SPECIAL ARTICLE

Neuroendocrinology in 2011 ♠, ♠ ♠

El año 2011 en Neuroendocrinología

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Received 24 January 2012; accepted 25 January 2012
Available online 29 June 2012

Introduction

This article intends to summarize what happened in 2011 in the field of neuroendocrinology, by reviewing the most outstanding and clinically relevant advances reported in this area. Contributions presented at the main meetings held at the end of 2010 and during 2011, including the European Neuroendocrine Association (ENEA) congress in Liege, the European Congress of Endocrinology (ECE) in Rotterdam, The Pituitary Society and Endocrine Society (ENDO) meetings held in Boston, and some of the articles appearing in the most relevant journals of neuroendocrinology, have been summarized and briefly discussed. The information presented at these meetings and reported in the selected articles demonstrates that basic, clinical, and translational research in neuroendocrinology is in good health. Since a detailed and comprehensive list of all or most of the titles, authors, and main findings of such articles would be tedious and of little value, we have instead selected a limited number of articles based on the views of members of the neuroendocrinology group of SEEN who are experts in the different subject areas in which this review has been divided. The purpose of this is to call attention to the novelty and/or relevance of selected subjects. In addition, some specific lines of research have been chosen for more detailed coverage because of their particular interest.

Translational research

There are three lines of research from which no immediate clinical or therapeutic applications are anticipated, but which deserve specific attention because of their originality and potential relevance.

Magmas: A potential new marker/molecular target in pituitary tumors?*

It is well known that, with few exceptions, pituitary tumors are benign, with a local invasiveness rate ranging from 5% to 35% depending on the case. Although various genes in which changes may lead to the development and
Maternally expressed gene 3: An interesting gene that generates a non-coding ribonucleic acid\textsuperscript{2,3}

This research line stems from the relatively recent finding that all cell types of the normal human pituitary gland express high levels of maternally expressed gene 3 (MEG3), while such expression is selectively lost in NFPTs. MEG3 is a "sealed gene" or "imprinted gene" expressed in the maternal line only and located in the DLK1-MEG3 locus of chromosome 14q32, a region which it has been proposed houses a tumor suppressor gene. Interestingly, MEG3 transcription does not result in a protein, but generates a long non-coding RNA whose adequate folding is critical for its function. The uniqueness of this molecule, almost the only one described for its exclusive association with NFPTs, its relationship to other conditions and relevant molecules (e.g. tumor suppressor p53), and the possibility it provides for opening up new research lines make it a extremely attractive target. Although its underlying mechanisms are unknown, MEG3 is known to suppress cell growth in vitro and tumor formation in vivo, increases p53 protein levels, and selectively activates p53 target genes. In fact, its absence in knock-out animal models causes perinatal death. The loss of MEG3 expression is related to the hypermethylation of its promoter and an intergenic differentially methylated region (IG-DMR) on which the characteristic MEG3 imprint appears to depend. It has therefore been suggested that MEG3 represents a new non-coding RNA that acts as a tumor suppressor in the pituitary gland. In fact, the rescue of MEG3 expression in a cell line used as a NFPT model (PDFS cells) not naturally expressing MEG3 decreased the tumorigenic capacity of these cells in athymic mice. It is also striking that normal PDFS cells (without MEG) are also less tumorigenic when mice drink water with doxorubicin, a compound that stimulates MEG3 expression. More recent studies have evaluated expression of 24 genes of the DLK1-MEG3 locus, paternally or maternally expressed, in 44 human pituitary adenomas (25 NFPTs, and 7 ACTH-secreting, 7 GH-secreting, and 5 PRL-secreting tumors). It was shown that the expression of 18 genes from the DLK1-MEG3 locus, 13 of them microRNAs, was reduced in NFPTs. In fact, one of these microRNAs is able to induce cell cycle arrest in G2/M in PDFS cells. Thus, the information available on this subject suggests that the DLK1-MEG3 locus, and particularly MEG3, may act as a tumor suppressor in human NFPTs.

Cell senescence: A relevant mechanism in pituitary adenomas. Role of clusterin and pituitary tumor transforming gene

As noted above, one of the most conspicuous properties of pituitary adenomas is that, while they are often aggressive and recurrent, they are almost always benign tumors. Reports of pituitary carcinomas are very rare, and metastatic pituitary carcinomas are even less common. This observation has attracted the attention of different research groups which have studied the unique trophic mechanisms underlying this endogenous self-control of proliferation in this type of tumor. The Melmed and Chesnokova\textsuperscript{4,5} group in particular have provided valuable evidence concerning the participation of specific molecular elements in this phenomenon. In recent presentations and reviews, they have also provided an overview of the phenomenon which we will try and summarize below. For example, these authors discovered that human somatotrophinomas usually show aneuploidy, DNA damage, and elevated expression of the Cdk inhibitor p21, which according to the authors leads to self-restraint in cell proliferation and to cell senescence, both characteristics of these tumors. Similarly, mechanisms restricting the growth of NFPTs appear to exist. In fact, a study of 36 NFPTs of gonadotrophic origin showed that while DNA damage was found in all of them, p21 expression was undetectable. This difference from somatotrophinomas is also relevant considering the high p16 expression in these adenomas and the fact that more than 90% of them have a marked cytoplasmic expression of clusterin, a protein little known in this setting which is in turn associated with induction of the p15 inhibitor in 70% of NFPTs and 26% of somatotrophinomas. Studies on models of pituitary tumor cells (mouse LsT2 and aT3 gonadotrope cells) and on the aGSU transgenic mouse model, which overexpresses the pituitary tumor transforming gene (PTTG) in targeted form in gonadotrope cells, causing non-functioning adenomas, revealed that studied cells and tumors also overexpressed clusterin and showed signs of early cycle cell arrest and senescence induced by oncogenes. Thus, induction of C/EBP has been shown. These are in turn able to activate the clusterin promoter, which subsequently activates the expression of p15 and p16, causing cell proliferation arrest in mouse gonadotrope cells. By contrast, when clusterin expression is suppressed, gonadotrope cell proliferation increases. Interestingly, when 12 pituitary carcinomas were analyzed, these authors found that nine of them had no clusterin, which supports the idea that this protein may contribute to limiting cell proliferation in benign pituitary adenomas and suggests the existence of mechanisms controlling growth and proliferation which may be specific for the different cell types of adenohypophysis.
Acromegaly

While acromegaly is an uncommon disease, there have been an increasing number of publications and communications to congresses on this condition in recent years. This is due to advances in early diagnosis, which may minimize the impact of comorbidities associated with this disease, which are often present in many cases at diagnosis, and also to advances in medical treatment and improved surgical procedures, which allow for earlier disease control.

The most relevant 2011 studies have been grouped in different categories: “Registers”, “Comorbidities”, “Advances in treatment”, and “Predictors of treatment response”. Introduced as a search term in PubMed, “acromegaly” came up with 238 publications on this topic between October 1, 2010 and September 10, 2011. The most novel and/or significant of these were reviewed. In addition, the 37 posters presented at ECE 2011 and the 41 posters presented at ENDO 2011 were consulted, as well as the corresponding oral communication sessions.

Registers of acromegaly

In the past 10 years, the national registers of acromegaly of different countries, analyzing patient characteristics, diagnostic methods, therapeutic management, and follow-up have been reported. No new register has been reported in the last year, but it should be noted that relevant data on the characterization and management of acromegaly over 20 years in Vancouver were presented at ENDO 2011. Mention should also be made of the Fieffe et al. publication concerning the prevalence of diabetes in the French acromegaly register.9 Some studies published in the past year emphasize the need for acromegaly screening in patients with diabetes.7,8

Comorbidities

Acromegaly has high healthcare costs because of its comorbidities and the need for lifetime monitoring. A review covering the last 12 years has shown that control of the disease improves its clinical signs and symptoms and decreases healthcare expenses.9

Software which makes it possible to distinguish the facial features of a person with acromegaly from those of a healthy person has been developed. This may facilitate the diagnosis of acromegaly and by achieving an early or earlier diagnosis, decrease the prevalence of its comorbidities.10

A study of 84 patients with acromegaly followed up for seven years11 showed a high vertebral fracture rate, regardless of bone mineral density values. Another study also showed a higher prevalence of vertebral fractures in diabetic males with acromegaly. In active cases, prevalence is high regardless of the presence of diabetes mellitus.12

Among cardiovascular comorbidity studies, two deserve special mention. One of them showed that arterial stiffness, rather than the presence of atherosclerosis, as previously thought, could be another cardiovascular risk factor in these patients.13 A second neuroradiological study conducted on 152 patients reported that GH increase is related to an increased number of intracranial sacular aneurysms in the circle of Willi.14

There are two particularly interesting studies on cancer and acromegaly, the first of which showed that the presence of micronuclei in lymphocytes is a marker of genetic damage. Values are more than doubled in patients with acromegaly and correlate to IGF-1 levels. This could be an index of genetic instability due to the effect of IGF-1.15 In the second study, the breast density of women with acromegaly was analyzed. These patients have a higher breast density as compared to controls, which correlates positively to IGF-1 and disease duration. Breast density is an independent risk factor for breast cancer, and acromegaly may therefore be an additional risk factor for developing breast cancer.16

An oral communication and two posters presented at the Rotterdam ESE congress deserve special comment. The oral communication assessed the role of mastocytes and their relation to IGF-1. Mastocytes are involved in breast gland development and controlled by IGF-1, which is in turn locally secreted in breast tissue and has a permissive role for estrogen action at gland level.17 One of the posters reported that cured acromegalic patients continued to have arterial stiffness,18 and the other poster that treatment of acromegaly improved fertility in women: 30% became pregnant following neurosurgery and 70% during medical treatment.19 At the ENDO congress, two posters from a Spanish group showed that acromegalic patients have verbal and visual memory problems, and patients with active disease perform worse in memory tests than cured patients.20,21

Advances in treatment of acromegaly

The most novel publications include a meta-analysis of the role of cabergoline in acromegaly.22 This study had considerable limitations due to variability in the groups and in the treatments received, and concluded that cabergoline has a modest efficacy in acromegaly. The positive effect is also seen in patients with normal PRL levels.

A study where 32 patients with de novo acromegaly were treated with octreotide 20 mg/28 days for six months before surgery has been published. Biochemical reduction was achieved in one third of patients, and significant tumor reduction in two thirds of patients.23 Another study reported the use of combined treatment with somatostatin analogs and pegvisomant with good results and few side effects.24

The results of the ACROSTUDY, conducted on 1288 patients treated with pegvisomant and mainly intended to assess treatment safety, were reported at both ENDO and ESE. Liver function impairment was found in less than 1% of subjects and consisted of transaminase increase to three times the upper limit of normal. An increased adenoma size was reported in 30 patients, or 3.2%.25

As regards treatment with somatostatin analogs, new presentations of octreotide hydrogel implants have shown good safety and efficacy results, and an oral octreotide formulation has been used in mice.26 The trial conducted by Mercado et al. is also of interest. These authors discontinued treatment with analogs in patients who had been controlled for at least two years, a strategy similar to that used in prolactinoma, but leading to a high biochemical recurrence rate in acromegaly.27
Two posters about the use of temozolomide in acromegaly and the use of botulinus toxin in rat models, a potential future treatment, were also presented.

Predictors of treatment response

As in any other condition, multiple factors may influence the choice of a given treatment in acromegaly. Such factors include: (a) the tumoral pathology of acromegaly itself and its pathogenetic and evolutive characteristics; (b) individual patient variability due to genetic factors, associated diseases and comorbidities, concomitant medication, and treatment compliance; (c) pharmacological factors such as pharmacokinetics and pharmacodynamics, interactions, adverse reactions, and cost–benefit ratio; and (d) understanding of the condition, training, and experience of the prescribing physician, and restrictions on pharmaceutical expense. Somatostatin analogs continue to be the first drug treatment option due to their efficacy, their safety, and to the experience which has been accumulated concerning their use. The variability in response to somatostatin analogs may be explained by the heterogeneity of the reported series, patient preselection, and the diversity of GH and IGF-1 testing methods. However, it also depends on the pre-defined criteria of efficacy or control, and on the expression and functionality of the different receptor subtypes (SSTR 1–5) present in adenoma.

Various factors possibly accounting for the individual response to somatostatin analogs have been proposed, including: age and sex, pre-treatment GH level, GH and IGF-1 levels at 3 and 6 months, acute GH suppression test, tumor mass (debulking before drug treatment or not), prior radiotherapy, octreoscan, histopathology (densely granulated tumors), immunohistochemical detection of subtype 2 receptor (SSTR2), the presence of the gsp oncogene, treatment dosage and duration. Up to 25% of patients are considered to be resistant to somatostatin analogs, resistance being defined as a lack of both biochemical control and antitumor effect. Puig-Domingo et al. reported a new response predictor based on MRI. After surgery failure, adenomas with a hyperintense image in T2 show a poorer response to treatment. These data have also subsequently been confirmed by other authors and data presented at ECE. Finally, genetic factors may also influence sensitivity to GH and, therefore, the pharmacodynamics of specific drugs in acromegaly. Thus, Bernabeu et al. reported that carriers of a deletion of exon 3 of the GH receptor (d3-GHR) are more responsive to treatment with pegvisomant, requiring a 20% lower dose for IGF-1 normalization, and achieve this faster. A better response may therefore be predicted in such patients. These findings have not yet been verified by other authors, and further studies are needed to confirm them. Finally, an interesting study by Marazuela et al. reported factors associated with tumor size changes in acromegalic patients treated with pegvisomant.

Cushing’s syndrome

With regard to Cushing’s syndrome (CS), it should be noted that in 2011, in the era of molecular medicine, a multitude of data have been reported about clinical and therapeutic aspects of the disease, such as presentation forms, comorbidities, and mortality, with less attention being paid to the evaluation of different diagnostic tests, the main focus of scientific debate in this field in previous years. The advent of pasireotide and its indication for the treatment of Cushing’s disease has revived interest in medical treatment of the condition, which was previously considered a third line approach after surgery and radiotherapy. In addition to studies reporting results achieved with pasireotide, and with temozolomide in more aggressive tumors, other reports have shown a renewed interest in more classical drugs, which are now being used in combination.

Novel drug treatments

(A) Pasireotide: new data about its efficacy and symptom control in the phase III trial have been reported, showing significant reductions in systolic and diastolic blood pressure, LDL cholesterol, and weight. Quantitative data regarding quality of life improvement were first reported. Two studies in healthy volunteers were designed to ascertain the mechanisms responsible for hyperglycemia during pasireotide treatment and its response to different hypoglycemic treatments. The SOM230B2216 study showed that after an oral glucose tolerance test and hyperglycemic clamp, pasireotide increased blood glucose and decreased insulin, GLP-1, GIP, and glucagon levels. During the euglycemic hyperinsulinemic clamp, pasireotide was shown not to affect insulin sensitivity. In the SOM230 B2124 study, the area under the blood glucose curve at seven days showed mean reductions of 2%, 10%, 15%, and 29% when pasireotide was administered together with metformin, nateglinide, vildagliptin, and liraglutide respectively as compared to the administration of pasireotide alone. DPP-4 inhibitors and GLP-1 agonists were confirmed as being the most effective treatment for resolving hyperglycemia induced by pasireotide.

(B) Among the relevant publications, mention should be made of the Castillo et al. study on the efficacy of pasireotide in dogs, which reported new data regarding the mechanism of action of the drug and showed its efficacy for tumor size control.

(C) Mifepristone: mifepristone is a corticoid receptor antagonist investigated for the treatment of CS in a phase III trial, the results of which were reported at ENDO 2011. The trial included 50 patients with CS (43 of them with Cushing’s disease) who were divided into two groups based on the presence of T2DM/glucose intolerance (GI) (n = 29) or high blood pressure with or without T2DM/GI (n = 21). Mifepristone dosage ranged from 300 to 1200 mg/d, and treatment lasted 24 weeks. Response was defined as the achievement of a 25% reduction in the area under the glucose curve in an oral glucose tolerance test or a 5 mmHg reduction in diastolic BP. A 60% response was seen in the first group, with a significant decrease in HbA1c value (7.4 ± 1.5 to 6.4 ± 1.2; [p < 0.001]), while the response rate in the HBP group was 38%. Side effects required treatment discontinuation in seven patients. The most common side effects included gastrointestinal events, fatigue, joint pain,
headache, edema, decreased HDL, hypokalemia, and increased endometrial thickness. The use of mifepristone in CS refractory to other treatments appears to induce significant clinical and metabolic improvement with an acceptable risk-benefit profile.

(D) Temozolamide: the French multicenter study,\(^{44}\) concluded that temozolamide may be helpful for the treatment of aggressive pituitary adenoma or carcinoma, but that MGMT activity does not predict treatment response. In one of the ENDO symposia, Raverot et al. reviewed the role of temozolamide by analyzing the responses reported in the 40 cases to have been published. Fourteen of the reported tumors secreted ACTH (6 carcinomas). Hormone and tumor response were seen in 61% and 64% of cases, respectively. Temozolamide is the first chemotherapeutic agent to show activity, inducing a 60% response, although some investigators think that this result may be overestimated.\(^{45,46}\) Temozolamide should be proposed in patients with carcinoma and aggressive tumors refractory to standard treatments. A response is seen within the first three months, but initial response does not guarantee long-term control of the tumor.

(E) Combination treatments: a study used a combination of mitotane, metyrapone, and ketoconazole\(^ {47}\) in 11 patients with advanced, SCTH-dependent CS (four cases of Cushing’s disease and seven ectopic) to assess the efficacy and safety of this triple combination. Significant clinical improvement with rapid decrease in free urinary cortisol levels from \(\mu g/24\) h (853–22,605) to 50 \(\mu g/24\) h (18–298) \((p < 0.001)\) was seen at 24–48 h of treatment start, and this effect was maintained during follow-up. In seven patients in whom ketoconazole and metyrapone were discontinued at 3.5 months, free urinary cortisol levels continued to be controlled on mitotane alone. Mitotane was effective at plasma levels lower than those recommended for the treatment of adrenal carcinoma. Five patients (four with microadenoma and one with initially occult ectopic ACTH secretion) underwent surgery and showed postoperative remission. Four of them recovered adrenal function after mitotane discontinuation, which represents an additional advantage as compared to bilateral adrenalectomy. The most common side effects were gastrointestinal (63%), followed by hypokalemia and significant increases in LDL cholesterol and GGT levels. Liver toxicity required a reduction of the ketoconazole dose in two patients and the discontinuation of this agent in one. When immediate etiological treatment is not possible in ACTH-dependent CS due to disease severity, combined treatment with mitotane, metyrapone, and ketoconazole is an effective alternative to bilateral adrenalectomy.

Clinical indicators, morbidity and mortality, and subclinical Cushing’s syndrome

A considerable number of all studies on CS published or presented at meetings during the past year have focused on morbidity and mortality associated with active disease, as well as on residual morbidity in patients in remission after hormone normalization, and in subclinical CS (SCS) states.

(A) Clinical indicators: the results of an analysis of the European Register on Cushing’s Syndrome (ERCUSYN)\(^ {48-50}\) were presented and published this year. The analysis included 481 patients with CS from 23 countries who were divided into four etiological groups: CS of pituitary (66%), adrenal (27%), ectopic (5%), and other (2%) origins. A higher proportion of males were found in the ectopic as compared to the other groups, patients in the adrenal group were older than those in the pituitary group, and weight gain was more frequent in females compared to males. The prevalence of lumbar osteoporosis and the incidence of vertebral and rib fractures were higher in males compared to females. The scores recorded in quality of life tests did not differ between the groups. The ERCUSYN project showed a heterogeneous clinical presentation, depending on sex and etiology.

(B) Morbidity and mortality: as regards morbidity and mortality, Clayton et al. found in their 50-year follow-up study\(^ {51}\) of 60 patients with CS a two-fold greater overall mortality as compared to the general English population (13 deaths, nine of them from cardiovascular disease). However, patients in remission had a much lower mortality, with high blood pressure and DM being factors of poorer prognosis. Vitale et al.\(^ {52}\) reviewed morbidity and mortality in 66 patients seen at a Naples hospital from 1980 and 2010. Overall mortality was 12%, while death rates from pituitary and ectopic CS were 7.5% and 50% respectively. The most common causes of death were myocardial infarction, cardiac failure, renal failure, and sepsis, and the most frequent systemic complications included dyslipidemia, high blood pressure, coagulopathy, and GI. Pivonello et al.\(^ {53}\) reported similar results and conclusions based on 5096 patients from centers worldwide. These studies suggest that careful monitoring is required of situations reported to predispose to mortality in CS.

Thromboembolic events: with regard to coagulopathy in CS, Manetti et al.\(^ {54}\) reported in their prospective study of 40 patients that they had a hypercoagulative phenotype as compared to healthy subjects, which was more marked in those not cured after surgery than in those in remission. Stuijver et al.\(^ {55}\) analyzed the incidence of venous thrombosis (VT) in 473 patients with CS before treatment and after surgery. Patients undergoing surgery for NFPTs were selected as controls. The risk of postoperative VT was 0% in ACTH-independent and 3.4% in ACTH-dependent tumors. A greater risk of VT therefore exists in CS, particularly in active disease and after surgery, and guidelines for thromboembolic prophylaxis are required.\(^ {56}\)

Changes in hippocampal volume and neurocognitive involvement: verbal and visual memory and hippocampal volumes were assessed by Remini et al.\(^ {57,58}\) in 33 patients with CS and 34 healthy subjects. Patients with CS had poorer results in REY5, Retention Index, Total Recall Scores, and Recognition-A and B, and delayed visual memory as compared to controls. Decreased hippocampal volumes were detected in the 16 patients
with pathologically low memory scores. Toffanin et al.\textsuperscript{59} reported a significant and selective increase in hippocampal volume 12 months after transsphenoidal surgery in eight women with Cushing's disease. Both studies reported the effects of endogenous hypercortisolism on the hippocampus and the best methods for detecting them. 

Quality of life: although van der Pas et al.\textsuperscript{60} detected improvements in quality of life tests in patients with Cushing's disease following biochemical remission, Tiemensma et al.\textsuperscript{61,62} and Wagenmakers et al.\textsuperscript{63} found that patients who had experienced CS had poorer quality of life than the general population even after long remission periods, regardless of CS etiology, type of treatment used, and residual hormone deficiencies, suggesting that sequelae from long-term exposure to severe hypercortisolism may be irreversible in some cases.

(C) Subclinical CS (SCS): in SCS, Eller-Vainicher et al.\textsuperscript{64} showed that the presence of two or more of the criteria of free urinary cortisol > 70 \( \mu \)g/24h, ACTH < 10 pg/mL, and plasma cortisol > 3.0 \( \mu \)g/dl after an overnight 1-mg dexamethasone suppression test was the best predictor for the improvement of various metabolic parameters following the removal of adrenal incidentaloma. In the first longitudinal study published in this field, Morelli et al.\textsuperscript{65} reported that SCS in adrenal incidentaloma is associated with an increased risk of vertebral fractures and progressive bone quality impairment.

Prolactinoma

A majority of studies published or presented at meetings in 2011 concerning prolactinoma were focused on aspects related to morbidity, the safety of dopamine agonists, and novel treatments.

Morbidity

(A) Risk of cancer in patients with hyperprolactinemia: based on the results of several experimental studies, it has been suggested that PRL may play a role in the tumorigenesis of several cancers, particularly breast cancer and prostate cancer. However, few data were available about the risk of cancer in patients with hyperprolactinemia. This year, Berinder et al.\textsuperscript{66} reported a study assessing the risk of cancer in a cohort of 969 patients with hyperprolactinemia as compared to a control group of 9618 subjects. These authors used the Swedish Cancer Register to collect information regarding the occurrence of cancer in these patients. They found an increased risk of cancer in patients with hyperprolactinemia (HR = 1.31). This was mainly due to the increased risks of upper gastrointestinal cancer in both sexes (HR = 3.69) and hematopoietic tumors in females (HR = 3.51). No increased risk of breast cancer was found in women (HR = 1.09) and, contrary to what had previously been suggested, a lower risk of prostate cancer was reported in men (HR = 0.40). One of the study limitations was that no adjustment could be made for confounding factors such as family history of breast cancer, smoking, alcohol consumption, or body mass index.

The results of this study stress the importance of the monitoring and treatment of these patients.

(B) Vertebral fractures: hyperprolactinemia may cause bone loss. However, few data are available regarding vertebral fractures in these patients. In a recent report, Mazzotti et al.\textsuperscript{67} assessed the prevalence of radiographic vertebral fractures in 78 women with prolactinoma and 156 healthy controls. Hyperprolactinemia was shown to be associated with a high prevalence of vertebral fractures, especially in postmenopausal women and in those with untreated hyperprolactinemia.

(C) Risk of hypercoagulability: the association between hyperprolactinemia and platelet aggregation is already known, but no data are available about coagulation changes. Erem et al.\textsuperscript{68} examined the relationship between PRL and various hemostatic parameters in 22 patients with recently diagnosed prolactinoma as compared to 20 healthy controls. Patients with prolactinoma showed increases in platelet count, fibrinogen, and PAI-1, and decreased TFPI levels. This hypercoagulability state may increase the risk of atherosclerosis and atherothrombotic complications.

Treatment

(A) Safety of treatment with dopamine agonists: few data are available in the literature regarding the safety of cabergoline during pregnancy and its effects on fetal development. A study by Staldercker et al.\textsuperscript{69} analyzed the potential adverse effects of treatment with cabergoline during the first trimester of pregnancy in 103 pregnancies of 90 women. No significant differences were found in frequency of miscarriage, preterm delivery, and neonatal malformations as compared to other studies and to the general population. They did find two developmental abnormalities in these children, epilepsy and generalized developmental disorder, but these are unlikely to be related to doses (very low) or to exposure time (short) to cabergoline. In any case, studies with larger series are needed to establish the safety of cabergoline during pregnancy and the subsequent development of these children.

(B) The reported association of valve disease and chronic cabergoline treatment in patients with hyperprolactinemia is uncertain, but no new data are available to confirm that the use of low doses, as given in prolactinoma, represents a clinically relevant problem. Gu et al.\textsuperscript{70} recently investigated whether echocardiographer expectations could influence the result. For this, they studied 40 patients with prolactinoma on cabergoline treatment (mean duration, nine years). Echocardiograms were randomly assigned to two groups of echocardiographers: those in group A were told "'patients were controls'", while those in group B were told "'patients were being treated with dopamine agonists, which may cause valve disease'". More cases of trivial regurgitation and valve thickening were reported by group B as compared to group A echocardiographers. There was a single case of moderate regurgitation associated with valve restriction, consistent with the effects of cabergoline. Thus,
long-term, low-dose cabergoline treatment rarely causes valve disease, but echocardiographer bias may lead to the findings of valve thickening and trivial regurgitation being overestimated. In a recent review, Colao et al.,71 in agreement with the above information, recommended the re-analysis of all studies associating cabergoline with valve disease with the participation of cardiologists blinded to the identity of patients and controls.

(C) Novel treatments: temozolamide may be helpful for the treatment of aggressive prolactinoma or carcinoma, but the efficacy margins are rather wide.72 New therapeutic targets for the treatment of prolactinoma, such as estrogen receptor alpha73 or epidermal growth factor receptor 2 (HER2)/ErbB2 are also being investigated.74

**Pituitary cancer**

Pituitary cancer in uncommon and has a dreadful prognosis. Little is known about its molecular pathogenesis and optimal treatment. The etiology of pituitary carcinoma is unknown, but they are thought to be most likely derived from initially benign tumors. Transformation may be induced by various molecular processes: chromosome aberrations, oncogenes, or changes in tumor suppressor genes. The mean time from the diagnosis of adenoma to its transformation into carcinoma is approximately seven years. The most significant prognostic markers for pituitary carcinoma include the measurement of protein derived from p53 gene mutation and the Ki-67 cell proliferation index. The presence of p53 > 3% and/or Ki-67 > 3% in a pituitary adenoma suggests aggressiveness and should alert clinicians to the possibility of carcinoma.75

Early surgery continues to be the treatment of choice for pituitary tumors. Radiotherapy appears to control the local growth of lesions subsequently diagnosed as carcinoma. It is however of little value when administered on already known carcinoma. RT may have a palliative role, but does not improve disease prognosis.76 Dopamine agonists, even when used at high doses, are not usually helpful in malignant prolactinomas. No benefit has been seen either in carcinomas secreting GH, ACTH, or TSH. Both octreotide and lanreotide have achieved variable results in carcinomas secreting GH, ACTH, and TSH, but in no case have they been able to control tumor growth. There has been no clinical experience with the use of pegvisomant in GH-secreting carcinomas, but it could normalize IGF-1 levels. No experience is available with pasireotide either. Several chemotherapy protocols have been used in pituitary carcinomas. They usually have little effect on tumor size and secretion, and only a minority of patients achieve a transient stabilization of their clinical situation.

Temozolamide is an alkylating agent which is especially useful in slowly growing tumors. They have successfully been used in some pituitary carcinomas, but their potential use should be validated in the future using multicenter, controlled, randomized studies. Treatment response appears to be inversely related to MGMT (O6-methylguanine-DNA methyltransferase) levels, which may be measured by immunohistochrometry, although this is controversial due to the inconsistent results of the few studies conducted to date.77,78

**Genetic study of pituitary adenomas**

The monoclonality and familial predisposition found in pituitary adenomas suggest the existence of underlying genetic factors, but the molecular processes implicated are unknown. It is not known whether genetic study should be performed in apparently sporadic pituitary adenomas. Approximately 1300 different mutations of the MEN1 (menin) tumor suppressor gene, located in chromosome 11q13, have been reported. Despite this, in 20–30% of cases of MEN1 no menin mutations are found, but the genetic change could be located in another chromosome.

Rats with MENX syndrome have a CDKN1B duplication of eight base pairs in exon 2 (G177fs) that modifies the mRNA responsible for transcription of protein p27 and have a phenotype similar to patients with MEN1 and MEN2. It is not known whether this mutation in humans predisposes to neuroendocrine tumors, but it has already been diagnosed in patients with MEN1, in families with neuroendocrine tumors, and in “isolated” primary hyperparathyroidism. The significance of the MENX model lies in the fact that it could be used in clinical trials aimed at achieving adequate treatments for neuroendocrine tumors.

The p27-dependent mechanism of tumorigenesis is unknown. p27 could alter stem cells, which would subsequently be responsible for cell proliferation and immaturity. It was thought that the PTTG gene could initially act as an oncogene, subsequently permitting the action of p27. However, a recent study conducted by Chen et al.79 has suggested that overexposure of PTTG mRNA present in pituitary adenomas is an epigenetic mechanism, rather than an essential factor in pituitary tumorigenesis.

Mutations in the protein regulating gene acting upon the aryl-hydrocarbon receptor (AIP) have been detected in 15–40% of familial isolated pituitary adenomas (FIPAs) and in sporadic adenomas without familial aggregation. When they are present, syndrome penetrance appears to be greater. Although treatment options are similar in sporadic or familial pituitary adenomas, it is important to recognize the latter because they usually occur in younger people and tend to be more aggressive, particularly FIPAs with AIP mutation. We do not have adequate information yet about who should be subject to genetic screening and what method should be used, but it appears very reasonable to search for AIP mutations in relatives (at least first degree relatives) of patients with FIPAs and in patients with pituitary adenomas which are apparently sporadic but aggressive and diagnosed before 30 years of age. If an AIP mutation was detected, biochemical monitoring could be performed and at least one pituitary MRI could be done on a regular basis. However, the optimal frequency of testing, the cost-effectiveness of such studies, and the risk of the development of pituitary adenomas in asymptomatic carriers are unknown.80-83

**Pituitary incidentalomas and non-functioning tumors**

Pituitary incidentaloma is a previously unsuspected lesion which is discovered when an imaging test is performed for another reason. Most of them are adenomas, and the highest percentage of them are non-functioning tumors (NFTs).
The diagnosis of these unsuspected lesions poses a number of questions to patients and endocrinologists that require answers which are based on documented information.

The prevalence of pituitary incidentaloma (10% in radiographic studies) is higher than the prevalence of pituitary adenoma reported in cross-sectional population studies (78–94/100,000), which raises the question of how many of them will cause problems. The new clinical guidelines of the ENDO for the diagnosis and treatment of pituitary incidentaloma and two meta-analyses of the natural history and surgical results of NFPTs have attempted to answer this question.84-86

The ENDO guidelines recommend: (1) initial evaluation including complete history and clinical examination, pituitary MRI if not previously available, hormone testing to rule out hypersecretion and hypofunction, and visual field examination if the lesion is in contact with or close to the chiasma; (2) surgery if there are carcinomatous defects, opthalmoplexy, or neurological compromise; proximity to chiasma or optic pathways; or pituitary apoplexy with visual involvement; (3) clinical monitoring of patients not meeting surgical criteria, including MRI and hormone and visual field evaluation, for lesions close to the chiasma at six months, and annually thereafter for macroadenomas. For microadenomas, MRI is advised at one year and less frequently thereafter.

In meta-analyses of NFPTs,85,86 the available reports reviewed provide a low quality of scientific evidence (retrospective series with heterogeneous groups and follow-up shorter than five years). However, they do reflect an extensive clinical experience and make it possible to draw some helpful conclusions for clinical practice. Most NFPTs cause few complications (growth, pituitary apoplexy, visual or hormone deficiencies) during their natural course. Complications are more common in bigger (>10 mm) and solid lesions. Surgery is associated with few complications and improves problems with vision, but not hormone deficiencies.87 Postoperative radiotherapy prevents recurrence in tumors that have not been completely removed.88,89

A prospective study in a pediatric population with craniopharyngioma associated a decreased risk of postoperative recurrence with total resection preserving hypothalamic structures or incomplete resection followed by adjuvant radiotherapy. Treatment with GH does not increase the risk of relapse.92,93 Increased mortality in adults with craniopharyngioma appears to be associated with the presence of hydrocephalus before treatment, comorbidities such as obesity, diabetes mellitus, HBP, obstructive sleep apnea syndrome, and diabetes insipidus associated with adipsia. No relationship was found with GH deficiency or replacement therapy.94

Hypophysisitis is a sellar lesion which is difficult to diagnose before surgery and its confirmation may eventually require histological study.95 IgG4-related hypophysitis has recently been described, and a number of criteria for its clinical diagnosis have been established. This would avoid the need for transsphenoidal surgery, as this condition responds to glucocorticoid treatment.96

Thyroid-secreting pituitary adenomas (TSH-omas) are also part of this group because of their rarity (less than 2% of pituitary adenomas) and their difficult diagnosis. The diagnosis of TSH-oma has improved in recent decades due to the use of ultrasensitive methods for TSH measurement, which allows for its detection at earlier stages.97 TSH-omas usually show an excellent response to treatment with somatostatin analogs in terms of both the restoration of euthyroidism and tumor volume.98 Both these circumstances have improved both surgical results and the prognosis of these lesions. It appears that a high ratio of SSTR5/SSTR2 receptors in these tumors may predict for a favorable response to somatostatin analogs. TSH hypersecretion could be related to aberrant expression of thyroxine receptor β isoform.99

New insights on the etiology of hypopituitarism and associated morbidity and mortality

The incidence of hypopituitarism, defined as a deficiency of one or more hormones of the anterior or posterior pituitary gland, has increased in the past decade. This is probably due, at least partly, to the fact that pituitary function is now being investigated in more pathological conditions than before. In addition, the publication of long-term follow-up studies of hypopituitarism on hormone replacement therapy has increased our understanding of the benefits of such treatment on morbidity and mortality in this disease.

Pituitary tumors, mainly non-functioning tumors, and their treatment continue to be the main cause of hypopituitarism, but new cases related to subarachnoid hemorrhage, head trauma,100–102 or radiotherapy for pituitary103 and non-pituitary tumors are now known to occur; these latter cases are recognized more often than the previous ones. All of this has led to an increase in the annual number of new cases, which is higher than that seen in the 1990s. On the other hand, mutations in different genes,104–107 autoimmune mechanisms,108 or perinatal complications often underlie idiopathic hypopituitarism.

The prevalence of metabolic syndrome and cardiovascular risk indices, as well as cardiovascular and cerebrovascular mortality, are increased in hypopituitarism.109
There is also an increased susceptibility to respiratory tract infections. The cause of hypopituitarism may influence vascular risk. Thus, the risks of metabolic syndrome and vascular disease remain high in successfully treated Cushing’s disease. On the other hand, radiotherapy may damage small vessels, thereby contributing to an increase in cerebrovascular risk. Replacement doses of hydrocortisone, levothyroxine, and sex hormones, difficult to monitor in hypopituitarism, may also increase vascular risk. GH replacement therapy may contribute to decreasing this risk because it improves body composition and lipid profile, modulates endothelial function, and improves cardiovascular risk indices. In long-term studies, treatment with GH was not associated with an increased incidence of diabetes. It was also not associated with an increased recurrence of pituitary disease or new neoplasms. However, these studies have not yet led to the obtaining of data regarding the impact of GH treatment on cardiovascular mortality.

**Neuroendocrine tumors**

The incidence of neuroendocrine tumors (NETs), while low, has multiplied in recent years, especially due to advances in imaging and endoscopic techniques, which allow for earlier detection. Despite this, diagnosis is made in most cases when the disease is already widespread. While the great novelty in this regard in 2010 was the new WHO classification, the most significant news in 2011 was the publication of encouraging results of clinical trials with sunitinib and everolimus in patients with advanced pancreatic NETs and carcinoid syndrome. The RADIANT-2 and 3 studies with everolimus showed longer progression-free survival, approximately six months, in groups with active treatment as compared to placebo. Similar results were reported in the sunitinib trial. In all three trials, most adverse effects were of grade 1–2 and were bearable on dose reduction.

Among the novelties related to NETs, advances in radionuclide therapy reported in studies by the Dutch group of the Erasmus Medical Center in Rotterdam should be stressed. Lu-DOTA-octreotate was used in 265 patients with advanced gastrointestinal or bronchial NETs. Progression-free survival was 40 months, with partial responses in 28% of patients and stabilization of progression in 50%. For the first time, a quality of life questionnaire was included. Patients improved in the social, emotional, insomnia, and anorexia domains, especially those with tumor reduction. The same group reported that treatment with labeled somatostatin analogs decreases severe hypoglycemic episodes in malignant insulinomas.

Several new trials are ongoing of everolimus associated with somatostatin analogs, tyrosine kinase inhibitors, antiangiogenic agents, or chemotherapeutic drugs such as temozolamide, which combined with aggressive surgery of primary tumor and metastases (resection, embolization, and chemoembolization, amongst others) or radionuclide treatment will probably improve the survival of patients with NETs in the longer term.

As regards news relating to pheochromocytoma and paraganglioma (PP), mention should be made of the dissemination in 2011 of the results of a study by the Dresden group including 365 patients. Eisenhofer presented at ECE and ENDO relevant data regarding the possibility of using the secretory pattern of such tumors, by measuring free plasma metanephrines, to start the genetic study of one or the other mutation. Patients with PP associated with hereditary syndromes have increases in different metanephrines depending on the syndrome, and particularly in methoxytyramine levels in paragangliomas with SDHB or SDHD mutations. As regards the morphological diagnosis of PP, the Timmers et al. group at the NIH in Bethesda also presented at both meetings a large study on 216 patients with PP who were evaluated in order to determine their sensitivity and specificity for the localization and staging of several diagnostic tests: 18F-FDG-PET, CT, MRI, and 123I-MIBG. In localized tumors, conventional techniques (CT/MRI) were more sensitive, while specificity was superior with PET as compared to MIBG. Uptake values in positron emission tomography which distinguished between normal and tumoral glands with 100% sensitivity and specificity were established.

**Novel treatments for neuroendocrine tumors**

Radical surgery of the primary tumor is the only curative treatment for NETs. However, since most NETs are diagnosed in an advanced stage, the effect of surgery is usually palliative, even if hepatic metastases are removed. The response of these tumors to drug treatment varies depending on their pathological characteristics. It is therefore very important to classify each tumor by its degree of differentiation (Ki-67 percentage, mitotic index) and dissemination stage in order to prescribe the most adequate treatment and achieve the best response while avoiding unnecessary toxicity.

Most NETs are well differentiated (low to intermediate degree of proliferation), which makes them poorly responsive to traditional cytotoxic chemotherapy. Thus, in well-differentiated NETs, cytotoxic chemotherapy should be reserved for phases where the tumor changes its clinical and biological behavior and accelerates its aggressiveness. Somatostatin analogs have been used for symptom control, and benefits have also been seen in terms of decreased progression due to their antiproliferative effect. As the vast majority of NETs show increased levels of growth factors and growth factor receptor expression, as well as dysregulation of post-receptor signaling pathways, drugs which act by inhibiting either these growth factors (monoclonal antibodies) or their receptors, or the signaling pathways of such factors or the m-TOR pathway have been designed. These drugs mainly cause stabilization or lack of progression, rather than tumor reduction.

Various Phase II and III studies with these drugs, either alone or in combination, have been started in recent years. The results of two Phase III studies (RADIANT-2 and 3) with everolimus (an m-TOR inhibitor) reported during 2011 are summarized below:

(a) RADIANT-2: this study recruited 429 patients with advanced NETs of a low to intermediate degree and symptoms attributed to carcinoid syndrome who had had radiographically documented tumor progression over the previous 12 months. The patients were
randomized to double-blind treatment with everolimus 10 mg/day plus octreotide LAR 30 mg/28 days (n = 216) or placebo + octreotide LAR 30 mg/28 days (n = 213). Mean progression-free survival was longer in the everolimus group (16.4 versus 11.3 months in the placebo group), but these differences did not reach statistical significance. The most commonly seen side effects included stomatitis, rash, fatigue, and diarrhea, and were mild to moderate in severity in most cases.\textsuperscript{115-117}

(b) RADIANT-3: this study recruited 410 patients with advanced pancreatic NETs of a low to intermediate degree who had had radiographically documented progression over the previous 12 months. The patients were randomized to double-blind treatment with everolimus 10 mg/day (n = 207) versus placebo (n = 210). Mean progression-free survival was 11 months in the everolimus group versus 4.6 months in the placebo group (a 6.4-month longer progression-free survival), which represents a 65% reduction in the estimated risk of progression of death. At 18 months, 34% and 9% of the patients were free of progression in the everolimus and placebo groups respectively. The most common adverse effects were mild to moderate in severity (grade 1 and 2) and included stomatitis (64% vs 17%), rash (49% vs 10%), diarrhea (34% vs 10%), fatigue (31% vs 14%), and infections (23% vs 6%). Discontinuation rates related to side effects were 13.3% and 2.0% in the active and placebo groups, respectively.\textsuperscript{118-120}

The results of a Phase III trial of treatment of NETs with sunitinib,\textsuperscript{121} a tyrosine kinase inhibitor of VEGFR and PDGFR, involved in angiogenesis and tumor cell proliferation, were also reported during 2011. In this study, 171 patients with advanced pancreatic NETs with radiographically documented progression over the previous 12 months were randomized to double-blind treatment with sunitinib 37.5 mg/day or placebo. The study was discontinued by an independent committee before the initially planned recruitment was completed because of evidence of differences between the groups. Mean progression-free survival was 11.4 months in the sunitinib group versus 5.5 months in the placebo group. Objective response rates were 9.3% versus 0% respectively. At study closure, there had been nine deaths (10%) in the sunitinib group and 21 (25%) in the placebo group. The most common adverse effects in the sunitinib group were diarrhea, nausea, vomiting, and asthenia.

Secretion pattern of pheochromocytomas/paragangliomas: Its value beyond biochemical diagnosis

Seventy-five per cent of pheochromocytomas/paragangliomas are sporadic. The remaining 25%, and up to 40% of cases starting in childhood, belong to various hereditary syndromes transmitted with an autosomal dominant pattern, such as multiple endocrine neoplasia type 2 (MEN 2A and MEN2B), von Hippel–Lindau disease, neurofibromatosis type 1(NF1), and familial paraganglioma–pheochromocytoma syndromes due to germline mutations in genes of subunits B–D of the mitochondrial enzyme succinate dehydrogenase and as components of Carney’s triad and Carney–Stratakis syndrome. At least 25% of patients with supposedly sporadic pheochromocytoma may have germline mutations in any of the genes that confer a susceptibility to the development of these tumors, even in the absence of a clear family history. However, the study of all of these genes is not considered to be indicated in all patients with pheochromocytoma or paraganglioma. A careful family history, the presence of other associated conditions in the patient or his/her relatives, and the biochemical pattern of catecholamine secretion may determine whether a genetic study should be made and what the most likely involvement is. Pheochromocytoma occurring in MEN2A syndrome and in neurofibromatosis type 1 is known to secrete both epinephrine and norepinephrine, while norepinephrine predominates in von Hippel–Lindau disease. All of them are virtually always adrenal, and a clear familial aggregation often exists. On the other hand, paragangliomas related to succinate dehydrogenase B mutations, which rarely have a positive familial history, secrete norepinephrine and often dopamine and have extra-adrenal localizations, in addition to a high prevalence of malignancy. Once the mutation is identified, a familial study should be done to identify those carriers amenable to monitoring.

Because of the variability in biochemical phenotype of these tumors, no parameter provides a 100% diagnostic accuracy. Assessment of O-methylated metabolites (metanephrine and normetanephrine) in 24-h urine or plasma is currently recommended. Plasma measurements should be taken after 30 min in a supine position. Because of the high sensitivity of free plasma metanephrines measured by enzyme immunoassay (>90%), a normal result makes it possible to rule out pheochromocytoma, except in cases of clear clinical suspicion or known genetic susceptibility. Some tumors, particularly those less differentiated, may predominantly secrete dopamine. Their detection therefore requires the measurement of dopamine, which shows a great variability and is difficult to interpret, or the measurement of its O-methylated metabolite, methoxytyramine. Neither of these measurements is included among the laboratory assessments normally performed in centers which are not highly specialized. The value of some plasma markers of neuroendocrine tumors such as chromogranin A or neuron-specific enolase has been postulated, but their usefulness in diagnosis or follow-up has yet to be established.

Recent studies have related different patterns of biochemical phenotype to certain mutations associated with familial paraganglioma syndrome. Since the investigation of several genes is often required to detect the molecular basis of the syndrome, Eisenhofer et al. tested\textsuperscript{124,125} plasma levels of metanephrines, normetanephrines, and methoxytyramine in 173 patients with the following diagnoses: 38 MEN2, 10 NF1, 66 von Hippel–Lindau, and 59 succinate dehydrogenase B or D gene mutations. All patients with MEN2 and NF1 had increased plasma metanephrine levels suggesting epinephrine production. Normetanephrine increases, reflecting norepinephrine secretion, were virtually the only findings seen in patients with von Hippel–Lindau disease. Increased levels of methoxytyramine, alone or associated with other metabolites, were found in 70% of patients with succinate
dehydrogenase B or D gene mutations. This biochemical pattern combining metanephrine and normetanephrine had a 99% value for discriminating MEN2 and NF1 from von Hippel-Lindau and succinate dehydrogenase B or D gene mutations. Methoxytyramine levels in turn made it possible to discriminate cases of succinate dehydrogenase B or D gene mutations from carriers of von Hippel-Lindau in an additional 78%. Thus, the incorporation of methoxytyramine testing into plasma naphrine measurements in selected cases may be a cost-effective tool prior to the search for the genetic cause in familial pheochromocytoma–paraganglioma syndrome. In addition, methoxytyramine may be the only useful biochemical marker in malignant pheochromocytomas/paragangliomas, or in apparently non-secreting paragangliomas associated with episodes of arterial hypertension.

Conflicts of interest

The authors state that they have no conflicts of interest.

Acknowledgements

We thank Novartis Oncology, and particularly Montserrat Gilabert, for their assistance in organizing the scientific meeting of the GNE of SEEN.

Translational research


Acromegaly


Cushing’s syndrome


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Prolactinoma

Pituitary carcinoma

Genetic study of pituitary adenomas

Pituitary incidentalomas and other pituitary tumors


Hypopituitarism


Neuroendocrine tumors


