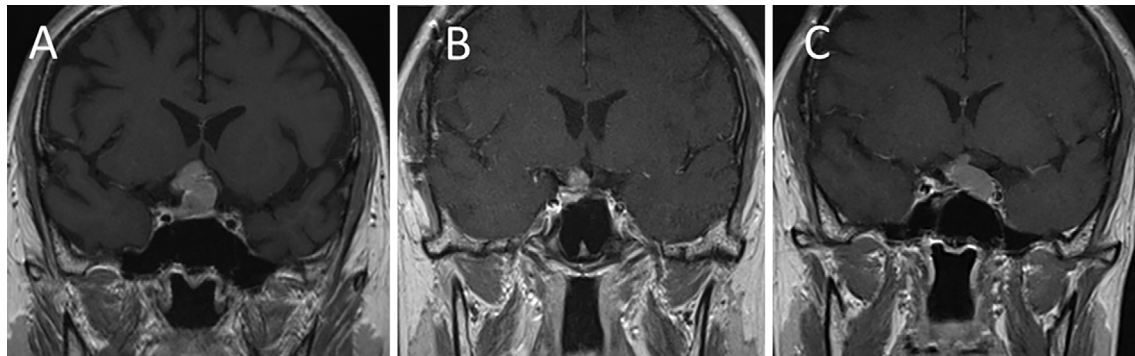
	8:00	11:00	16:00	23:00
ACTH (pg/mL)	184	217	222	210
Cortisol (μg/dL)	30.2	28.7	24.1	25.7

Table 2. Plasma ACTH and Serum Cortisol Response to 1- and 8-mg Dexamethasone Suppression Tests before the First TSS. the Serum Cortisol Level was Not Suppressed in Either the 1- or 8-mg Dexamethasone Suppression Tests.

Dose of dexamethasone	1 mg	8 mg
ACTH (pg/mL)	148	180
Cortisol (μg/dL)	42.5	42.5

Table 3. Corticotropin-releasing Hormone (CRH) Loading Test before the First TSS. ACTH Responses were Observed in the CRH Loading Test.

	0 min	15 min	30 min	60 min	90 min	120 min
ACTH (pg/mL)	185	208	220	177	153	184
Cortisol (μg/dL)	32.5	45.4	39.2	41.9	37.9	40.9

**Figure 1.** Pituitary contrast-enhanced coronal T1-weighted magnetic resonance imaging. A: The initial examination showing a large tumor extending into the suprasellar region from the sella. B: The examination before the second TSS showing a recurrent tumor extending from the remnant portion of the right cavernous sinus to the suprasellar region surrounding the right internal carotid artery. C: The examination before the third TSS showing the recurrence of the tumor from the left cavernous sinus invasion, which was not observed at the time of the second TSS.

Case Report

A 28-year-old man without a remarkable medical history had been noted to have hypertension and a moon face during a medical checkup 1 year prior. After a closer endocrine examination, he was diagnosed with Cushing's disease and referred to our hospital for surgery.

His ACTH level was high at 183.5 pg/mL, his serum cortisol level was 30.2 μg/dL, his 24-hour urine free cortisol level was 590.8 μg/day, and his circadian rhythm of serum cortisol was lost. Although the serum cortisol level was not suppressed in either the 1- or 8-mg dexamethasone suppression test, an ACTH response was observed in a corticotropin-releasing hormone (CRH) loading test (Table 1-3). Head magnetic resonance imaging (MRI) revealed

a pituitary macroadenoma extending to the suprasellar region (Fig. 1A).

Transsphenoidal surgery (TSS) and subsequent craniotomy were performed 11 days later because of incomplete removal of the portion extending into the suprasellar region. This tumor was pathologically shown to be a unique ACTH-producing adenoma consisting of tumor cells with a pale eosinophilic cytoplasm and some perivascular arrangements. According to immunostaining, the anterior pituitary was ACTH positive, with very faint CAM5.2 cytokeratin immunostaining. The expression of both SSTR2A and SSTR5 was low on immunostaining (Fig. 2A, B, C, D and H). Additional immunohistochemical staining was sporadic for p53, negative for epidermal growth factor receptor (EGFR) expression, and positive for BRAF V600E expression (Fig. 3A, B and C). The Ki-67 labeling index was 0.7%.

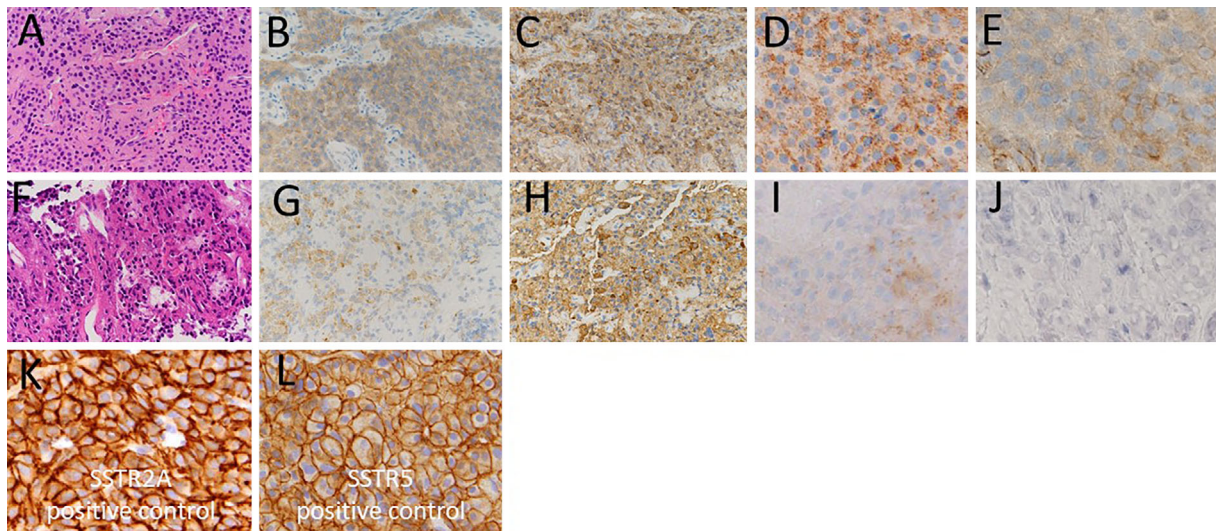


Figure 2. Pathological findings in a high-power field (400-fold magnification) after the first TSS (A-E) and third TSS (F-J). Hematoxylin and Eosin staining (A and F) revealed tumor cells with a pale eosinophilic cytoplasm and some perivascular arrangements. Anti-cytokeratin (CAM5.2) staining (B and G) was very faint. There was no accumulation of cytokeratin filaments surrounding the nucleus, which is a characteristic of Crooke's cell adenoma (CCA). CAM5.2 staining revealed that this tumor was not a CCA. Staining for ACTH (C and H) was positive. On staining for SSTR2A (D and I), the cell membrane showed no staining, indicating a low SSTR2A expression. On staining for SSTR5 (E and J), the cell membrane showed no staining, indicating a low SSTR5 expression. There was almost no difference between before and after pasireotide treatment and radiotherapy. (K): Staining of a SSTR 2A-positive control showed strong staining of the cell membrane; this is an image of a pituitary tumor of pit-1 lineage. (L): Staining of a SSTR5-positive control showed strong staining of the cell membrane; this is an image of a corticotroph adenoma.

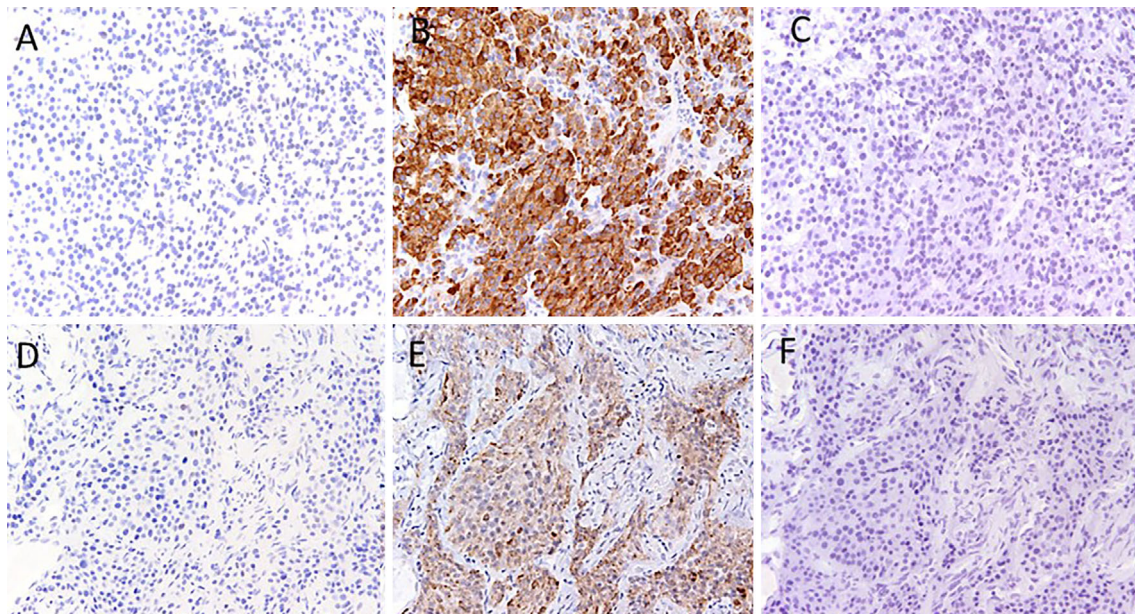


Figure 3. Pathological findings in a high-power field (400-fold magnification) after the first TSS (A-C) and third TSS (D-F). Staining for p53 (A and D) was sporadic. Staining for BRAF V600E (B and E) was positive. Staining for EGFR (C and F) was negative.

Electron microscopy revealed a mild accumulation of large secretory granules that corresponded to those of a densely granulated corticotroph adenoma. There was no accumulation of cytokeratin filaments surrounding the nucleus,

which is a characteristic of Crooke's cell adenoma (CCA). The honeycomb Golgi structure, which is often associated with a sparsely granulated adenoma, was not recognized. As a special finding, mitochondrial filling was observed in the

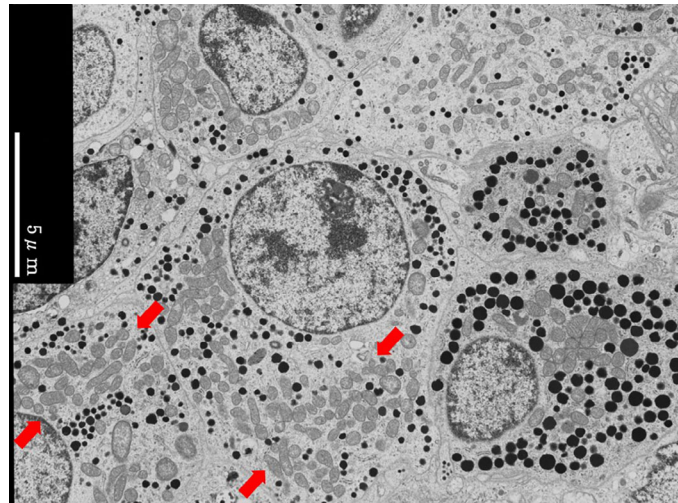


Figure 4. Electron microscopy findings after the first TSS revealed a mild accumulation of large secretory granules, and as a special finding, mitochondrial filling was observed in the cytoplasm (arrows).

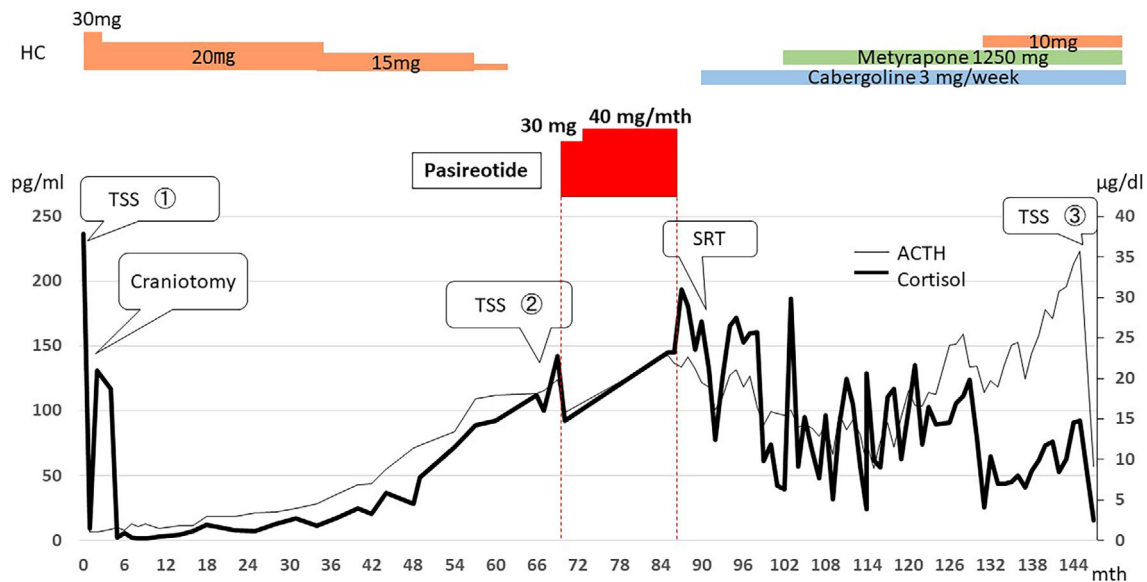


Figure 5. The 12-year clinical course of this case from the first operation to the third operation. HC: hydrocortisone, TSS: transsphenoidal surgery

cytoplasm in many adenoma cells (Fig. 4). These observations present a special organizational finding that is not well described in the 2017 WHO classification. Therefore, we pathologically diagnosed the patient with corticotroph adenoma with oncocytic changes. After craniotomy, the patient suffered from panhypopituitarism and central diabetes insipidus and was treated with hydrocortisone, levothyroxine, and desmopressin.

The patient was diagnosed with Cushing's disease recurrence six years after the first surgical intervention due to elevated baseline ACTH and cortisol levels. Although he underwent TSS a second time, the tumor, which showed a wide area of invasion into the right cavernous sinus, was not completely removed (Fig. 1B). Postoperative MRI showed that the remaining tumor resulted in a serum ACTH level of

88.0 pg/mL and a cortisol level of 16.3 μ g/dL (Fig. 5). The pathological findings of the tumor were similar to those obtained at the first surgery, except for a higher Ki-67 labeling index of 5.1%. Immunostaining also revealed a low expression of SSTR2A and SSTR5. No *USP8* mutations were detected by reverse transcription polymerase chain reaction (RT-PCR). The patient started pasireotide treatment; however, the tumor was resistant to pasireotide. The ACTH and serum cortisol levels further increased despite 4 months of pasireotide administration (ACTH 133.5 pg/mL; serum cortisol, 31.0 μ g/dL).

Pasireotide treatment was terminated after 17 months of administration. Treatment with cabergoline was started instead. Stereotactic radiotherapy (SRT) was performed three months after the start of cabergoline treatment. However, the

ACTH and cortisol levels continued to rise. Metyrapone was added a year after SRT. Although the serum cortisol level normalized after the start of metyrapone treatment, the tumor continued to grow (Fig. 1C). Therefore, TSS was performed a third time 4 years after the execution of SRT (12 years after the first operation). Although the tumor was strongly fibrotic, it was completely removed. The histopathological features were similar to those of the tumor observed after the second TSS procedure (Fig. 2F, G, H, I, J). The tumor was sporadic for p53, negative for EGFR expression, and positive for BRAF V600E expression (Fig. 3D, E, F). After the third TSS procedure, the ACTH level decreased to 30.7 pg/mL, and the serum cortisol level decreased to 2.47 µg/dL (Fig. 5).

Discussion

This study reports a case of Cushing's disease with an invasive macroadenoma of the pituitary. Despite treatment twice with TSS and SRT, Cushing's disease repeatedly recurred. Pathologically, this was a case of a corticotroph adenoma with oncocytic changes. The expression of SSTR2A and SSTR5 was low, and no *USP8* mutations were observed. The tumor was sporadic for p53, negative for EGFR expression, and positive for BRAF V600E expression. In addition, in this case, the tumor did not respond to pasireotide.

Corticotroph adenomas are pathologically classified into three subtypes in the WHO classification: densely granulated corticotroph adenoma with large and abundant secretory granules on electron microscopy, sparsely granulated corticotroph adenomas with small and few secretory granules, and a special type of CCAs with an abundant cytokeratin filament accumulation (6). Densely granulated corticotroph adenomas with large and abundant secretory granules on electron microscopy are usually functioning corticotroph adenomas with hypercortisolism and Cushing's symptoms. In contrast, sparsely granulated corticotroph adenomas with small and few secretory granules are silent corticotroph adenomas lacking hypercortisolism and Cushing's symptoms (7). Tumors that have a Ki-67 positive rate >3% were classified as atypical adenomas according to the previous WHO classification (8). However, this evaluation was insufficient to predict malignant clinical behavior, and the category of atypical adenoma was removed from the latest WHO classification (6). Instead, the evaluation method including the surrounding invasiveness to the Ki-67 labeling index has become widely used (9). In this evaluation, CCAs and sparsely granulated corticotroph adenomas are considered adenomas with a poor prognosis (10). However, the tumor in the present case, which was rich in mitochondria, could not be classified into one of these three subtypes of corticotroph adenomas. We previously reported on the histological subtype of this corticotroph adenoma, which has a high recurrence rate (11).

In the present case, the tumor was immunohistochemically sporadic for p53, negative for EGFR expression, and

positive for BRAF V600E expression (Fig. 3). Several studies have shown that *USP8*, *USP48*, and *BRAF V600E* are frequently mutated in pituitary corticotroph adenomas, causing Cushing's disease by enhancing the promoter activity and transcription of the gene encoding proopiomelanocortin (POMC), which is the precursor of ACTH (12-14). The deubiquitinase gene *USP8* has been found to be mutated in 35-62% of corticotroph adenomas, resulting in sustained EGFR and mitogen-activated protein kinase pathway activation and subsequent ACTH overproduction (12, 13).

USP48 and *BRAF* mutations have been detected in a corticotroph adenoma with wild-type *USP8*. Similar to *USP8* mutants, both *USP48* and *BRAF* mutants enhance the promoter activity and transcription of the gene encoding POMC, which is the precursor of ACTH, providing a potential mechanism for ACTH overproduction in corticotroph adenomas (14). In the present case, immunostaining was positive for BRAF V600E expression. BRAF V600E immunohistochemistry in pituitary adenomas is reportedly not associated with the *BRAF V600E* mutation (15). However, the *BRAF* mutation may have been the causative gene mutation in this case. In recent years, molecular-targeted treatments have attracted attention. The effect of the *BRAF* inhibitor vemurafenib on ACTH secretion has been evaluated in primary human corticotroph adenomas. The results support the potential efficacy of vemurafenib in the treatment of corticotroph adenomas with the *BRAF V600E* mutation (14). Therefore, vemurafenib might be effective in the present case with the *BRAF* mutation. Immunohistochemical staining for p53 is one of the conventional methods of distinguishing between adenoma and cancer (16).

Pasireotide is currently the only pituitary-directed medical therapy approved in the EU, USA, and Japan for the treatment of Cushing's disease. Recently, Pivonello et al. reported that pasireotide normalized urinary cortisol levels in at least 68% of Cushing's disease patients with very mild-to-moderate disease (17). It has been reported that SSTR5 expression in immunostaining is correlated with the effectiveness of pasireotide in somatotroph adenomas (18). The method for evaluating the pathological immunostaining of SSTR2A expression proposed by Volante et al. (19) is based on the results of an octreotide scan and is routinely used to assess the SSTR5 expression (20). Recently, a report revealed that the SSTR2A expression on immunostaining, rather than the SSTR5 expression on immunostaining, is correlated with the efficacy of pasireotide for treating somatotroph adenomas (21). However, because corticotroph adenomas have a low SSTR2A expression and a high SSTR5 expression (6, 22), the SSTR5 expression may be more important concerning the efficacy of pasireotide than the SSTR2A expression. In addition, among somatotroph adenomas, densely granulated adenomas have a high rate of SSTR2A expression, but sparsely granulated adenomas have a low rate of SSTR2A expression and a high rate of SSTR5 expression (23). In contrast, in corticotroph adenomas, the correlation between the pathological classification and the fre-

quency of SSTR expression is unclear. We previously reported that, in 209 cases of corticotroph adenomas, *USP8* mutations were exclusively detected in non-CCA patients, and clinically well-behaved presentations were more common in patients with mutated tumors than non-mutated tumors. In addition, mutated tumors can express SSTR5 well (22).

In addition, Castellnou et al. reported that among 39 functioning corticotroph adenomas, 24 were SSTR5-positive, and 15 were SSTR5-negative. Negative SSTR5 expression was less frequent in women than in men. *USP8* mutations were less frequent in SSTR5-negative tumors than SSTR5-positive tumors. This report did not mention the histopathological features of functioning corticotroph adenomas. Furthermore, Castellnou et al. reported the response to pasireotide in five patients with Cushing's disease. Among the five patients, one was SSTR5-negative, and four were SSTR5 positive, as confirmed by immunohistochemical staining. An improvement in hypercortisolism and Cushing's symptoms was observed in most of the patients, including SSTR5-negative patients. Thus, there may be no correlation between the expression of SSTR5 in corticotroph adenomas and the efficacy of pasireotide. However, the small number of patients did not allow us to draw a firm conclusion (24).

Pasireotide is currently indicated for refractory Cushing's disease. However, patients with refractory Cushing's disease often do not express SSTR5 and are likely to be resistant to pasireotide. In the present case, the tumor showed no *USP8* mutations and low levels of SSTR2A and SSTR5. Unfortunately, pasireotide was ineffective. Because SSTR5 immunostaining is readily available for a routine pathological diagnosis, the SSTR5 expression should be investigated before pasireotide is administered. However, regarding the correlation between the results of SSTR5 immunostaining and the effect of pasireotide, further research is needed.

Conclusion

A case of a corticotroph adenoma with repeated recurrence and no sensitivity to pasireotide is reported. This tumor had low levels of SSTR2A and SSTR5 and no *USP8* mutations. This case report is not an analysis of many cases but a report of only one case. We thus cannot clearly conclude that a low SSTR5 expression would indicate pasireotide inefficacy in other cases. In addition, BRAF V600E immunohistochemistry was positive in this case. However, BRAF V600E immunohistochemistry in pituitary adenomas is reportedly not associated with the BRAF V600E mutation (15). Therefore, whether or not the causative gene of this case was the BRAF V600E mutation is unclear.

Although a further investigation is necessary, the analysis of the SSTR5 expression may support the prediction of the efficiency of pasireotide for Cushing's disease.

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

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