European Journal of Endocrinology (2007) **156** 203–216 ISSN 0804-4643

CLINICAL STUDY

Pathohistological classification of pituitary tumors: 10 years of experience with the German Pituitary Tumor Registry

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Abstract

In 1996, the German Registry of Pituitary Tumors was founded by the Pituitary Section of the German Society of Endocrinology as a reference center for collection and consultant pathohistological studies of pituitary tumors. The experiences of the first 10 years of this registry based on 4122 cases will herein be reported. The data supplement former collections of the years 1970–1995 with 3480 surgically removed tumors or lesions of the pituitary region. The cases were studied using histology, immunostainings and in some cases also molecular pathology or electron microscopy. The adenomas were classified according to the current World Health Organization classification in the version of 2004. From 1996 on 3489 adenomas (84.6%), 5 pituitary carcinomas (0.12%), 133 craniopharyngiomas (3.2%), 39 meningiomas (0.94%), 25 metastases (0.6%), 22 chordomas (0.5%), 115 cystic non-neoplastic lesions (2.8%), and 46 inflammatory lesions (1.1%, 248 other lesions or normal tissue (6.0%)) were collected by us. The adenomas (100%) were classified into densely granulated GH cell adenomas (9.2%), sparsely granulated GH cell adenomas (6.3%), sparsely granulated prolactin (PRL) cell adenomas (8.9%), densely granulated PRL cell adenomas (0.3%), mixed GH/PRL cell adenomas (5.2%), mammosomatotropic adenomas (1.1%), acidophilic stem cell adenomas (0.2%), densely granulated ACTH cell adenomas (7.2%), sparsely granulated ACTH cell adenomas (7.9%), Crooke cell adenomas (0.03%), TSH cell adenomas (1.5%), FSH/LH cell adenomas (24.8%), null cell adenomas (19.3%), null cell adenoma, oncocytic variant (5.8%), and plurihormonal adenomas (1.3%). Following the WHO classification of 2004, the new entity 'atypical adenoma' was found in 12 cases in 2005. Various prognostic parameters and clinical implications are discussed.

European Journal of Endocrinology **156** 203–216

Introduction

Clinically relevant pituitary tumors presenting with disturbances of hormonal secretion or mass effect are rare, with an estimated prevalence of 200/1 000 000 and an incidence of 2/100 000 per year (1–3). However, pituitary tumors are increasingly detected incidentally in 3.7–20% of computed tomography (CT) and approximately 10% of magnetic resonance imaging (MRI) scans of the central nervous system (CNS) performed for unrelated reasons (4, 5). Furthermore, about 10% of all pituitaries in unselected series of postmortem examinations show small adenomas (6, 7). Most of these are presumably hormonally inactive adenomas with no clinical significance during lifetime of the patient. The optimal management of such incidentalomas is still unclear (8). The management of clinically relevant pituitary tumors has changed quite dramatically in recent years. New medical treatment and radiation options became available in addition to advanced neurosurgical techniques. Optimal advice to the patient about these possibilities necessitates a precise characterization of the tumor. Whereas pituitary tumors are clinically differentiated mainly on the basis of their size and hormonal secretion, pathology offers further classification using sophisticated techniques. The present studies on the comparison of different treatment options mostly rely on the clinical diagnosis from endocrine characteristics. However, future studies may need to address differences in tumor behavior based on the pathogenesis and the pathohistological evaluation of the tumor. Furthermore with advancing diagnostic endocrine tests and radiological evaluation techniques, these procedures may need to be evaluated in comparison with the subclassification offered by pathology. Nowadays, pathohistological classification of pituitary

DOI: 10.1530/eje.1.02326
Online version via www.eje-online.org

adenomas according to the WHO criteria is very sophisticated and requires a high methodological standard. A registry for pituitary tumors that classifies the tumors by such methods may allow a better standardization of the diagnosis and therapy. Furthermore comparison with other countries could provide new insight in specific ethnic and behavioral differences in the pathogenesis of pituitary tumors. Lastly, such a registry may act as a reference center to enable improvements in the pathohistological diagnosis of pituitary tumors in local centers. With these aims in mind, the Pituitary Section of the German Society of Endocrinology founded the German Pituitary Tumor Registry offering co-operation with endocrinologists, neurosurgeons, and pathologists. Between 1996 and 2005, 4122 surgical cases were registered here and are presented in this review. In addition to the analysis summarizing 10 years of experience with the registry, this review may aid the establishment of registries in countries in which such an institution has not yet been founded.

Structure of the German Pituitary Tumor Registry

Tissue specimens obtained during surgery of pituitary or other sellar region lesions were mainly collected from ten centers (Table 1, defined by $>1\%$ of all contributors), with additional individual problematic tumors submitted to the registry for counsel by many other centers. Samples were sent to the Institute of Pathology of the Marienkrankenhaus Hamburg, Germany, as formalin-fixed or glutaraldehyde-fixed samples or as paraffin-embedded specimens. Clinical information could be entered into a standardized questionnaire provided to all centers. Detailed analyses of the registry are presented at the yearly meeting of the German Pituitary Group. The German Pituitary Tumor Registry was partly funded by unrestricted grants from Novartis Pharma GmbH, Ipsen Pharma GmbH, Ettlingen, Germany, Pfizer Pharma GmbH, Karlsruhe, Germany, and NovoNordisk Pharma GmbH, Mainz, Germany.

Table 1 Numbers of tumors sent from different centers to the German Registry of Pituitary Tumors, 1996–2005 ($N=4122$).

Center	Number (N)	Percentage (%)
Hamburg	1285	31.2
Erlangen–Nuremberg	935	22.7
Göttingen	658	16.0
Bremen	239	5.8
Munich	233	5.6
Karlsruhe	102	2.5
Erfurt	94	2.3
Gießen	75	1.8
Münster	48	1.2
Wuppertal	43	1.0
All others	410	9.9

Methods used for histopathological characterization

If enough material was available and the fixation was suitable, specimens were postfixed in osmium tetroxide and embedded in Epon 812 for semithin toluidine blue-stained sections or electron microscopy. Evaluation by electron microscopy was necessary in 22 problematic cases. Paraffin sections were stained with hematoxylin–eosin and periodic acid schiff (PAS), and used for immunostaining with monoclonal antibodies (MAB) against the specific pituitary hormones, such as growth hormone (GH; Sigma Immunochem, 1:200, Marseille, France, avidin-biotin-peroxidase complex (ABC)), PRL (1:400, Immunochem, ABC), thyroid-stimulating hormone (TSH; Immunotech, 1:5000, ABC), luteinizing hormone (LH; Immunotech, 1:40 000, ABC), and follicle-stimulating hormone (FSH; Immunotech, 1:40 000, ABC). For adrenocorticotrophic hormone (ACTH), a polyclonal antibody was used (Zymed, San Francisco, CA, USA, 1:200, ABC). The glycoprotein α-subunit was detected with a specific MAB (Immunotech, 1:1500, ABC). Cell proliferation was assessed by staining with an MAB for Ki-67 (MiB-1; Zymed, 1:150, ABC) and counting of mitoses per high-power visual fields. S100 expression as a marker for folliculostellate cells was analyzed by staining with MAB (Dako, Glostrup, Denmark, 1:1500, ABC). Expression of the p53 protein was evaluated in a subgroup of tumors suspected of being atypical adenomas using an MAB (Novocastra, Newcastle upon Tyne, UK, 1:80, ABC). For positive controls of pituitary hormones, normal postmortem pituitaries were used.

Principles of classification

According to the 2004 WHO classification (9), pituitary tumors are defined as neoplasms located in the sella turcica. Adenomas (84.6% of all cases in our registry; Table 2) deriving from adenohypophysial parenchymal cells are classified as typical adenomas or atypical adenomas. In very rare cases, they represent pituitary carcinomas (0.12% of all cases in our registry; Table 2). In contrast to typical adenomas, atypical adenomas are defined by (a) their invasiveness, (b) a Ki-67 (MiB-1) proliferation index of 3% or more, and (c) an extensive nuclear staining for p53 protein. Pituitary carcinomas are characterized by the presence of metastases. Brain invasion as the only criterion of malignancy is generally not accepted. Pituitary adenomas are also classified according to the size. Microadenomas are <10 mm in size, whereas macroadenomas have an estimated diameter of at least 10 mm. Most importantly, pituitary adenomas are classified by their similarity to normal parenchymal cells and the expression of specific pituitary hormones.

Table 2 Tumors of the pituitary and sellar regions in the German Registry of Pituitary Tumors, 1996–2005 ($N=4122$).

Tumor type	Number (N)	Percentage (%)
Pituitary adenoma	3489	84.6
Pituitary carcinoma	5	0.12
Craniopharyngioma, adamantinous	121	2.9
Craniopharyngioma, papillary	12	0.3
Meningioma	39	0.94
Chordoma	22	0.5
Metastasis	25	0.6
Squamous carcinoma	2	0.05
Gangliocytoma (with adenoma)	14 (13)	0.34 (0.31)
Chondrosarcoma	7	0.17
Other sarcomas	2	0.05
Granular cell tumor	7	0.17
Neurinoma	3	0.07
Astrocytoma	6	0.15
Pituicytoma	3	0.07
Ganglioglioma	1	0.02
Neurocytoma	1	0.02
Suprasellar germinoma	6	0.15
Gliomatous tumor, not classified	1	0.02
Histiocytosis of Langerhans	2	0.05
Neuroendocrine tumor, not classified	1	0.02
Malignant lymphoma	1	0.02
Fibroma	2	0.05
Hemangioma	3	0.07
Hamartoma	3	0.07
Fibrous dysplasia	4	0.1
Rathke's cyst	76	1.8
Colloid cyst	15	0.36
Arachnoidal cyst	9	0.22
Epidermoid cyst	10	0.24
Cyst, not classified	5	0.12
Mucocele	2	0.05
Plasma cell granuloma	1	0.02
Granulation tissue	3	0.07
Lymphocytic hypophysitis	14	0.34
Granulomatous hypophysitis	6	0.14
Granulomatous hypophysitis in generalized disease	1	0.02
Tuberculous hypophysitis	1	0.02
Peritumorous hypophysitis	2	0.05
Abscess	10	0.24
Chronic inflammation, not classified	9	0.22
Necrosis	2	0.05
Fibrosis or scar	17	0.4
Hyperplasia of ACTH cells	4	0.1
Hyperplasia of prolactin cells	6	0.15
Hyperplasia of GH cells	2	0.05
Hyperplasia of FSH/LH cells	1	0.02
Castration cells	2	0.05
Crooke cells (without adenoma)	76	1.84
Normal pituitary	53	1.3
No diagnosis (insufficient specimens)	13	0.32
Sum	4122	100

Proliferation markers for pituitary tumors

For evaluation of the proliferative activity of the pituitary tumor, both counting of mitoses and immunostaining of nuclei for proliferation markers may be used. Mitoses are rarely seen in non-invasive adenomas (3.9% of cases), but more frequently demonstrated in invasive adenomas (21.4%) and carcinomas (66.7%) (10). Concerning the mitotic index, the highest values ($0.09\pm0.035\%$) are found in pituitary carcinomas, without any clear differences between invasive ($0.013\pm0.005\%$) and non-invasive ($0.02\pm0.002\%$) adenomas (10). Therefore, a critical index cannot be defined. In an own study (11), the mitotic index correlated to DNA aneuploidy of adenomas. Ki-67 (MiB-1) as the most important proliferation marker is expressed in the G1, S, G2, and M phases of the cell cycle. In an early study of 1987 (12), biopsy specimens of 31 pituitary adenomas representing all major endocrine types harbored immunoreactive nuclei to Ki-67 ranging from 0.1 to 3.7%. Eleven hormonally inactive adenomas demonstrated Ki-67 values in the lower range (0.1–1.0%), whereas six acromegalic patients presented with Ki-67 levels in the upper range (1.1–1.5%). The percentages of Ki-67-positive cells in 12 prolactinomas and two adenomas from patients with Cushing's disease covered the entire range (0.1–3.7%). Preoperative bromocriptine treatment of prolactinomas did not influence Ki-67 expression. In a more recent study (13), Ki-67 (MiB-1) was positive in 139 of 159 adenomas (87%). The Ki-67 index ranged from 0.16 to 15.48% (mean $\pm$ S.D. $=1.22\pm2.09\%$) and was higher in ACTH-secreting adenomas. Invasive pituitary adenomas had a significantly higher Ki-67 index ($2.01\pm3.15\%$) than non-invasive adenomas with or without suprasellar extension ($1.12\pm1.87\%$). The index was not significantly different in the subgroup of adenomas with invasion of the cavernous sinus compared with groups with other types of invasion. Focusing on hormonally inactive pituitary adenomas, a study of our own material revealed a mean labeling index (LI) for MiB-1 of 0.12 (S.D. 0.29) in adenomas growing <1.5 mm/year, and 0.34 (S.D. 1.05) in adenomas growing more than 1.5 mm (14). For non-invasive adenomas, the mean MiB-1 LI was 0.03 (S.D. 0.057), for invasive adenomas, it was 0.126 (0.273), and for strongly invasive adenomas 0.212 (S.D. 0.393).

The mean MiB-1 LI was lower in null cell adenomas (LI 0.12, S.D. 0.25) than in FSH/LH adenomas (LI 0.63, S.D. 1.28). However, all these data did not reach statistical significance. Further markers (proliferating cell nuclear antigen (PCNA), Topoisomerase IIa, p27, and others (15, 16)) may be used to analyze proliferation, but are generally not thought to be superior to Ki-67 (MiB-1), although in one of our own studies (14), PCNA was a more valuable marker of rapidity of tumor growth and confirmed the significance of p53 protein expression for invasive adenomas.

p53 expression as a marker of pituitary adenomas

The product of the tumor suppressor gene p53, p53 protein was found to be useful for the identification of recurrent pituitary adenomas in childhood and adolescence. Its expression correlates with invasive behavior (17), with immunostaining of p53 limited to invasive

adenomas in our own studies (14). Whereas, we did not find any correlations to the clinical growth rate, p53 expression correlated significantly with the numbers of MiB-1-positive nuclei ($P=0.002$) and PCNA-positive nuclei ($P=0.0027$).

Invasiveness as a marker of pituitary adenomas

Enclosed adenomas have a clear delineation to the remaining pituitary tissue, the sellar floor bone, the cavernous sinus, and the diaphragm. In contrast, invasive adenomas are defined by their growth into one or more of these surrounding tissues, mostly in the form of small adenoma cell nests. Although defined as benign tumors, nearly 50% of pituitary adenomas invade surrounding tissues (18). Invasion of sellar bone may easily be demonstrated in surgical specimen, invasion of the dura is best found histologically in special dural surgical specimens (19). The frequency of dural invasion was found to increase with the size of the pituitary adenoma, as measured on MRI (19). Invasion should be demonstrated preoperatively by neuroimaging with MRI (20). The rate of invasiveness differs between the various pituitary adenoma types (Table 3). By far, the lowest rate of invasive adenomas was observed for Cushing's disease (13%), followed by GH- and PRL-expressing adenomas, null cell, and plurihormonal adenomas (31–52%) (21). In contrast, the other types (ACTH cell adenomas in Nelson's syndrome, inactive ACTH cell adenomas, FSH/LH cell adenomas, and TSH cell adenomas) demonstrated invasion of surrounding structures in the majority of tumors investigated (82–100%; Table 3) (22). Invasive pituitary adenomas are generally characterized by a higher Ki-67 proliferation index (13, 14). In addition, invasive adenomas may demonstrate p53-positive nuclei, which were found to be absent in enclosed adenomas (14, 23, 24). The molecular mechanisms controlling cell proliferation and invasion are largely unknown, but are believed to be separate from each other (25). Invasiveness of the pituitary adenomas was established as a prognostic factor. It is feasible that the invasion of surrounding tissue may hinder complete surgical resection and for this reason, residual tumor tissue after pituitary surgery was more frequently demonstrated in patients with invasive adenomas significantly than in those with non-invasive adenomas (19). Furthermore, the survival rate at 6 years postsurgery was slightly but significantly decreased for patients with dural invasion (19).

Tumor size as a marker of pituitary adenomas

Both the proliferation rate and the ability to invade into the surrounding tissue will determine the size of a pituitary tumor at the time of diagnosis. However, due to the endocrine hyperfunction, hormonally active adenomas are usually diagnosed at an earlier stage than hormonally inactive tumors, the latter being diagnosed mostly due to the effect of local pressure exerted by the already existing large tumor. In our registry (Table 3), 15–86% of macroadenomas occur among hormonally active tumors compared with 95–100% in the group of typical hormonally inactive tumors. Other factors like treatment strategies may also influence the size of the tumor at the time of operation. Interestingly, the tumor size at diagnosis may point to specific aspects in the pathogenesis of certain pituitary adenomas. Careful studies of the case history of patients with

Table 3 Size and rate of invasiveness of surgically resected pituitary adenomas (18, 21).

Adenoma type	Rate of macroadenomas (>1.0 cm)	Rate of invasiveness
Densely granulated GH cell adenoma	86%	52%
Sparsely granulated GH cell adenoma		
Densely granulated prolactin cell adenoma	74%	50%
Sparsely granulated prolactin cell adenoma	50%	
Mixed GH/prolactin cell adenoma	74%	31%
Mammosomatotrope adenoma	50%	
Acidophilic stem cell adenoma	100% (?)[a]	100% (?)[a]
Densely granulated ACTH cell adenoma		
Sparsely granulated ACTH cell adenoma		
Morbus Cushing	15%	13%
Nelson's syndrome	100%	82%
Inactive	100%	82%
Crooke cell adenoma	75%	85%
TSH cell adenoma	100%	100%
FSH/LH cell adenoma	95%	95%
Null cell adenoma	95%	42%
Null cell adenoma, oncocytic variant	95%	?[b]
Plurihormonal adenoma	75%	52%
Silent adenoma (subtype 3)	100%	100%

[a]Series too small for significant data.
[b]No reliable data.

microprolactinomas who refused treatment revealed a low risk of approximately 6.5% up to 10% for progression to macroprolactinomas (26–29). These may therefore be considered two separate disease entities. Clearly, tumor size is a relevant prognostic marker for the success of any treatment. Surgical outcome was found to be better in patients with microadenomas of various types as compared with macroadenomas of these types (30–32). Primary medical treatment of GH-secreting pituitary adenomas with somatostatin analogs was more efficacious in microadenomas compared with macroadenomas (33). Generally, larger tumors recur more frequently than smaller adenomas after surgery. The highest recurrence rate (19%) was found in patients with inactive macroadenomas with a follow-up of more than 2 years (34).

Expression of specific hormones as markers of pituitary tumors

The WHO classification of 2004 (9, 35, 36) is based on the structural similarities with normal parenchymal cells and the immunohistochemical demonstration of hormone content. Thereby, pituitary adenomas may express more than one or two hormones. The bihormonal–bicellular character of the combination of GH and PRL is problematic due to the common stem cells, the existence of mammosomatotrope cells in the normal pituitary and the transition between both the cell types (37, 38). Nevertheless, three different bihormonal GH- and PRL-secreting adenoma types were again specified in the new WHO classification (9). The combination of FSH and LH is considered as bihormonal but not as bicellular since both hormones are always produced in the same cell type (39). Plurihormonal adenomas may be monomorphous showing only one cell type or may be plurimorphous showing more than one cell type. Some plurihormonal adenomas do not reveal any real similarities with normal pituitary cells. Two types of monohormonal (densely or sparsely granulated) GH-secreting adenomas are distinguished, three types of adenomas producing GH and PRL, and two types secreting PRL only (Table 4). Adenomas producing

Table 4 Pathohistological classification of pituitary adenomas.

Adenoma type	Number[a] (%)	Hormone expression by immunocytochemistry	Most important structural criteria	Clinic
(1) Densely granulated GH cell adenoma	314 (9.2)	GH	Acidophil	Acromegaly, very rarely inactive
(2) Sparsely granulated GH cell adenoma	215 (6.3)	GH	Weakly acidophil, fibrous bodies	Acromegaly, very rarely inactive
(3) Sparsely granulated prolactin cell adenoma	302 (8.9)	Prolactin	Chromophobic, elongated cells	Hyperprolactinemia
(4) Densely granulated prolactin cell adenoma	11 (0.3)	Prolactin	Acidophil	Hyperprolactinemia
(5) Mixed bicellular GH/prolactin cell adenoma	176 (5.2)	GH/prolactin	Densely or sparsely granulated GH-cells like (1) or (2) and prolactin cells like (3) or (4)	Acromegaly with hyperprolactinemia
(6) Mammosomatotrope adenoma	39 (1.1)	GH/prolactin	Like (1), unimorphous cell type	Acromegaly with hyperprolactinemia
(7) Acidophil stem cell adenoma	6 (0.2)	Prolactin/GH	Oncocytic like (14), fibrous bodies like (2), giant mitochondria	Hyperprolactinemia, often slight acromegaly
(8) Densely granulated ACTH cell adenoma	245 (7.2)	ACTH	Basophilic, strongly PAS-positive	Morbus Cushing, may be inactive (‘silent adenoma, type 1’)
(9) Sparsely granulated ACTH cell adenoma	270 (7.9)	ACTH	Chromophobic or slightly basophilic, slightly PAS-positive	Morbus Cushing, may be inactive (‘silent adenoma, type 2’)
(10) Crooke cell adenoma	1 (0.03)	ACTH	Crooke cells (intracytoplasmatic hyalin ring, paranuclear accumulation of secretory granules and vacuoles)	Inaktive, possibly Morbus Cushing[b]
(11) TSH cell adenoma	50 (1.5)	TSH	Medium-sized elongated chromophobic cells, partly PAS-positive, globular inclusions	Hyperthyroidism, hypothyroidism
(12) FSH/LH cell adenoma	857 (25.2)	FSH and/or LH	Elongated or slightly vacuolated, chromophobic, often oncocytic parts like (13)	Inactive
(13) Null cell adenoma	673 (19.8)	–	Small uniform chromophobic cells, often oncocytic parts like	Inactive
Null cell adenoma, oncocytic variant	198 (5.8)	–	Large, slightly acidophilic cells, demonstration by electron microscopy or semithin sections	
(14) Plurihormonal adenoma, incl. ‘silent adenoma, subtype 3’	46 (1.3)	GH/prolactin/TSH/FSH/LH, TSH/FSH/LH, prolactin/ACTH usw.	Uniform or plurimorphous	Often acromegaly, not uniform ‘silent adenoma, subtype 3’: mostly strongly invasive

[a]Collection in the German Registry of Pituitary Adenomas of the years 1996–2005 ($N=3403$; without unclassified adenomas ($N=74$), atypical adenomas ($N=12$), and pituitary carcinomas ($N=5$)).
[b]No significant results due to small number of cases.

ACTH can be active or inactive, densely or sparsely granulated, and very rarely of the Crooke cell type. Adenomas secreting TSH may be either pure TSH cell adenomas or belong to the group of plurihormonal adenomas. Pituitary adenomas expressing hormones may differ in their ability and quantity of hormone secretion, as well as in the functional integrity of the hormones secreted. Without typical clinical symptoms, such pituitary adenomas are termed silent. Typical inactive tumors are the FSH/LH cell adenomas and the null cell adenomas. In our series, 66.5% are monohormonal, 6.5% are bihormonal for GH and PRL, 1.3% are plurihormonal, and 25.6% do not express any hormones (Table 4). Even with such a sophisticated classification based on the experiences of many specialized pathologists around the world (9), a small percentage of adenomas (2.1% in our collection) cannot be classified. Technical problems related to small specimens, artifacts, and necroses of the cells are one obvious reason, while very unusual structures may prevent classification in other samples. In acromegaly, the most important prognostic factor was found to be the invasiveness of the adenoma which can be predicted by MRI and definitely proved by a thorough intraoperative inspection with adequate visualization methods. Therefore, the differentiation of resectable and incompletely resected or non-resectable adenomas is clinically relevant (31). This decision is made by the surgeon and not on the basis of tissue investigations.

Adenomas secreting only GH

The *densely granulated GH cell adenoma* (9.2% of adenomas in our collection; Table 4) has a diffuse pattern. The cells are slightly pleomorphic, large- or medium-sized, partly angular, and mostly strongly acidophilic. The nuclei show small- to medium-sized nucleoli and moderate chromatin. Binucleated cells can be observed. Mitoses are very rare, and $<3\%$ of nuclei are MiB-1-positive. Immunostaining for GH is strong and diffuse. In the electron microscope (6, 40), the similarity of adenoma cells with normal GH cells is evident. Medium-sized (300–450 nm) secretory granules are numerous. The rough endoplasmic reticulum is moderately developed and the Golgi fields are spherical. In general, densely granulated GH cell adenomas are slow-growing and usually well-demarcated tumors, with a relatively low rate of invasion (40). In contrast, the *sparsely granulated GH cell adenoma* (6.3% of adenomas in our collection; Table 4) shows a more diffuse growth pattern. The cells are medium-sized or large, and slightly acidophilic. The nuclei are often pleomorphic and rich in chromatin. The cytoplasm exhibits a paranuclear spherical clear zone with strong keratin expression. These fibrous bodies are the hallmark of this adenoma type. The GH immunoreaction is weaker than in the densely granulated type, and may be negative in some cases (41). In the latter case, the staining should be repeated, possibly using other GH antibodies. Furthermore, *in situ* hybridization may be used in cases of strong clinical suspicion to detect GH mRNA, which will be positive in sparsely granulated GH cell adenomas (42). Some ($<10\%$) cells may be positive for PRL. The number of MiB-1-positive nuclei is between 0 and 3%. Evaluation of the ultrastructure reveals a different pleomorphism of nuclei organelles (6). The rough endoplasmic reticulum may be more prominent and forming nebenkerns. The secretory granules are sparse, mostly small and pleomorphic. The fibrous bodies are composed of dense aggregates of type II filaments (43). Increased hormone secretion (44), proliferation (45), and recurrence after surgery (46) of sparsely granulated GH adenomas in comparison with densely granulated adenomas have been claimed by some authors, but disputed by others (47).

Alterations after treatment with somatostatin analogs Treatment with somatostatin analogs results in tumor shrinkage in 40–50% of patients, especially in those with receptors for somatostatin (48). Frequent histological findings are perivascular and interstitial fibroses, an increased acidophilia of cells, stronger GH immunoreaction, and a lower MiB-1 index (49).

Prolactin-secreting adenomas

The *densely granulated PRL cell adenoma* is a very rare adenoma type (0.3% in our collection; Table 4). It contains elongated acidophilic cells with elongated nuclei and increased chromatin. Immunoreaction for PRL is strong and diffuse. The electron microscope reveals large, in part densely arranged secretory granules with exocytoses and a strongly developed rough endoplasmic reticulum (6). The *sparsely granulated PRL cell adenoma* (8.9% in our collection; Table 3) has a medullary-diffuse, trabecular or pseudopapillary growth pattern and is composed of large, often elongated chromophobic cells with oval large moderately chromatin-rich nuclei. Calcifications of psammoma body type can be found. Amyloid derived from PRL (endocrine amyloid) is demonstrable in up to 48% of adenomas (50, 51). Immunostaining for PRL is often concentrated in the Golgi areas (so-called Golgi pattern) (6, 40). The MiB-1 index is low. In the electron microscope, we find slightly lobated nuclei, a very strongly developed rough endoplasmic reticulum with long membranes partly forming nebenkerns, large Golgi fields, and sparse, mostly small (100–300 nm) secretory granules. A characteristic feature is the demonstration of exocytoses into the intercellular space (52). Although prolactinomas are the most frequent pituitary adenomas, their frequency in surgical material is decreasing due to their preferential medical treatment. PRL hypersecretion is always

present in these patients and clearly correlates with the size of the adenoma (53).

Effects of treatment with dopamine agonists
Dopaminergic drugs induce a strong shrinkage of adenoma cells in most cases correlating to the strong decrease of PRL levels (40, 54). The cytoplasm is reduced and the rough endoplasmic reticulum and the Golgi fields are strongly decreased (55). The number of single necrotic cells and, after long-term treatment, the amount of fibroses are increased (56).

Adenomas secreting both GH and prolactin

The *bicellular–bihormonal GH/PRL cell adenoma* (5.2% in our series; Table 4) is composed of sparsely or densely granulated cells that correspond to the sparsely or densely granulated cells of the GH cell adenomas, and of sparsely granulated PRL cells that we find otherwise in sparsely granulated PRL cell adenomas. Additionally, some adenoma cells may contain both GH and PRL (52). The ultrastructure exhibits adenoma cells of the GH cells type and of the PRL cell type (57). In contrast, the monocellular bihormonal *mammosomatotrope adenoma* (1.1% in our collection; Table 4) is composed of medium-sized to large strongly acidophilic cells in a medullary or diffuse arrangement resembling the densely granulated GH cell adenoma. The immunoreaction for GH is strong in most cells, whereas the immunoreaction for PRL is mostly weak in the same cells. The MiB-1 index is very low. The ultrastructure is very similar to densely granulated GH cell adenomas, although the secretory granules are larger and more irregular (57). Exocytoses into the intercellular space are demonstrable. The *acidophil stem cell adenoma* (0.2% in our collection; Table 4) is a monomorphous monohormonal adenoma secreting PRL or a monomorphous bihormonal adenoma secreting mostly PRL and less GH. The growth pattern is diffuse. The cells are slightly pleomorphic, weakly acidophilic, and PAS-negative. Large clear cytoplasmic vacuoles are demonstrable which correspond to giant mitochondria. Furthermore, the mitochondria are increased in number comparable with oncocytic adenomas. Fibrous bodies are found similar to the sparsely granulated GH cell adenomas. Immunostaining for PRL is variable and the reaction for GH is weak. The MiB-1 index is not increased. The ultrastructure shows giant mitochondria, increased numbers of mitochondria, fibrous bodies, and other structures of sparsely granulated GH cell adenomas but also structures of PRL cell adenomas. The acidophil stem cell adenoma is more aggressive than other adenomas, always invasive (40), and mostly resistant to drug treatment (6). Clinically, GH–PRL adenomas with the exception of acidophil stem cell adenomas are characterized by acromegaly or gigantism as well as varying degrees of hyperprolactinemia (40). An increased frequency and higher levels of PRL secretion have been demonstrated in GH–PRL adenomas compared with GH adenomas (58). The authors suggest that PRL levels above 200 ng/ml in combination with autonomous GH secretion indicate indeed GH–PRL adenomas.

ACTH-secreting adenomas in Cushing's disease or Nelson's syndrome

Densely granulated ACTH cell adenomas represent 7.2% of all adenoma types (Table 4). They show a medullary, diffuse, or sinusoidal growth pattern. The cells are monomorphic, partly elongated, basophilic, and show a dense PAS-positive granulation. Cellular or nuclear pleomorphism is generally low but may be demonstrable in some cases. Immunostaining reveals strong reactions for ACTH. Other peptides derived from proopiomelanocortin may also be found, and in some cases, α-subunit or galanin (59, 60). Electron microscopy shows monomorphic spherical or ovoid nuclei with distinct nucleoli, a moderately developed rough endoplasmic reticulum, spherical medium-sized Golgi fields and numerous slightly pleomorphic secretory granules with diameters between 200 and 450 nm. In Cushing's disease, cytokeratin filaments are numerous and may form bundles near the nuclei, probably in reaction to the hypercortisolism. In Nelson's syndrome, these cytofilaments are lacking or sparse (61). *Sparsely granulated ACTH cell adenomas* occupy 7.9% of our registry (Table 4). Due to their low granulation, sparsely granulated ACTH cell adenomas are only weakly basophilic and weakly PAS-positive or chromophobic. Cellular pleomorphism is more frequent in the sparsely granulated than in the densely granulated type. Immunostaining is nearly identical with the densely granulated ACTH cell type, although the reactions especially for ACTH are weaker and may be negative. In the latter case, when clinical suspicion appears justified, more than one antibody for ACTH should be tried for differentiation from other adenoma types, especially from the inactive adenomas of the null cell type. The electron microscopy reveals far less numerous and often smaller secretory granules than in the densely granulated ACTH cell adenomas, whereas the organelles structures are similar or slightly irregular. Clinically, ACTH cell adenomas are associated with pituitary-dependent Cushing's disease. Most adenomas are of small size (Table 2). Due to the very small size of some adenomas, tissue that represents the true nature of the lesion may be lost during surgical procedures, so that the specimens contain only pituitary tissue with Crooke cells (about 50 cases in our series; Table 2) or the adenoma tissue submitted is too small for cutting enough slides for immunohistological classification. This is the main reason for 74 unclassified tumors (2.2%; Table 4) in our collection. Adenomas associated with Nelson's syndrome are usually large

with an aggressive, invasive behavior, and a high rate of recurrences after surgery (44, 62). In contrast to most other hormonally active pituitary adenomas, there is no relationship between the severity of hypercortisolism, the plasma ACTH levels, and the tumor size (58).

Peritumorous pituitary in active ACTH cell adenomas In the presence of an active ACTH cell adenoma in Cushing's disease, the adenohypophysis shows characteristic alterations of the ACTH cells that were first described by Crooke (63) and called Crooke cells. These cells are larger than normal ACTH cells and exhibit an intracytoplasmic hyaline ring, paranuclear vacuoles, and secretory granules beside the vacuoles and along the cellular membrane. In the electron microscope, the hyaline ring is composed of dense keratin filaments and the vacuoles are large lysosomes that take up the secretory granules (64, 65). These cells develop in hypercortisolemic states of all types (drugs, adrenal Cushing's syndrome, active ACTH cell adenomas, and paraneoplastic ACTH syndrome). If Crooke cells are not demonstrable in pituitaries with ACTH cell adenomas, these adenomas are considered inactive adenomas (64, 66) or represent adenomas in Nelson's Syndrome. In our collection, we found Crooke cells in all cases of active ACTH cell adenomas where sufficient specimens and paraadenomous pituitary tissue were available.

Clinically inactive (silent) ACTH cell adenomas

Two subtypes of inactive ACTH cell adenomas are identified. Furthermore, the rare Crooke cell adenoma may be inactive. The *silent densely granulated ACTH adenoma is also designated as subtype 1 adenoma* (52). The incidence in our collection of clinically silent and inactive adenomas is 1.1% (Table 5). It is histologically and ultrastructurally indistinguishable from active densely granulated ACTH cell adenomas. The incidence of spontaneous hemorrhagic necroses is higher than in the active ones (40). The second inactive ACTH adenoma is the *silent sparsely granulated adenoma* and is called *subtype 2 adenoma* in the Kovac's classification (52). Its incidence in our collection of clinically silent and inactive adenomas is 4.4% (Table 5). Most patients are men. The tumor shows very similar structures to the active sparsely granulated ACTH cell adenoma and is indistinguishable from that if clinical data or adjacent tumor-free tissue for the identification of Crooke cells are not available. The ultrastructure of the organelles may be slightly more pleomorphic than in the active ones. *Crooke cell adenomas* are very rare (0.03% of all adenoma types in our registry; Table 4). They are composed of Crooke cells with typical cytoplasmatic structures (hyaline ring of densely arranged microfilaments, large lysosomes, secretory granules around the lysosomes, and at the cell periphery). Some adenoma cells are not completely transformed to Crooke cells showing less microfilaments. Whereas, ACTH hyperfunction is observed in 65%, 35% are clinically inactive (67). The clinical course is variable, with some tumors demonstrating an aggressive behavior, and others only being found as small incidental inactive adenomas in postmortem pituitaries (68). In a recent study, invasiveness was found in 72% of these adenomas (67).

Table 5 Clinically silent and inactive pituitary adenomas (surgical specimens of sellar region, 1991–2005; *N*=2012).

Adenoma type	Number	Percentage (%)
Null cell adenoma	678	33.7
Null cell adenoma, oncocytic variant	216	10.7
FSH/LH cell adenoma	865	43.0
Prolactin cell adenoma, sparsely granulated	31	1.5
Prolactin cell adenoma, densely granulated	3	0.15
GH cell adenoma, sparsely granulated	19	0.94
Mixed GH/prolactin cell adenoma	1	0.05
Acidophil stem cell adenoma	1	0.05
ACTH cell adenoma, sparsely granulated	89	4.4
ACTH cell adenoma, densely granulated	22	1.1
TSH cell adenoma	18	0.9
Plurihormonal adenoma	36	1.8
Unclassified adenoma	33	1.6
Sum	2011	100

TSH cell adenoma

TSH cell adenomas are not frequent (1.5% in our collection; Table 4). By far, most of the adenomas of this type are invasive macroadenomas (69, 70). Light microscopy reveals sinusoidal growth pattern. The cells are medium-sized and chromophobic. They may harbor globular PAS-positive inclusions representing lysosomes. Immunostaining for TSH is varying, mostly slight, but may be negative (71), although others found a conclusive immunoreactivity for TSH (40). The ultrastructure is characterized by distinct similarities to normal TSH cells with often prominent rough endoplasmic reticulum, globoid Golgi complexes, and sparse small (150–250 nm), rod-shaped or spherical or irregular secretory granules (71). TSH cell adenomas are hyperfunctioning for TSH in about 42% of patients, but may also be found in euthyroid patients. They can develop in patients with long-standing hypothyroidism and may be accompanied and/or preceded by TSH cell hyperplasia (72). High levels of TSH and peripheral thyroid hormones in these patients must be differentiated from inappropriate secretion of TSH due to peripheral resistance to thyroid hormones.

Non-functioning pituitary adenomas

Patients with non-functioning pituitary tumors have no obvious symptoms related to a hormone excess except for mild hyperprolactinemia in some cases. The latter is thought to result from compression of the pituitary stalk by the tumor, leading to interference with the PRL-inhibiting dopamine transport. Due to the absence of hormone-related symptoms, non-functioning adenomas are usually large at the time of diagnosis. However, with an increasing frequency of radiological evaluations of the CNS for unrelated reason, pituitary adenomas are detected more frequently than in the past. Most of these incidental findings are non-functioning adenomas (7). In our registry, 12.6% of all clinically inactive adenomas are silent adenomas, which are characterized by the insufficient secretion of active hormones into the circulation, despite their expression (Table 5). Most of these are ACTH cell adenomas (Table 5), which are described earlier. Next in frequency are plurihormonal adenomas, sparsely granulated PRL cell adenomas, TSH cell adenomas, and sparsely granulated GH cell adenomas (Table 5). In contrast to these, 87.4% of clinically inactive adenomas are adenomas of the null cell type or FSH/LH type. Importantly, other sellar masses should be considered in the differential diagnosis of these adenomas, as described later. FSH/LH adenomas are the most frequent non-functioning adenoma type representing 25.2% in our total collection (Table 4) and 43% of inactive adenomas (Table 5) respectively. They exhibit a mostly sinusoidal growth pattern, and may develop pseudorosettes and microfollicles. The cells are relatively large, partly cylindrical PAS-negative and chromophobic. Some very small PAS-positive granules may be demonstrable. Immunostaining varies in proportions and intensities. In most of these adenomas, both FSH and LH are demonstrable, with isolated expression of FSH second, and LH third. In addition, α-subunit may be expressed. Evaluation of the ultrastructure reveals that many adenomas are similar to oncocytic adenomas or null cell adenomas, whereas others show distinct similarities with normal gonadotrope cells. The rough endoplasmic reticulum may be dilated, but the structures of so-called castration cells are not found (73). Secretory granules are sparse and very small. FSH–LH cell adenomas of female patients often exhibit honeycomb Golgi fields with characteristic vacuolar transformations (52, 74), and these may also be found in ACTH cell adenomas (75). Elevated levels of gonadotropins in the blood circulation are found in approximately 25% of patients with non-functioning pituitary adenomas. However, the levels of LH and FSH in the blood circulation do not correlate with the expression of these hormones (76). Null cell adenomas represent 25.6% of our collection (Table 4) and 33.7% of all inactive adenomas respectively (Table 5). A sinusoidal or diffuse growth pattern is characteristic for this adenoma type. Some pseudorosettes may be present. The small- to medium-sized monomorphic cells are chromophobic. Generally, hormones are not demonstrable but focal staining (in not more than 5–10% of cells) for FSH, LH, or α-subunit may be found. In one series (77), occasional cells immunopositive for GH, PRL, and ACTH were additionally demonstrable in 18% of null cell adenomas. The variably strong expression of chromogranin A (78) can be used if difficulties in the differential diagnosis to lymphomas or meningiomas exist. The electronmicroscope (6, 52) reveals polyhedral small- to medium-sized cells with irregularly outlined nuclei and sparse organelles. Rough endoplasmic reticulum and Golgi fields are poorly developed. The secretory granules are small and spherical, and randomly distributed. The number of mitochondria varies and transitions to oncocytes are not rare but should be limited to less than 50% of adenoma cells (79, 80). Oncocytic adenomas are a variant of the null cell adenoma (WHO classification: null cell adenoma, oncocytic variant) and like the null cell adenomas are presumably derived from gonadotropic cells. In our collection, 5.8% of adenomas are such null cell adenomas of the oncocytic variant (Table 4). In the group of inactive adenomas, they represent 10.7% (Table 5). They show a diffuse or sinusoidal architecture and rarely pseudorosettes. The cells are medium-sized or large, weakly acidophilic or chromophobic. The nuclei may be rich in chromatin. Immunostainings for pituitary hormones are negative, although sparse FSH, LH, or α-subunit-positive cells may be demonstrable. Chromogranin A is expressed (78). Semithin sections of Epon-embedded specimens reveal a typical granular or cloudy structure basing on densely arranged mitochondria. In the electron microscope, by far most adenoma cells show many, in part densely arranged mitochondria. The cristae may be slightly pleomorphic. The rough endoplasmic reticulum is sparse and the Golgi areas are small. Secretory granules are sparse and of different size. In our collection, 14 adenomas (0.5%) express α-subunit only, but no complete pituitary hormones like FSH or LH, as determined by immunocytochemistry. They had been designated as α-subunit-only adenomas (81), but structurally they represent null cell adenomas and should be included in null cell adenoma type (Table 4).

Plurihormonal adenomas

With improved methods of immunostaining and the increased use of monoclonal antibodies, the incidence of plurihormonal adenomas is lower than expected from former studies with polyclonal antibodies (41). The plurihormonal adenoma type I corresponds light microscopically to densely granulated GH cell adenomas but expresses TSH, α-subunit, and often FSH, LH, or PRL, in addition to GH. Ultrastructurally (57), the tumor reveals structures of densely granulated GH cell adenomas, or plurimorphous structures with predomination of the GH cell type.

Table 6 Classification of atypical adenomas (surgical specimens of sellar region, 2005; *N*=12/451).

Adenoma type	Number
Atypical densely granulated GH cell adenoma	1
Atypical sparsely granulated GH cell adenoma	2
Atypical sparsely granulated prolactin cell adenoma	1
Atypical densely granulated ACTH cell adenoma	1
Atypical sparsely granulated ACTH cell adenoma	2
Atypical FSH/LH cell adenoma	1
Atypical null cell adenoma	3
Atypical plurihormonal adenoma	1
Sum	12

The plurihormonal adenoma type II is structurally very similar to gonadotrope adenomas but expresses either TSH or GH, or PRL, in addition to FSH, LH, and α-subunit. Evaluation of the ultrastructure reveals plurimorphism (57). Another plurihormonal adenoma is the silent subtype 3 adenoma (9, 74, 82, 83) being immunoreactive not only for GH, PRL, and TSH but also for other hormones. In the electron microscope, this adenoma type shows characteristics of glycoprotein hormone-producing adenomas. The broad cytoplasm contains high amounts of rough and smooth endoplasmic reticulum and distinctly large Golgi fields. The sparse 100–200 nm secretory granules often accumulate in cytoplasmic processes (9). This unusual tumor exhibits aggressive behavior and poor prognosis due to its highly infiltrative growth, rapid progression, and high recurrence rate (9).

Atypical adenomas

In the WHO classification of 2004 (35, 36, 84), a new adenoma entity was included designating a borderline or uncertain behavior. Although this may also be true for the subtype 3 adenoma, the atypical adenoma was defined as an invasive tumor with elevated mitotic index, an MiB-1 labeling index $>3\%$, and an extensive nuclear immunostaining for p53. Brain invasion is not an agreed upon criterion of malignancy. It differs from pituitary carcinoma only in the lack of metastases. Atypical adenomas account for 2.7% (12/451) tumors in our registry, as evaluated for 2005 (Table 6). Different types of atypical adenomas can be distinguished in our collection, corresponding to the 'typical' adenomas (Table 6). Most frequent atypical adenomas were sparsely granulated GH cell adenomas, sparsely granulated ACTH cell adenomas, and null cell adenomas, but for significant data of incidence the number is too small. Atypical adenomas were found to have a poorer prognosis due to decreased operability by a higher degree of invasiveness, larger size, and accelerated growth (9).

Pituitary adenomas in combination with neuronal choristoma (PANCH)

In rare cases (0.29% (14/4891) in our collection of the years 1991–2005), ganglion cell-containing tumorous lesions (gangliocytomas or neuronal choristoma) adjacent to a typical pituitary adenoma were demonstrable (Table 7). Often, these neuronal tumors are situated cap-like over the adenomas. The border between both tumors is mostly ill-defined. The neuronal lesions are composed of various numbers of ganglion cells. These are large and mature and contain abundant cytoplasm and Nissl granules. They can be immunostained for synaptophysin, neuron specific enolase, and neurofilament. In acromegaly, GHRH is demonstrable (85, 86). In the very rare cases with ACTH hyperfunction, CRH was expressed (87). Some accompanying glial cells are demonstrable (86). The ultrastructure reveals slightly lobated nuclei and a broad cytoplasm with numerous microvesicles. The rough endoplasmic reticulum is sparse but partly dilated. Small- to medium-sized secretory granules are sparse. The mitochondria may be (86) numerous. Induction of adenoma formation by production of hypothalamic-releasing hormones in developmental lesions, i.e., neuronal choristomas has been discussed for the pathogenesis of PANCH (86–88). Alternatively, it has been suggested that the ganglionic lesions are the result of metaplasia from adenoma cells (89). Most cases of PANCH have demonstrated a benign behavior like ordinary adenomas (40).

Pituitary carcinomas

Pituitary carcinomas are defined by the demonstration of metastases (9). Whether or not extensive invasion of the brain by a pituitary tumor is a further criterion for carcinomas is a matter of controversy (9). Pituitary carcinomas are a very rare neoplasm (about 100 tumors published to date). Most are ACTH- or PRL-secreting tumors. GH-positive (90) or inactive tumors develop rarely into carcinomas (91). Most pituitary carcinomas develop from invasive relapsing adenomas. The tumor, its

Table 7 Intrasellar gangliocytomas in combination with pituitary adenomas (surgical specimens of sellar region, 1991–2005; *N*=4891).

Adenoma type	Gangliocytoma type	Hyperfunction	Number
Sparsely granulated GH cell adenoma	GHRH gangliocytoma	Acromegaly	7
Sparsely granulated mixed GH/prolactin cell adenoma	GHRH gangliocytoma	Acromegaly	4
Sparsely granulated prolactin cell adenoma	Gangliocytoma with prolactin expression	Hyperprolactinemia	2
Densely granulated ACTH cell adenoma	CRH gangliocytoma	Morbus Cushing	1

Table 8 Pituitary carcinomas (surgical specimens of sellar region, 1970–2005; *N*=7602).

Carcinoma type	Hyperfunction	Number
Sparsely granulated ACTH cell carcinoma	Morbus Cushing	7
Sparsely granulated prolactin cell carcinoma	Hyperprolactinemia	3
Densely granulated GH cell adenoma	Acromegaly, later inactive	1
Sparsely granulated GH cell adenoma	Acromegaly	1

connective tissues, and surrounding structures may be altered by previous surgery and/or radiation therapies in a way that enables tumor cells to invade vessels for metastatic spread. The light and electron microscopical structures of pituitary carcinomas are not much different from that of atypical adenomas. They show a higher index of Ki-67 and p53 protein, and a lower expression of p27 (92) in the primary tumor and in its metastases. Ras mutations can be found in PRL cell carcinomas (93). Increased PCNA index and c-erbB-2 membrane staining were demonstrated in sellar tumors and its metastasis (94). The structures of the metastases are mostly identical to the features of the primary tumor (91, 95). In our series of 7602 pituitary lesions since 1970, 12 carcinomas (Table 8) were collected. Seven could be classified as ACTH cell carcinomas, three as PRL cell carcinomas, and two as GH cell carcinomas (90, 91, 95).

The prognosis of pituitary carcinomas is generally poor, although patients with long-term survival have been described (96). Due to the small number of cases, comparative studies of different treatment options are lacking.

Differential diagnosis of pituitary adenomas

Pituitary adenomas have to be differentiated from other tumors of the sellar region (Table 1), especially from meningiomas, chordomas, craniopharyngiomas, gliomas, lymphomas, metastases, and germinomas. In most cases, the correct diagnosis is easily established using hematoxylin–eosin-stained sections, but in some tumors additional immunostaining may be necessary (97, 98). Synaptophysin and neuron-specific enolase are expressed in all types of pituitary adenomas but chromogranin A or keratins as important and common markers for endocrine epithelial tissue may be negative.

Conclusions

The classification of pituitary adenomas represents different types with specific characteristics. A large national registry such as ours includes sufficient numbers of samples, so that all types of pituitary adenomas including very rare entities are present in a significant number. Furthermore, other entities to consider for the differential diagnosis of sellar masses are entered. In the long term, such registries may allow further correlation of pathohistological characterization with clinical data and prognosis to improve the diagnosis and therapy even for rare types of pituitary adenomas (15). A national registry like our institution that is open to all pathologists and neurosurgeons to send specimens for consultant studies is therefore important in improving the patient's care and answering scientific questions.

Acknowledgements

This work was mainly financed by Novartis Pharma GmbH (Nuremberg), Novo Nordisk Pharma GmbH (Mainz), Pfizer Pharma GmbH (Karlsruhe), and Ipsen Pharma GmbH (Ettlingen).

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Received 25 July 2006
Accepted 2 November 2006