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A Case of Growth Hormone Producing Pituitary Adenoma with Crystal-Like Structures: Bihormonal Stained Amyloid Deposition

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Abstract

Amyloid deposition may adopt a crystal-like structure in functioning pituitary adenomas. We report a case of growth hormone（GH）-producing pituitary adenoma with crystal-like structures that are positive, by immunohistochemistry, for GH and prolactin（PRL）. A 53-year-old Japanese woman presented with change in facial appearance, headache, hypertension, and enlargement of hands and feet. Hormonal analysis showed elevated amounts of GH and insulin-like growth factor-1. A slightly elevated PRL concentration indicated pituitary "stalk effect". Magnetic resonance imaging revealed a mass arising from the pituitary gland. Preoperative diagnosis was acromegaly, and transsphenoidal resection was performed. Histopathologically, neoplastic acidophils and chromophobes were arranged in diffuse arrays with occasional intracytoplasmic fibrous bodies. Between tumor cells we found numerous crystal-like structures（≤40 μm in diameter）. The tumor was composed of an admixture of GH-positive cells and focal PRL-positive cells. Congo Red stain and electron microscopy revealed crystal-like amyloid deposits that were GH- and PRL-positive. Clinicopathologically, a diagnosis of sparsely granulated somatotroph adenoma with increased PRL production due to stalk effect was determined. Crystal-like structures showed a heterogenous or bihormonal staining pattern, which might be indicative of bihormonal amyloid formation.

Key words：**Pituitary Adenoma, Growth Hormone, Crystal, Amyloid, Bihormonal**

Introduction

Crystal-like structures are one type of amyloid deposition observed in pituitary adenoma. Previous reports concerning this type of amyloid are less common [1)-6)]. Here, we report a case of growth hormone（GH）-producing pituitary adenoma with crystal-like structures that are immunohistochemically positive for GH and prolactin（PRL）. This is the first report concerning bihormonal stained, crystal-like amyloid.

Clinical summary

A 53-year-old Japanese woman presented with changes in facial appearance, headache, hypertension, and tightness of rings and shoes. Physical examination showed increased prominence of forehead, thickening

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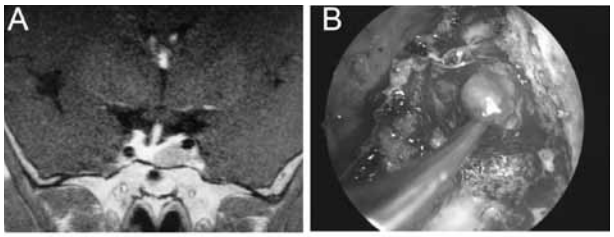

Fig. 1 A magnetic resonance imaging (MRI) scan of the brain. **A** A mass arising from the pituitary gland was of iso-intensity on T1 gadolinium-enhanced MRI, relative to gray matter. **B** The tumor was a soft, grey-white nodule.

of nose, lips, and tongue, and thickened skin on palms of hands and soles of feet. Hormonal analysis revealed elevated concentrations of GH 45.50 ng/ml (normal, 0.28-1.64 ng/ml), insulin-like growth factor-1 (IGF-1) 364 ng/ml (normal, 37-266 ng/ml), and PRL 54.90 ng/ml (normal, 5.18-26.53 ng/ml). Adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH) and leuteinizing hormone (LH) serum concentrations were in the normal range. Oral glucose tolerance test revealed that GH reduction was insufficient (Before: 55.4 ng/ml, 120 minutes: 25.9 ng/ml). A magnetic resonance imaging (MRI) scan of the brain showed an expansion of sella turcica and deviation of the infundibulum to the right. A mass arising from the pituitary gland was of iso-intensity on T1 gadolinium-enhanced MRI, relative to gray matter (Fig. 1A). We preoperatively diagnosed acromegaly, and we performed a transsphenoidal resection. The tumor

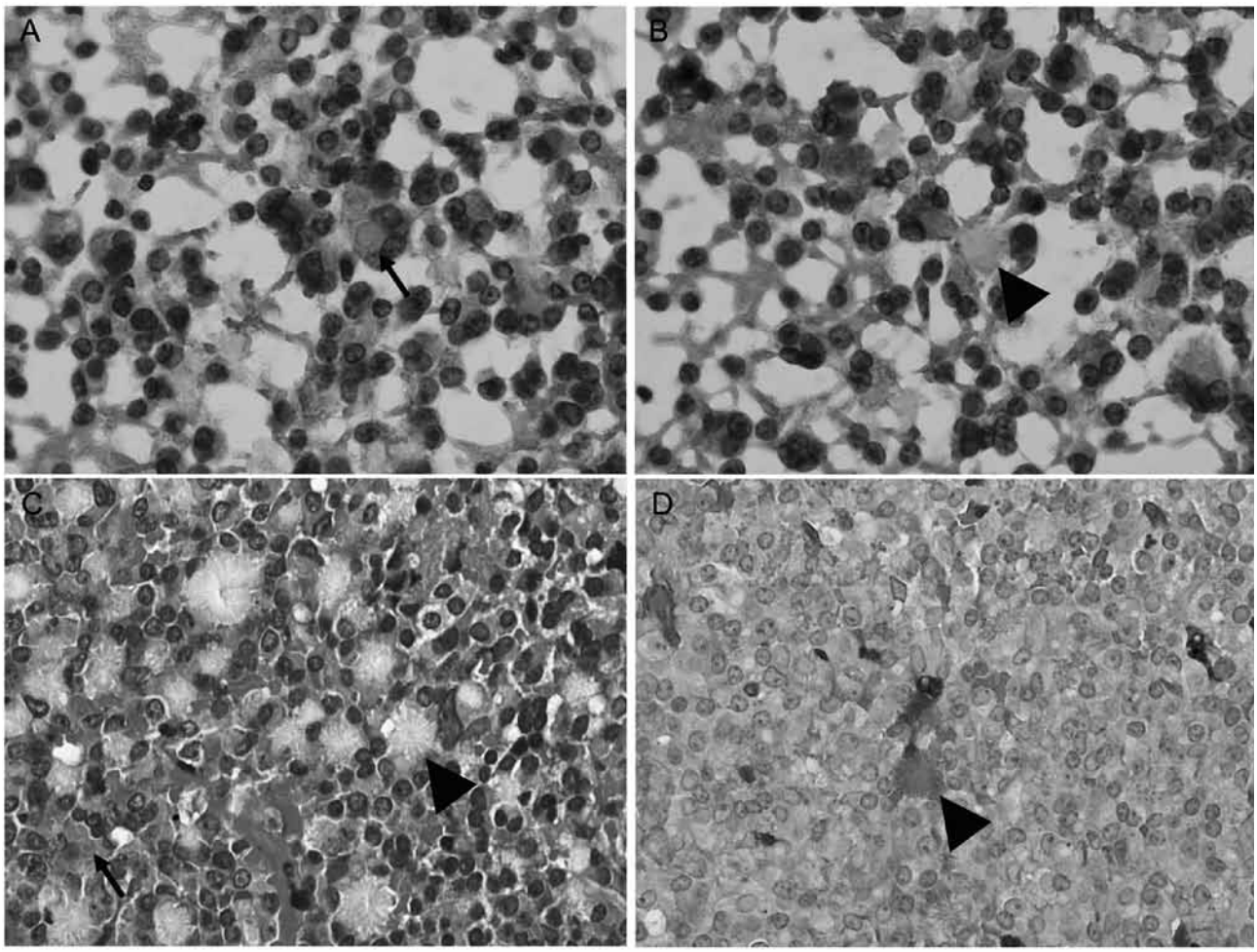

Fig. 2 **A-B** An intraoperative hematoxylin and eosin staining (H&E) touch preparation. **A, B** The tumor is composed of atypical polygonal cells with round-to-oval nuclei. *Arrow*, fibrous body. *Arrow head*, crystal-like structures in the intercellular spaces. **C** Postoperative H&E sections. Neoplastic acidophils and chromophobes were arranged in diffuse structures. *Arrow*, fibrous body. *Arrow head*, crystal-like structures. **D** Pearse's periodic acid-Schiff (PAS) stain. The tumor cells are chromophobic. *Arrow head*, crystal-like structures were PAS-positive. Magnification **A, B** × 400; **C, D** × 200.

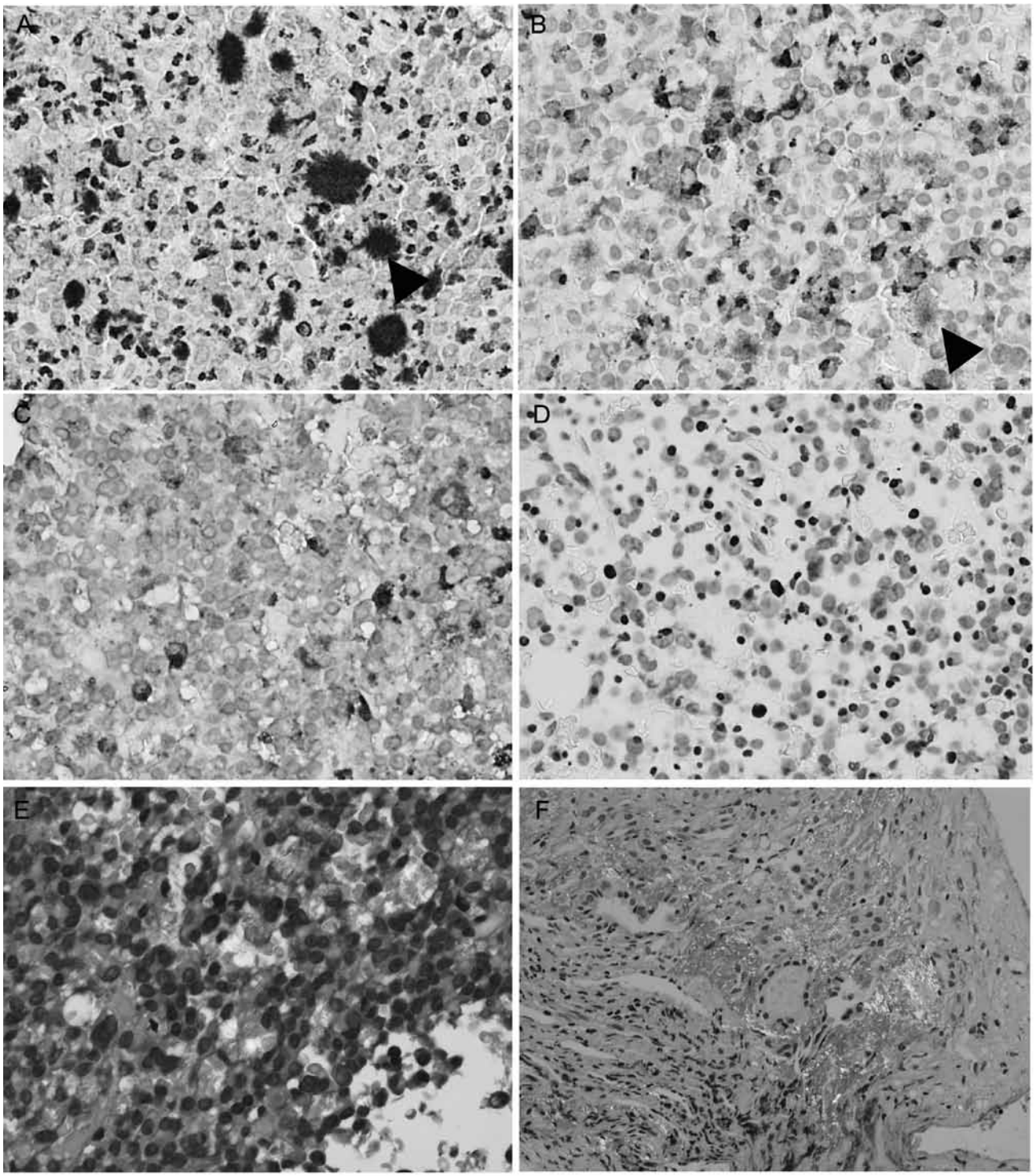

Fig. 3 Immunohistochemical and special stains. **A** Growth hormone（GH）stain. The tumor cells were positive. *Arrow head*, crystal-like structures were GH-positive. **B** Prolactin（PRL）stain. The tumor cells were focally positive. *Arrow head*, crystal-like structures were weakly PRL-positive. **C** GH and PRL double stains. GH-positive cells and PRL-positive cells were separate［GH（red）, PRL（brown）］. **D** AE1/AE3 stains. Fibrous bodies were dot-like immunoreactivity pattern. **E-F** Congo red stain. Crystal-like structures（**E**）and the stroma（**F**）were positive and revealed apple green in the polarized microscopy. Magnification **A-F** × 200.

was a soft, gray-white nodule (Fig. 1B). The postoperative course showed decreased GH (6.65 ng/ml), IGF-1 (301 ng/ml) and PRL (5.5 ng/ml). Medical therapy was started to normalize GH and IGF-1 concentrations.

Histopathological Findings

An intraoperative touch preparation with hematoxylin and eosin staining (H&E) was prepared using a specimen fragment. Intraoperative H&E staining of a tumor touch preparation showed proliferation of atypical polygonal cells with round-to-oval nuclei (Fig.2A, B). The tumor cells had lightly eosinophilic cytoplasms and fibrous body was partially found (Fig.2A, arrow). Near the tumor cells we found crystal-like structures (Fig.2B, arrow head). The surgically resected specimen was fixed with 10% formalin and processed into paraffin blocks for histopathological examination. Tissue sections were cut 4-μm thick and stained with H&E, Congo Red, and Pearse's periodic acid-Schiff (PAS) stain. Congo Red staining was analyzed under polarized light. We also performed immunohistochemistry using primary antibodies specific for GH, PRL, ACTH, TSH, FSH, LH, and pancytokeratins (AE1/AE3). Postoperative H&E sections showed proliferation of neoplastic acidophils and chromophobes arranged in diffuse structures. The tumor cells had round nuclei with mild pleomorphism (Fig.2C), and fibrous bodies were noted (Fig.2C, arrow). Between the tumor cells, we found numerous crystal-like forms (Fig.2C, arrow head). These structures (≤40 μm in diameter) consisted of filamentous materials. The tumor cells were chromophobic, as tested with Pearse's PAS (Fig.2D), and the crystal-like were PAS-positive (Fig.2D, arrow head). Immunohistochemically, the tumor cells were diffusely and strongly positive for GH (Fig.3A), and PRL-positive cells were also seen in approximately 10% of tumor cells (Fig.3B). Intriguingly, double staining revealed that GH-positive cells and PRL-positive cells

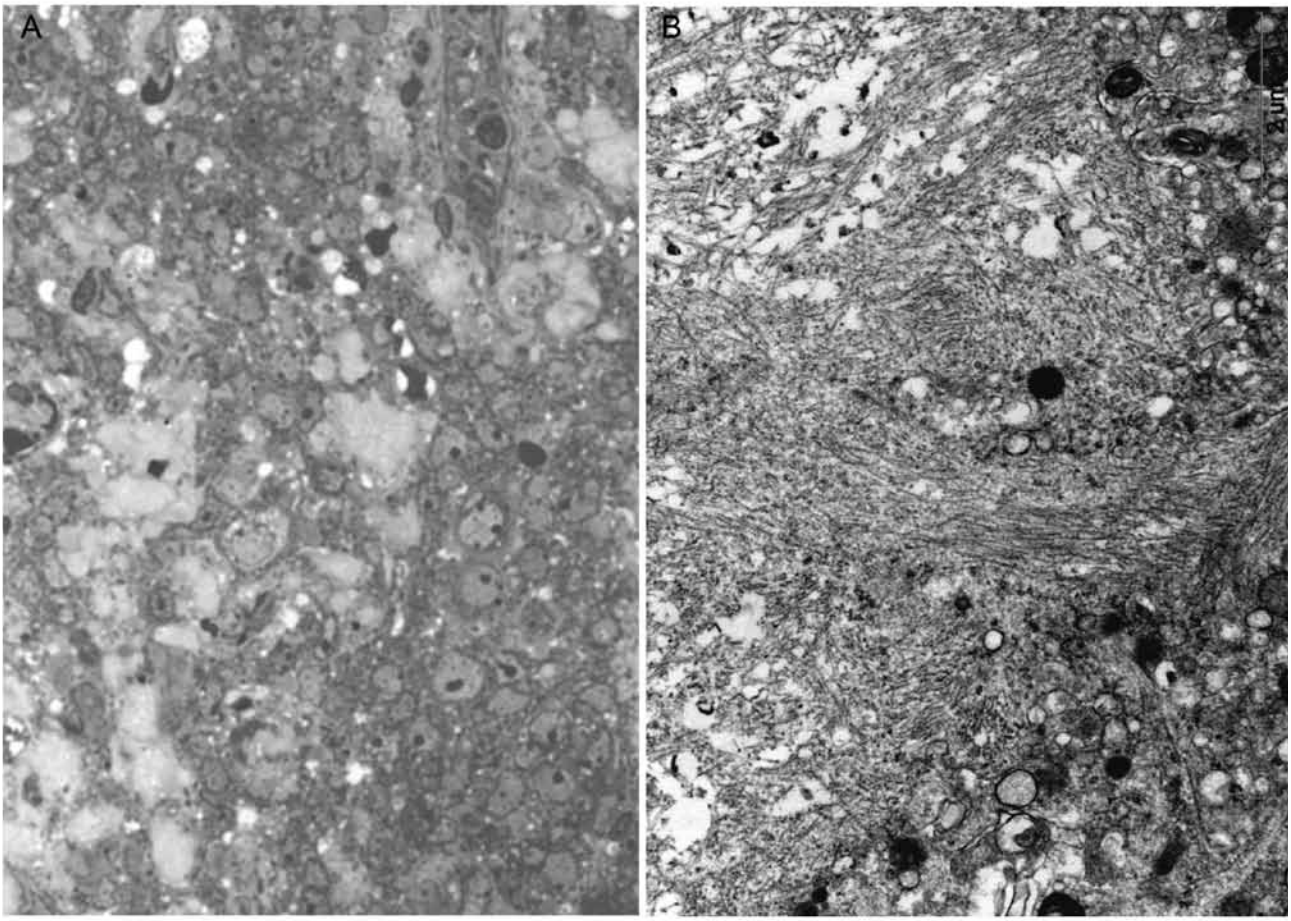

Fig. 4 **A** Microscopic findings from Epon-embedded sections. Crystal-like structures located between the adenoma cells. **B** Electron micrograph. Crystal-like structures were composed of bundles of fibrils approximately 10-13 nm in thick and were morphologically identical to the amyloid fibril. Magnification **A** × 400; **B** × 15000.

were separated [Fig.3C, GH (red), PRL (brown)]; however, the tumor cells were negative for TSH, ACTH, LH, and FSH. AE1/AE3-positive staining formed a dot-like pattern in fibrous bodies (Fig.3D). The crystal-like structures were strongly positive for GH (Fig.3A, arrow head), and weakly positive for PRL (Fig.3B, arrow head). Congo Red staining showed birefringence both for crystal-like structures (Fig.3E) and for the stroma (Fig.3F). The crystal-like forms also exhibited apple green-yellow birefringence in polarized light. For electron microscopic study, the tissues were postfixed and embedded in Epon. Our findings from Epon-embedded sections showed the crystal-like structures to be between the adenoma cells (Fig.4A). In the electron micrographs, crystal-like structures were composed of bundles of fibrils approximately 10-13 nm thick (Fig.4B), morphologically identical to amyloid fibrils reported previously [3].

Discussion

Through microscopic inspection, varinous types of amyloid deposition pattern have been reported [6]. But the reported cases of crystal-like, stellate or spheroid amyloid deposition are less common [1)-8)]. Here, we reported a case of GH-producing pituitary adenoma with crystal-like structures that were positive for GH and for PRL, immunohistochemically.

GH-producing adenomas may be described as either densely or sparsely granulated, whereas bihormonal GH- and PRL-producing adenomas may be mixed GH-PRL cell adenomas, mammosomatotropic adenomas, or acidophilic stem cell adenomas [9]. With our patient, the serum PRL concentration (54.90 ng/ml) was slightly elevated, and the "stalk effect" was mostly considered clinically. It has been hypothesized that suprasellar tumors induce hyperprolactinemia by compressing the pituitary stalk, resulting in impairment of dopamine delivery to the pituitary and, consequently, disinhibition of the lactotrophs [10]. Saeger et al. [11] reported that some (<10%) cells might be positive for PRL in adenomas secreting only GH. In our case, approximately 80% of adenoma cells were GH-positive, intermingled with approximately 10% of PRL-positive cells. Combining all our observations, we make a diagnosis of sparsely granulated somatotroph adenoma with increased PRL production due to stalk effect for our patient.

With regard to amyloid deposition and immunohistochemical staining or electron microscopic findings, previous reports revealed that crystal-like depositions were GH-positive [2),3)]. Previously, by electron microscopy, adenoma cells had numerous vesicles and vacuoles filled with amyloid fibrils, some of which were continuous with the extracellular space. Secretory granules are also reportedly present in vesicles that also contained amyloid fibrils and the simultaneous release of the granules and amyloid fibrils suggested that degeneration of secretary granules was involved in the formation of amyloid [1)]. In this case, crystal-like deposition was GH- and PRL-positive. Electron microscopy showed bundles of amyloid fibrils in the extracellular space. Orita et al. [12] reported a case of GH-negative and PRL-positive amyloid deposition in the GH-secreting pituitary adenoma. Moreover, there were no correlations between the extent or patterns of the deposits and either age, immunohistological hormone content, or localization [4)]. They found spherical, stellate and filamentous deposits with no correlation to the type of adenoma or the immunohistological findings. Recently, Maji et al. [13] reported that peptides and protein hormones in secretory granules of the endocrine system were stored in an amyloid-like cross β-sheet-rich conformation, although their immunohistochemical studies were performed on mouse non-tumoral pituitary tissue. There was almost complete colocalization between Thio-S-detected amyloids and the hormones ACTH, β-endorphin, PRL, and GH in the interior lobe. Saeger et al. [11] reported that amyloid from the same precursor could appear in different forms, depending on environmental factors.

In conclusion, we presented a case of GH producing pituitary adenoma with crystal-like structures. Clinicopathologically, our case was diagnosed as sparsely granulated somatotroph adenoma, and the crystal-like structures were GH- and PRL-positive, indicative of bihormonal amyloid formation.

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