Virchows Arch (1996) 427:613–617

CASE REPORT

Hidehiro Oka · Toru Kameya · Hironobu Sasano
Motohiko Aiba · Kalman Kovacs · Eva Horvath
Yukifumi Yokota · Nobuyuki Kawano · Kenzo Yada

Pituitary choristoma composed of corticotrophs and adrenocortical cells in the sella turcica

Received: 17 October 1995 / Accepted: 13 December 1995

Abstract A pituitary tumour composed of well-differentiated corticotrophs and adrenocortical cells is reported. Sections of the tumour revealed a mixture of small round cells with amphophilic or basophilic periodic acid-Schiff (PAS)-positive cytoplasm and large spherical and oval cells with abundant, granular, partly vacuolated PAS-negative cytoplasm. The small cells contained type 1 cytokeratin-positive microfilaments, numerous 250–500 nm endocrine-type secretory granules immunoreactive for adenocorticotropic hormone (ACTH) and β-lipotropin. The large cells possessed ample cytoplasm filled with abundant vesicular smooth endoplasmic reticulum, numerous mitochondria possessing tubulovesicular cristae and frequent dense bodies. They lacked the features of pituitary endocrine cells or folliculostellate cells and were found to contain a panel of steroidogenic dehydrogenases and hydroxylases. The tumour was classified as a choristoma, in which two distinct cells types, corticotrophs and adrenocortical cells, were mixed. We suggest that, under continued ACTH stimulation, uncommitted stem cells may differentiate into adrenocortical cells. Alternatively, the presence of adrenocortical cells may be the result of heterotopia.

H. Oka · T. Kameya (✉)
Department of Pathology, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa, Japan
Tel: (81)427-78-9020; Fax: (81) 427-78-8441

H. Sasano
Department of Pathology, Tohoku University School of Medicine, Sendai, Japan

M. Aiba
Department of Pathology, Tokyo Women's Medical College, Tokyo, Japan

K. Kovacs · E. Horvath
Department of Pathology, St. Michael's Hospital, Toronto, Canada

Y. Yokota
Department of Paediatrics, Kitasato University School of Medicine, Kanagawa, Japan

H. Oka · N. Kawano · K. Yada
Department of Neurosurgery, Kitasato University School of Medicine, Kanagawa, Japan

Key words Pituitary gland · Choristoma · Corticotroph · Adrenocortical cell

Introduction

Heterotopic adrenocortical cells are found in the retroperitoneal space, the broad ligament, and the male or female gonads [5]. Aberrant adrenocortical rests or adrenocortical adenomas have also been identified in a wide variety of other non-steroidogenic tissues, including kidney [4], lung [3], the spinal canal [9] and, exceptionally, on the leptomeningeal surface in the cranium [12, 22]. However, the emergence of adrenocortical cells in the sellar region has not previously been reported.

Our case, a pituitary tumour composed of well-differentiated corticotrophs and adrenocortical cells, located in the sella turcica, might be classified as a pituitary choristoma, and neither this nor a teratoma with a combination of corticotrophic and adrenocortical cells is found in the literature. The histogenesis of this unusual composite lesion is obscure. We suggest that, under continued adrenocorticotropic hormone (ACTH) stimulation, uncommitted stem cells or mesenchymal cells in the pituitary gland may differentiate into adrenocortical cells, or alternatively, the presence of adrenocortical cells may be the result of misplacement to an abnormal location during embryonic life.

Case report

A 16-year-old man was admitted to the Kitasato University Hospital with growth retardation of 3 years' duration. There were no hormonal disturbances (acromegaly, Cushing's disease, or impotence), and visual disturbances were not evident. Preoperative endocrinological testing revealed slightly low serum levels of pituitary hormones including growth hormone (GH; 0.3 ng/ml), follicle stimulating hormone (FSH; 1.7 mIU/ml), luteinizing hormone (LH; 0.5 mIU/ml) and thyroid stimulating hormone (TSH; 0.1 μU/ml). Free T3 (2.2 pg/dl) and free T4 (1.2 ng/dl) were within the normal range. Blood cortisol and testosterone levels were also normal. Plain CT scans showed isodensity, and contrast-enhanced

CT showed an enhancement effect. MRI demonstrated a tumour measuring approximately 1.5 cm with low signal intensity on T1-weighted images, and mixed (iso and high) signal intensity on T2-weighted images. Slight suprasellar extension was apparent. The diagnosis of pituitary adenoma was made and the tumour was removed by the trans-sphenoidal approach. Growth retardation was improved by 2.5 years of GH administration after surgery.

Materials and methods

The surgically removed tissue consisted of 5–6 soft fragments, which were divided and fixed in 10% formalin, freshly prepared 4% paraformaldehyde and Bouin's solutions for light microscopy. Five-micrometre-thick sections were stained with haematoxylin and eosin, periodic acid-Schiff (PAS) and Watanabe's silver impregnation technique for the demonstration of the reticulin fibre network.

Several pieces of tissue were fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide, dehydrated and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with JEM1200EX and Philips 410-LS transmission electron microscopes.

Immunohistochemical studies were performed on 5-μm-thick paraffin sections using the streptavidin-biotin-peroxidase-conjugated detection system (Dako Japan, Kyoto). Sources of primary antisera and monoclonal antibodies used and their sources are summarized in Table 1. To prove the specificity of immunostaining, the primary antisera were replaced with non-immune sera. In addition, the antisera were absorbed with an excess of homologous antigen of pituitary hormones (donated by NIADDKD or purchased from Peptide Institute, Osaka). The staining was judged positive only when the non-immune serum or absorption with homologous antigen yielded negative results.

Results

Histological sections of the tumour revealed a mixture of small round cells with amphophilic or basophilic cytoplasm and large spherical and oval cells with abundant, granular, partly vacuolated acidophilic cytoplasm (Fig. 1). The small cells were weakly to moderately positive and the large cells were negative on PAS staining. The reticulin fibres, delineated by silver impregnation, surrounded a mixture of the two cell types which formed groups and were intermingled. The numerical ratio of the two cell types varied considerably from area to area of the tumour (Fig. 1). No major cellular or nuclear pleomorphism was noted and no mitotic figures were seen. The margin of the tumour bordering the non-tumorous adenohypophysis could not be examined. By electron microscopy (Fig. 2), two distinctly different cell types were recognized. The two cell types were closely associated and were occasionally attached by small adherent junctions. In some sites a narrow strip of connective tissue intervened (not shown in the figure). The small, well-granulated cells contained bundles of type 1 microfilaments in the perinuclear cytoplasm. Secretory granules were spherical, oval or drop-like, homogeneously dense without halo, measuring from 250 to 500 nm in diameter. These features corresponded to those of densely granulated functioning corticotroph cell adenomas or non-tumour corticotrophs in the anterior lobe or the basophilic cells in the intermediate and posterior lobes or

Table 1 Immunostaining results (– negative, + occasionally positive, ++ most cells positive, +* strongly positive aggregate in paranuclear area; *NIADDKD* National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases)

Antigen	Source of antiserum	Working dilution	Two cell types	
			Small	Large
Epithelial membrane antigen	Dako Japan, Kyoto	×200	–	–~+
Keratins (mol.wt. 56–64 kDa)	Dako Japan, Kyoto	×1000	+	–
Human cytokeratin (CAM5.2)	Nippon Becton-Dickinson, Tokyo	×2	++	–
Neurofilament (NF) (68, 160 and 200 kDa)	Amersham, UK	×200	–	–
Glial fibrillary acidic protein (GFAP)	Amersham, UK	×200	–	–
S100 protein	Dako Japan, Kyoto	×4	–	–
Vimentin	Amersham, UK	×200	–~+*	–
α_1-Antitrypsin	Lipshaw, Mich.	×500	–	++
α_1-Antichymotrypsin	Lipshaw, Mich.	×500	–	++
Mitochondrial 65 kDa protein[18]	Chemicon International (monoclonal antibody mAb 1273), Calif.	×20	–~+	+~++
Porcine pituitary ACTH[1–39]	ImmunoNeclear, Minn.	×500	++	–
Fragment of β-lipotropin or β-MSH (synthetic)	UCB-Bioproducts, Belgium	×800	++	–
Growth hormone	Dako, Calif.	×500	–	–
Prolactin	Dako, Calif.	×1000	–	–
Follicle stimulating hormone (FSH β)	NIADDKD, Md.	×1000	–	–
Luteinizing hormone (LH β)	NIADDKD, Md.	×1000	–	–
Thyroid stimulating hormone (TSH β)	NIADDKD, Md.	×600	–	–
3β-hydroxysteroid dehydrogenase (3β HSD)	[16]	×500	–	++
Cytochrome $P450_{11}$	[19]	×450	–	++
Cytochrome $P450_{SCC}$	[19]	×500	–	++
Cytochrome$P450_{17}$	[15]	×500	–	++
Cytochrome$P450_{C21}$	[21]	×400	–	++

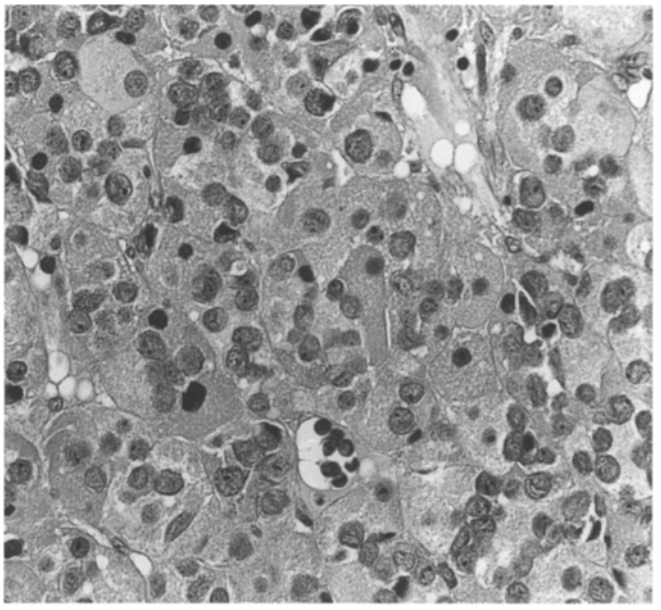

Fig. 1 Histology of the tumour. The small round well-granulated cells with amphophilic or basophilic cytoplasm and the large spherical or oval cells with abundant, granular, partly vacuolated cytoplasm form groups and were close to each other

to those of silent subtype 1 corticotroph adenomas [8]. The large cells which enmeshed the small corticotroph cells possessed ample cytoplasm packed with abundant vesicular smooth endoplasmic reticulum (SER), numerous mitochondria mostly with vesicular or tubulovesicular cristae, frequent lysosomal dense bodies and occasional lipid vacuoles. These cells strongly resembled stimulated steroid hormone producing "compact" cells with highly increased SER and decreased lipid vacuoles [13] and with no feature of amine/peptide-producing cells and of folliculostellate cells. No transitional forms appeared to be present between the two cell types.

The results of immunostaining (summarized in Table 1 and Fig. 3) clearly demonstrated that the small cells were corticotrophs storing abundant ACTH and β-lipotropin and possessing no steroidogenic enzymes. The large cells were not immunoreactive for ACTH or any other adenohypophysial hormones and were immunohistochemically indistinguishable from stimulated adrenocortical cells as shown by immunohistochemistry for 3β-hydroxysteroid dehydrogenase and a panel of cytochrome P450 steroid synthetic enzymes [14, 15, 17, 19], indicating the presence of a set of steroidogenic enzymes, although some of these enzymes have also been shown to be present in normal and/or tumour tissues of salivary, mammary, and prostate glands, pancreatic duct and kidney. Therefore, their presence cannot be absolutely specific to classical steroidogenic cells, even if they are shown in our tumour by immunohistochemistry. Mitochondrial protein [20] was positive in the large cells. Only the small cells were positive for human cytokeratin (CAM5.2) and occasionally positive for vimentin. Both

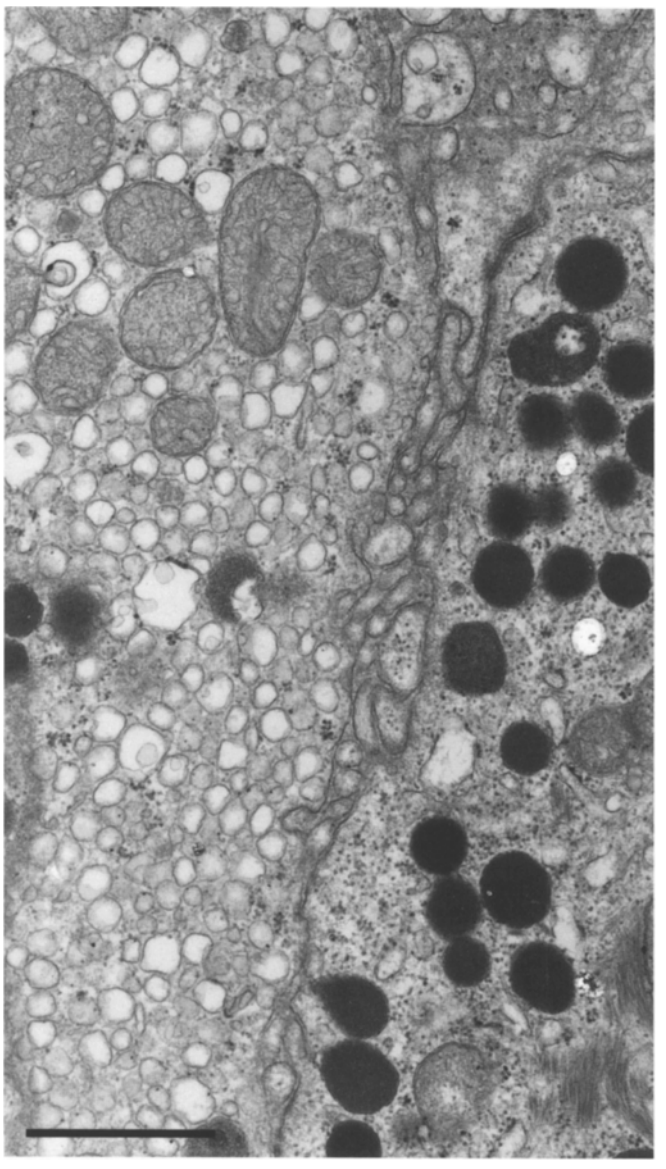

Fig. 2 Electron microscopy demonstrates that the small cells closely resemble densely granulated corticotroph cells and that the large cells closely resemble stimulated steroid hormone producing compact cells with greatly increased vesicular smooth endoplasmic reticulum, numerous mitochondria of tubulovesicular crista type and frequent dense bodies (*bar* represents 5 μm)

cell types were negative for S100 and glial fibrillary acidic protein, immunohistochemical markers of folliculostellate cells. Based on our findings, the tumour was classified as an endocrinologically silent, pituitary choristoma composed of corticotrophs and adrenocortical cells. No comparable has been described before.

Discussion

The cytogenesis of this unique tumour remains obscure. It appears that it is a random mixture of two cell types which proliferate in the sella. Immunohistochemistry and

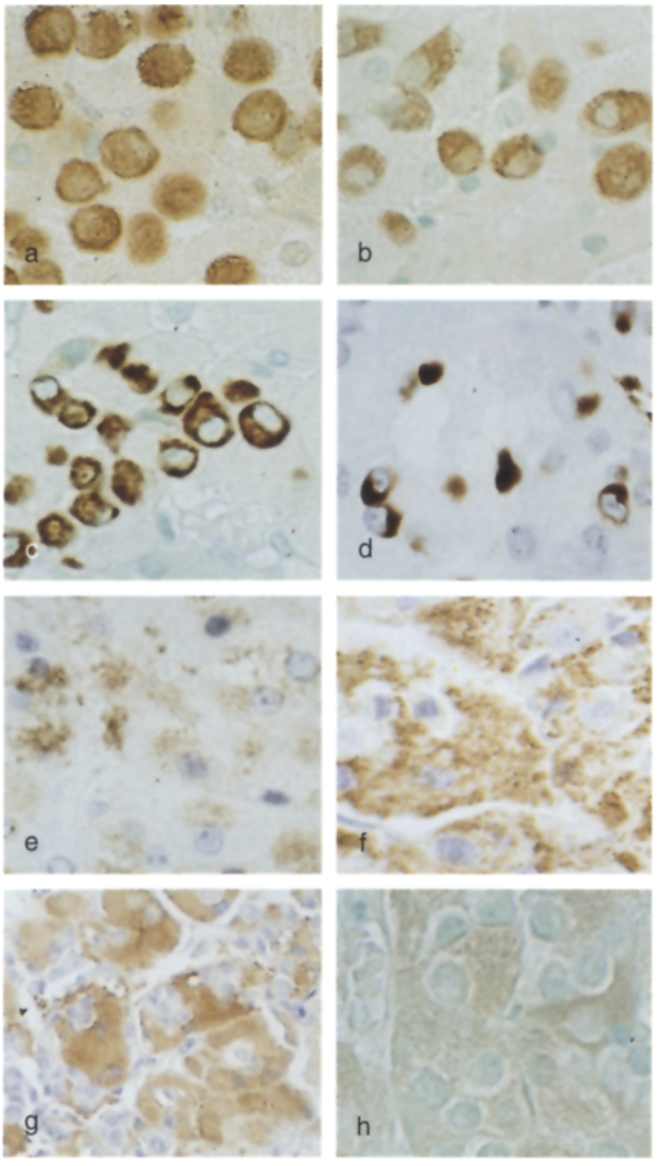

Fig. 3 Immunohistochemical detection of **a** ACTH, **b** β-lipotropin, **c** CAM 5.2 cytokeratin, **d** vimentin, **e** α_1-antichymotrypsin, **f** mitochondrial protein, **g** 3β-hydroxysteroid dehydrogenase (3β HSD), **h** $P450_{17\alpha}$ cytochrome enzyme. ACTH, β-lipotropin, CAM 5.2 and vimentin were restricted to the small cells, while α_1-antichymotrypsin, mitochondrial protein, 3β HSD, $P450_{17\alpha}$ and other $P450_{11\beta, SCC \text{ and } C21}$ steroidogenic enzymes (the last three not shown) were largely restricted to the large cells

electron microscopy proved that the lesion consisted of corticotrophs and stimulated adrenocortical cells. It is intriguing that the tumour was clinically silent causing no Cushing's disease, although a major component of the tumour represented corticotrophs. The origin of the adrenocortical cells in the sella and the reason for their co-existence with corticotrophs is not clear. Since a close relation exists between ACTH and adrenocortical cells, it is tempting to speculate that the two cell types interact with each other in "paracrine" fashion and not in "endocrine" fashion as in the normal hypophyseal-adrenocortical axis. The adrenal cortex has its origin in the mesoderm beneath the mesothelium of the coelomic cavity near the cranial pole of the mesonephros on each side [1]. In some cases, heterotopic adrenocortical tissues, exceptionally linked with the adrenal medulla, are found in the retroperitoneal space and the broad ligament, as well the male or female gonads [5]. Aberrant adrenocortical rests or adrenocortical adenomas have also been identified in a wide variety of other non-steroidogenic tissues, including kidney [4], lung [3], the spinal canal [9] and the leptomeningeal surface in the cranium [12, 22]. Organogenesis of an intracranial adrenal gland was explained by Wiener et al. [22] as representing either misplaced blastomere or conversion of fatty tissue to adrenocortical cells. A teratoma with a combination of corticotrophs and adrenocortical cells has not been reported so far in the sellar region or elsewhere. The most plausible explanations are that either uncommitted stem cells such as undifferentiated mesenchymal cells are present in the pituitary region which under prolonged ACTH stimulation can differentiate into adrenocortical cells or adrenocortical cells migrate to a wrong place, the sellar region, during an early embryonic stage. The emergence of adrenocortex-like cells in the sellar region has not been previously reported. Increased large basophilic or chromophobic vacuolar cells immunopositive for ACTH are well known in the pituitary of untreated Addison's disease but they possess no features of adrenocortical cells [6, 18]. The large cells in our case show no resemblance to corticotrophs and no transitional forms between corticotrophs and adrenocortical cells could be identified. Studies in man [10] and experimental animals [2, 7, 11] lead to the hypothesis that continued paracrine ACTH stimulation by corticotroph adenoma cells may transform uncommitted stem cells including mesenchymal cells or adipose cells to adrenocortex-like cells. The response to stress in the adrenal cortex and brown fat is obviously mediated by ACTH. Groat [7] observed the differentiation of ovarian and other mesenchymal cells into adrenocortex-like cells in the adrenalectomized ground-squirrels of both sexes. Thus, it is conceivable that the adrenocortical tissue can differentiate from various sources [11]. No differentiation to adrenocortical cells occurred in hypophysectomized animals, suggesting that the change was due to ACTH stimulation compensatory for adrenocortical ablation. Our case is, by definition, a pituitary choristoma, which has not been reported in the literature. Its cytogenesis is obscure and further studies are required to elucidate the mechanisms responsible for its formation.

Acknowledgement This work was supported in part by a grant-in-aid from the Ministry of Education, Science and Culture, Japan (05670179).

References

1. Arey LB (1965) Developmental anatomy. A textbook and laboratory manual of embryology, 7th edn. Saunders, Philadelphia, pp 518–519

2. Blumenthal HT (1955) Aging processes in the endocrine glands of various strains of normal mice-relationship of hypophyseal activity to aging changes in other endocrine glands. J Gerontol 10:253–267
3. Bozic C (1974) Ectopic fetal adrenal cortex in the lung of a newborn. Virchows Arch [A] 363:371–374
4. Chin L, Brody RI, Morales P, Black VH (1994) Immunocytochemical characterization of intrarenal adrenal tissue. Urology 44:429–432
5. Cotran RS, Kumar V, Robbins SL (1989) Pathologic basis ofdisease, 4th edn. Saunders, Philadelphia, p 1251
6. Crooke AC, Russel DS (1935) Pituitary gland in Addison's disease. J Pathol Bacteriol 40:255–283
7. Groat RA (1994) Formation and growth of adrenocortical-like tissue in the ovaries of the adrenalectomized ground squirrel. Anat Rec 89:33–41
8. Horvath E, Kovacs K, Killinger DW, Smyth HS, Platts ME, Singer W(1980) Silent corticotropic adenoma of the human pituitary gland. Am J Pathol 98:617–638
9. Kepes JJ, O'Boynick PO, Jones S, Baum D, McMillan J, Adams ME (1990) Adrenal cortical adenoma in the spinal canal of an 8-year-old girl. Am J Surg Pathol 14:481–484
10. Laqueur GL, Harrison MB (1951) Glandular adipose tissue associated with cytotoxic suprarenal contraction and diabetes mellitus. Am J Pathol 27:231–244
11. MacFarland WE (1945) The vital necessity of adrenal cortical tissue in a mammal and the effects of proliferations of cortical cells from dormant coelomic mesothelium. Anat Rec 93: 233–249
12. Meyer AW (1917) A congenital intra-cranial, intra-dural adrenal. Spolia anatomica, addenda II. Anat Rec 12:43–50
13. Reibord H, Fisher ER (1968) Electron microscopic study of adrenal cortical hyperplasia in Cushing's syndrome. Arch Pathol 86:419–426
14. Sasano H, Sasano N (1988) Extraadrenal immunohistochemical distribution of steroid 21-hydroxylase in human. Tohku J Exp Med 154:21–28
15. Sasano H, Mason JI, Sasano N (1989) Immunohistochemical study of cytochrome P-$450_{17\alpha}$ in human adrenocortical disorders. Hum Pathol 20:113–117
16. Sasano H, Mason JI, Sasano N, Nagura H (1990) Immunolocalization of 3β-hydroxysteroid dehydrogenase in human adrenal cortex and its disorders. Endocr Pathol 1:94–101
17. Sasano H, Nagura H, Harada N, Goukon Y, Kimura M (1994) Immunolocalization of aromatase and other steroidogenic enzymes in human breast disorders. Hum Pathol 25:530–535
18. Scheithauer BW, Kovacs K, Randall RV (1983) Pituitary gland in untreated Addison's disease: a histologic and immunocytologic study of 18 adenohypophysis. Arch Pathol Lab Med 107: 484–487
19. Sugano S, Ohnishi T, Hatae N, Ishimura K, Fujita H, Yamano T, Okamoto M (1985) Monoclonal antibodies against bovine adrenal cytochrome P-$450_{11\beta}$ and cytochrome P-450_{SCC}. Their isolation, characterization and application to immunohistochemical analysis of adrenal cortex. J Steroid Biochem 23: 1013–1021
20. Weiss LM, Michael JG, Warhol MJ, Mehta P, Bonsib SM, Bruder E, Santos E, Mederios LJ (1991) Immunocytochemical characterization of a monoclonal antibody directed against mitochondria reactive in parraffin-embedded sections. Mod Pathol 4:596–601
21. White PC, New MI, Dupont B (1984) Cloning and expression of cDNA encoding a bovine adrenal cytochrome P-450 specific for steroid 21-hydroxylation. Proc Natl Acad Sci USA 81: 1986–1990
22. Wiener MF, Dallgaard SA, Manhasset LI (1959) Intracranial adrenal gland. AMA Arch Pathol 67:228–233