



The Pituitary Society presents the

X 10th International Pituitary Congress

JUNE 5-7 2007

*Immediately following
the Endocrine Society's
2007 Meeting*

THE FAIRMONT CHICAGO



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PROGRAM AND ABSTRACTS

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Welcome

Dear Colleague,

Following a long-standing tradition, the Pituitary Society has reached a milestone - the Tenth International Pituitary Congress. As with previous Congresses, this one offers the opportunity for worldwide experts and fellows in training in Pituitary problems, ranging from basic molecular biology to clinical practice, to gather in a quiet atmosphere in order to hold an in-depth analysis of the most relevant Pituitary aspects. We hope you can take advantage of the program and site of the meeting to enhance interaction and free exchange of ideas with the other attendees.

We on the Program Organizing Committee have selected a group of speakers of international stature that are currently addressing the most relevant basic and clinical problems in the pituitary area. We have also continued the Hot Topic sessions as they were well received last year.

Finally we would like to thank all our sponsors for their continued support.

Welcome again to two days of excellent science and companionship.

The Program Organizing Committee



Marcello Bronstein (Co-chair)



Ezio Ghigo (Co-chair)

Nira Ben-Jonathan
Lawrence Frohman
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Gloria Tannenbaum
Michael Waters



Symposium Schedule

Arrival: Tuesday, June 5, 2007

3:00 - 10:00pm

Hospitality Welcome Desk and Registration

Day 1: Wednesday, June 6, 2007

7:30 - 8:30am

Continental Breakfast

8:30am

Opening

8:45 - 9:30am

PLENARY LECTURE 1

(Chairs: David L. Kleinberg, USA / Mary Lee Vance, USA)

Control of the onset of puberty

John Marshall (USA)

9:30 - 11:00am

SYMPOSIUM 1

Neuroendocrinology of aging

(Chairs: Steven W.J. Lamberts, The Netherlands / Rolf Gaillard, Switzerland)

Aging brain and neuroendocrine system

Roy G. Smith (USA)

Endocrine genetics of aging

Elisabeth F.C. Van Rossum (The Netherlands)

The gonadal axis and male aging

Frederick Wu (UK)

11:00 - 12noon

POSTER VIEWING/COFFEE BREAK

12:15 - 1:45pm

MEET THE PROFESSORS (LUNCH SEMINARS)

MP1 Pitfalls in diagnosis and treatment of Cushing's syndrome

Xavier Bertagna (France)

Giorgio Arnaldi (Italy)

MP2 Management of aggressive pituitary tumors

Ashley Grossman (UK)

Michael Buchfelder (Germany)

MP3 GH replacement therapy adolescence and adulthood

Ken Ho (Australia)

Paul Saenger (USA)

MP4 Acromegaly: What's new in diagnosis and treatment

John A.H. Wass (UK)

Ariel L. Barkan (USA)

MP5 Craniopharingioma

Edward R. Laws (USA)

Niki Karavitaki (UK)

MP6 Imaging and diagnosis of pituitary masses

Nina Musolino (Brazil)

Kalmon Post (USA)

MP7 New technology in lab

Nelson Horseman (USA)

Ameae Walker (USA)

MP8 Bring on your difficult clinical cases

William Chandler (USA)

Brooke Swearingen (USA)

Francesco Cavagnini (Italy)

Ilan Shimon (Israel)

2:00 - 3:30pm

SYMPOSIUM 2

GH and PRL signalling

(Chairs: Vincent Goffin, France / Michael J. Waters, New Zealand)

Role of STAT5 in PRL feedback

David R. Grattan (New Zealand)

Multiple gene transcription mechanisms mediate GH action

Jessica Schwartz (USA)

Post-receptor signalling defects as a cause of short stature

Vivian Hwa (USA)

3:30 - 4:30pm

POSTERS/COFFEE BREAK

4:45 - 6:15

SYMPOSIUM 3

Neuroendocrine aspects of obesity and appetite

(Chairs: Aart J. van der Lely, The Netherlands / Cesar

Boguszewski, Brazil)

Hypothalamic circuitry and appetite control

Shane Hentges (USA)

The hypothalamic melanocortin system and the regulation of energy balance and neuroendocrine function

Sharon Wardlaw (USA)

Ghrelin action

Steven Grinspoon (USA)

Ghrelin and obestatin interplay

Matthias Tschoep (USA)

7:00 - 10:00pm

Rooftop Reception at the Mid-America Club



Day 2: Thursday, June 7, 2007

7:30 - 8:30

CONTINENTAL BREAKFAST

8:30 - 9:15am

PLENARY LECTURE 2

(Chairs: Davide Carvalho, Portugal / Anne Klibanski, USA)

Pituitary cell to cell communication

Eduardo Artz (Argentina)

9:15 - 9:45am

COFFEE BREAK/POSTERS

9:45 - 12:45pm

WORKSHOP

Approach to the patient with pituitary insufficiency

(Chairs: Marcello D. Bronstein, Brazil / Ezio Ghigo, Italy)

PATHOPHYSIOLOGY

Natural history of pituitary dysfunction after traumatic brain injury

Christopher Thompson (Ireland)

Non-functioning pituitary tumors

Yona Greenman (Israel)

DISCUSSANTS:

Gianluca Aimaretti (Italy), Vivien Bonert (USA), Felipe Casanueva (Spain), Andrea Giustina (Italy)

DIAGNOSIS

Assessment of HPA axis in patients with pituitary disease

Richard N. Clayton (UK)

General pituitary insufficiency

Mary Lee Vance (USA)

DISCUSSANTS:

Julio Abucham (Brazil), Mirtha Guitelman (Argentina), Vera Popovic (Serbia Montenegro), Christian Strasburger (Germany)

THERAPY

Corticoid steroid replacement

Paul Stewart (UK)

Androgen replacement in hypopituitarism

Karen Miller (USA)

GH replacement

Mark Hartman (USA)

DISCUSSANTS:

Beverly M.K. Biller (USA), Rudolf Fahlbusch (Germany), Monica Gadelha (Brazil)

1:00 - 2:45pm

HOT TOPICS - LUNCH SESSION

(Chairs: David Clemmons, USA / Lawrence Frohman, USA)

HT1: Identification and isolation of murine pituitary tumor progenitor cells

HT2: Evidence for a local natriuretic peptide system in pituitary GH3 somatotropes

HT3: Specific, siRNA mediated depletion of PTAG is associated with an attenuated apoptotic response in pituitary cells

HT4: Growth factor amplification by upregulation of epidermal growth factor substrate (Eps8) in human pituitary tumors

HT5: Molecular mechanism of glucocorticoid resistance in Cushing disease

HT6: Reduced sympathetic metabolites in urine of obese patients with Craniopharyngioma

3:00 - 4:30pm

SYMPOSIUM 4

New aspects in the regulation of HPA axis

(Chairs: Gunther Stalla, Germany / John Carmichael, USA)

Early life programming of the HPA axis

Stafford Lightman (UK)

Mineralocorticoid antagonists influence on HPA axis in humans

Emanuela Arvat (Italy)

Relevance of dopamine and somatostatin in the control of HPA axis

Maria Chiara Zatelli (Italy)

4:30 - 5:00pm

COFFEE BREAK / POSTERS

5:00 - 6:00pm

SYMPOSIUM 5

Roles for autocrine and paracrine hormone action

(Chairs: Nelson D. Horseman, USA / Nira Ben Jonathan, USA)

Growth hormone: autocrine/paracrine roles

Steve Harvey (Canada)

Role of prolactin in cancer

Nadine Binart (France)

6:00 - 7:30pm

Outstanding Young Investigator Presentation

Sponsored by an unrestricted educational grant from Eli Lilly and Company

AIP inhibits cell proliferation - genetic studies and the effects of mutations occurring in familial acromegaly

Márta Korbonits

Poster Prizes

Presidential Address

Hypophyseal artistic and social consequences of early induced hypogonadism

John Wass (UK)

Closing

8:00 - 9:00pm

COCKTAIL RECEPTION

9:00 - 12midnight

GALA DINNER



Social Events

HOSPITALITY WELCOME DESK

After you register, join your colleagues for a soft drink and snack to refresh yourself following your journey.

WEDNESDAY, JUNE 6TH

RECEPTION AT THE MID-AMERICA CLUB, OVERLOOKING CHICAGO SKYLINE ON THE 80TH FLOOR OF THE AON CENTER.

The Mid-America Club is a premier business and social dining club in the Chicagoland area. The Club provides unsurpassed service, world-class cuisine and sophisticated amenities topped off by unparalleled 360 degree panoramic views of the city. The opening reception will provide attendees with a large selection of passed hors d'oeuvres in addition to hot and cold appetizer stations. The meal and entertainment are included in the registration fee for the Congress.

THURSDAY, JUNE 7TH

GALA DINNER CELEBRATING THE MILESTONE OF THE 10TH YEAR OF THE INTERNATIONAL PITUITARY CONGRESS.

The dinner will be held in the International Ballroom of the Fairmont Chicago. This night promises to be festive and entertaining continuing our tradition of gala celebrations. Attendance is included in the registration fee for the Congress.

Certificate of Attendance

A certificate of attendance will be given to all attendees upon registration in Chicago.



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ABSTRACTS

Young Investigator Award

AIP inhibits cell proliferation - genetic studies and the effects of mutations occurring in familial acromegaly

Márta Korbonits¹, Chrysanthia Leontiou¹, Maria Gueorguiev¹, Jacqueline van der Spruy², Richard Quinton¹, Sevda Hassan³, Francesca Lolli¹, Suzanne Jordan¹, Gordon Peters⁴, Graeme Bolger⁵, Paul Chapple¹, Ashley Grossman¹, Lawrence Frohman⁶

¹Barts and the London medical School, London, United Kingdom, ²University of Newcastle, Newcastle, United Kingdom, ³Institute of Ophthalmology, London, United Kingdom, ⁴CRUK London Research Institute, London, United Kingdom, ⁵Comprehensive Cancer Center, University of Alabama, Birmingham, United States, ⁶University of Illinois at Chicago, Chicago, United States

We have previously suggested that the gene for familial isolated somatotrophinoma is located on 11q13 and mutations have been identified in some of the familial acromegaly kindreds in the AIP gene located in this region. We have identified five AIP mutations in 21 families with familial acromegaly. No mutations were identified in further screening of gDNA from 33 sporadic cases (including 5 giants) and cDNA from 49 sporadic pituitary adenomas. Double immunofluorescent staining and subsequent laser-scanning confocal microscopy identified that in normal pituitary AIP was expressed in all GH cells and a subset of prolactin cells but not in corticotroph, thyrotroph and gonadotroph cells. Cytoplasmic AIP immunostaining was observed in both familial and sporadic adenomas. In sporadic adenomas real-time RT-PCR, immunoblotting and immunostaining showed that, in addition to sporadic somatotroph and lactotroph adenomas, corticotrophinomas and non-functioning adenomas also expressed AIP; this is of particular interest as we did not detect AIP expression in normal corticotrophs and gonadotrophs. It has previously been suggested that AIP can function as a tumour-suppressor gene. To investigate this hypothesis we over-expressed the AIP protein in HEK293 cells by transfection with pCI-neo-AIP plasmid and measured proliferation. We observed a 46±5% reduction in proliferation in cells over-expressing AIP. Furthermore, infection of human fibroblasts with the pBABE-AIP retroviral vector resulted in arrested cell growth over the timescale of the experiment (Day 7:45±2%). A number of different AIP interacting proteins have been identified. To investigate the effects of the clinically occurring mutations we have identified on AIP protein-protein interactions we assessed their effect on the interaction between AIP and phosphodiesterase-4A5 using a beta-galactosidase filter two-hybrid assay. All of the mutations we studied prevented the interaction between AIP and phosphodiesterase-4A5. In summary, our data suggest that AIP regulates cell proliferation and the mutations we have identified disrupt some of AIP's functions. As the majority of familial acromegaly kindreds do not harbour a mutation in the coding region of AIP, we hypothesise that in addition to AIP and menin, there is a third gene, located in the 11q13 region that participates in the pathogenesis of pituitary adenomas.

ABSTRACTS
Hot Topics



HT1

Identification and isolation of murine pituitary tumor progenitor cells

Ines Donangelo, Shiri Gutman, Kolja Wawrowsky, Vera Chesnokova, Shlomo Melmed

Cedars-Sinai Research Institute, Los Angeles, CA, United States

Introduction: The capacity to sustain tumor formation and growth resides in a small proportion of tumor stem/progenitor cells. Here we report the identification and isolation of cells with progenitor cell characteristics in murine pituitary tumors. **Methods:** Pituitary tumor cells derived from Rb+/- and \pm GSU.PTTG \times RB+/- mice were cultured in a system that allows formation of nonadherent spheres. Expression of pituitary hormones, and stem cell markers in pituitary tumor spheres (PTS) was determined by immunocytochemistry, FACS and qRT-PCR. Proliferation was assessed by detecting BrdU incorporation in cells composing PTS. The differentiation potential of PTS was investigated by culturing PTS on adherent surface with GnRH or CRH. **Results:** A small fraction (\sim 0.001%) of plated cells formed PTS, which express stem cell markers Sca1 and Prominin1 (CD133), and are negative for pituitary hormones. PTS exhibit a relatively high expression of Notch1 and Prop1, and low expression of Lhx3 compared to their respective uncultured pituitary tumor cells. PTS exhibited self-renewal capacity, as demonstrated by ability of cells derived from dissociated spheres to grow into new spheres. BrdU incorporated into cells within PTS, indicating that they grow as a result of cell proliferation. PTS were dissociated and re-plated for up to 6 passages, and the total number of cells in culture (both PTS and surrounding aggregates) progressively decreased. However, the rate of sphere formation, i.e. the PTS number / plated pituitary tumor cell number ratio, increased with every passage suggesting that sphere-forming cells possess extended longevity compared to nonsphere-forming cells. Sca1^{pos} pituitary tumor cells, but not Sca1^{neg} cells, had the ability to form PTS, indicating that sphere-forming ability depends on the presence of Sca1. PTS plated with 1 μ M GnRH or 10nM CRH express immunoreactive LH or ACTH, respectively, indicating differentiation potential. **Discussion:** PTS exhibit characteristics of progenitor cells, notably self-renewal capacity and differentiation potential. Identification of pituitary tumor progenitor cells may be important for unravelling determinants of tumor formation and recurrence.

HT2

Evidence for a local natriuretic peptide system in pituitary GH3 somatotropes

Iain Thompson, Kim Jonas, Annisa Chand, Caroline Wheeler-Jones, Rob Fowkes

Royal Veterinary College, London, United Kingdom

The third member of the mammalian natriuretic peptide family, C-type natriuretic peptide (CNP), has previously been shown to be expressed at high tissue concentrations in the anterior pituitary. Recent evidence from CNP knock-out mice, or those lacking the CNP-specific receptor guanylyl cyclase-B (GC-B) suggest major roles for CNP in bone formation, female reproductive function and pituitary growth hormone secretion. We have used the rat somatotrope-derived GH3 cell line to examine the potential existence of a local pituitary natriuretic peptide system. RT-PCR analysis of GH3 cell cDNA's revealed expression of CNP, GC-B and GC-A (guanylyl cyclase-A), as well as the α -subunit of the soluble guanylyl cyclase. The presence of pharmacologically active GC-B receptors was confirmed by cGMP-enzymeimmunoassays, which revealed CNP dose-dependently caused a significant increase in cGMP accumulation (10⁻⁸ to 10⁻⁶M, P<0.05), and GC-B protein expression was confirmed by Western blotting. Interestingly, the nitric oxide donor sodium nitroprusside failed to alter cGMP accumulation (P>0.5), and RT-PCR analysis failed to demonstrate expression of the β -subunit of soluble guanylyl cyclase. Subcellular localization of CNP expression was performed by electronmicroscopy of rat and mouse anterior pituitaries, and revealed immunogold labelling for CNP over secretory granules of gonadotropes and somatotropes. To determine the physiological role of CNP in somatotropes, we examined the effects of CNP on cell proliferation and downstream signaling pathways in GH3 cells. CNP failed to significantly alter cell proliferation in GH3 cells, as determined by tritiated thymidine incorporation, MTT assay, flow cytometry or cell counting (P>0.5), but dramatically enhanced the phosphorylation status of MAPK-family proteins (ERK, JNK and p38-MAPK) and downstream effectors (p90-RSK), by a mechanism independent of cGMP-accumulation yet GC-B-dependent. These data reveal a potential autocrine feedback pathway exists in somatotrope-lineage cells, and describe for the first time a novel paradigm for GC-B signaling independent of cGMP generation. The biological implications for these findings are currently being elucidated.



HT3

Specific, siRNA mediated depletion of PTAG is associated with an attenuated apoptotic response in pituitary cells

Farsana Rowther, William E. Farrell

University of Keele, Stoke-on-Trent, United Kingdom

We recently identified a novel, pituitary derived pro-apoptotic mediator, pituitary tumour apoptosis gene (PTAG), and showed loss in a significant proportion of primary human pituitary tumours. Furthermore, enforced expression of PTAG, in the mouse corticotroph cell line, AtT20, significantly augmented bromocriptine induced apoptosis. In this investigation we employed a further pituitary cell line model, the rat somatotroph cell line, GH3, that expresses endogenous PTAG. Our studies were designed to determine if we could recapitulate the drug induced, augmented apoptotic response, apparent in AtT20 cells. In addition, this model allowed us to determine the consequences of siRNA mediated depletion of endogenous PTAG on apoptotic responses. Similar to our studies in AtT20 cells, enforced expression of PTAG in GH3 cells significantly augmented bromocriptine mediated apoptosis. In WT, GH3 cells, siRNA depletion of endogenous PTAG levels significantly attenuated bromocriptine induced apoptosis. In GH3 cells, expressing endogenous PTAG, bromocriptine challenge was associated with increased activation of the proximal caspases -8 and -9. To determine upstream/downstream effectors in this apoptotic response we investigated the role of p38 kinase and JNK kinase mediated pathways. No decrease in apoptosis was apparent in cells co-incubated with bromocriptine and a specific p38 inhibitor (SB202192). In contrast, inhibition of the JNK kinase pathway (SP600125) attenuated the drug-induced apoptotic response in these cells. These studies demonstrate the importance of PTAG in sensitising pituitary cells of different lineage to drug induced apoptosis. GH3 cells, expressing endogenous PTAG will be a useful model for further studies, where, depletion studies will allow investigation of other mediators in PTAG augmented responses. In contrast to other published data, we show, for the first time, that bromocriptine induced apoptosis is mediated through JNK kinase whereas the p38 pathway is without measurable effect.

HT4

Growth factor amplification by upregulation of epidermal growth factor substrate (Eps8) in human pituitary tumors

Mei Xu¹, Lynnette Shorts-Cary¹, Bette K-Demasters², Kevin Lillehei³, Margaret E Wierman⁴

¹Dept Medicine, UCDHSC, Aurora, CO, United States, ²Dept Pathology, UCDHSC, Aurora, CO, United States, ³Dept Neurosurgery, UCDHSC, Aurora, CO, United Kingdom, ⁴Research Service, VAMC, Denver, CO, United States

Growth factors and their receptors are thought to play a role in the molecular pathogenesis of human pituitary tumors. We hypothesized that downstream components of growth factor signaling pathways might also be differentially expressed in tumors compared to normal pituitary. RNA was isolated from pituitary tumors obtained at time of resection and normal pituitaries at time of autopsy (4-18h postmortem). Microarrays (Affymetrix Human Genome U133 Plus 2.0) were performed on 10 gonadotrope cell tumors and 9 normal pituitaries. Analysis was performed using pairwise comparisons (significant 2-fold changes). Eps8 was upregulated in the pituitary tumors compared to normal pituitaries (5.5-fold), with no changes in other Eps8 isoforms. This differential expression pattern of Eps8 was confirmed by RTPCR and immunoblot. Eps8 is a protein normally activated in response to growth factor signaling such as epidermal growth factor (EGF). In fibroblasts, Eps8 can bind to Sos-1 and Abi-1 to activate PI3K to modulate cell movement and mitogenesis. In pituitary tumors, Eps8 mRNA was upregulated in an ACTH tumor, and multiple prolactinomas, null cell and GH tumors, suggesting the overexpression was not gonadotrope specific. Eps8 was also expressed in immortalized mouse gonadotrope cell lines (alphaT3 and L_T2). Eps8-LBT2 cells stably overexpressing Eps8 proliferated more robustly (4-fold at 7d) than vector control cells in growth factor replete conditions, consistent with a putative role of Eps8 to augment growth factor signaling. Eps8 overexpression also protected cells from serum withdrawal induced apoptosis as assessed by caspase 3 cleavage. Eps8-LBT2 cells formed more colonies in a soft agar assay compared to vector controls (93 vs 8 at 21d). EGF signaling through ERK was augmented in the presence of Eps8 overexpression (25-fold), which was blocked in the presence of PD98059. In summary, Eps8 is a downstream component of EGF signaling that is overexpressed in human pituitary tumors compared to normal pituitary. Overexpression of Eps8 in gonadotrope cell lines promotes cell proliferation and antiapoptosis. Its ability to amplify endogenous growth factor signaling may promote or sustain pituitary tumorigenesis. (supported by VA Merit to MEW).

HT5

Molecular mechanism of glucocorticoid resistance in Cushing disease

Steve Bilodeau¹, Sophie Vallette-Kasic¹, Dominique Figarella-Branger², Thierry Brue², André Lacroix³, France Berthelet³, Dalia Batista⁴, Constantine Stratakis⁴, Jeanette Hanson⁵, Björn Meij⁵, Jacques Drouin¹

¹Laboratoire de génétique moléculaire, Institut de recherches cliniques de Montréal (IRCM), Montréal, Québec, Canada, ²Laboratoire Interactions cellulaires neuroendocriniennes (ICNE), Marseille, France, ³Department of Medicine, Research Center, Hotel-Dieu du Centre hospitalier de l'Université de Montréal (CHUM), Montréal, Québec, Canada, ⁴Section on Endocrinology and Genetics (SEGEN), Developmental Endocrinology Branch (DEB), National Institute of Child Health and Human Development (NICHD), Bethesda, Maryland, United States, ⁵Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands

Cushing's disease is characterized by POMC-expressing adenomas and the loss of glucocorticoid (Gc) negative feedback on the pituitary POMC gene. The mechanism of this Gc resistance remains elusive. Repression of POMC transcription by GR has been ascribed to a mechanism known as trans-repression that is also implicated in many anti-inflammatory actions of Gc. In corticotroph cells, trans-repression is exerted between GR and the orphan nuclear receptors NGFI-B, or two related factors Nurr1 and NOR1.

While investigating the mechanism of trans-repression between GR and NGFI-B, we found that Brg1, the catalytic subunit of the Swi/Snf complex, is an essential component in the trans-repression mechanism. In fact, Brg1 interacts with both GR and NGFI-B to stabilize a trans-repression complex. Thus, cells deficient for Brg1 do not support trans-repression between GR and NGFI-B. In the Brg1-containing complex, HDAC2 appears to be required for an effective trans-repression and HDAC inhibitors completely block Gc-mediated repression of POMC gene. Using co-immunoprecipitation and chromatin immunoprecipitation (ChIP), we have characterized the formation, organization and recruitment of this complex at the POMC promoter in vivo.

In view of the essential function of Brg1 and HDAC2 in Gc repression of POMC, we hypothesized that their deficiency may cause Gc resistance in Cushing's disease. Using a panel of human and dog corticotroph adenomas, we have indeed shown that near 50% of tumors have misexpression of either Brg1 and HDAC2; some showing very low/undetectable protein while others exhibit mislocalisation (cytoplasmic rather than normal nuclear expression). Aberrant expression of Brg1 and HDAC2 provides the first molecular explanation for hormone resistance in Cushing disease.

HT6

Reduced sympathetic metabolites in urine of obese patients with childhood craniopharyngioma

Hermann L. Muller¹, Ursel Gebhardt¹, Donald Hunneman², Christian Roth²

¹Dep. of Pediatrics, Klinikum Oldenburg gGmbH, Oldenburg, Germany, ²Dep. of Pediatrics, University Hospital Gottingen, Gottingen, Germany

In patients suffering from craniopharyngioma (cp), a tumor of low histological malignancy located in pituitary and hypothalamic regions, severe posttreatment obesity is a major problem. In the present study, we aimed to test the hypothesis that hypothalamic damage leads to reduction of the overall sympathetic tone and physical activity. We measured the major catecholamine metabolites, homovanillic acid (HVA) and vanillylmandelic acid (VMA), which arise from epinephrin and norepinephrin and their precursor dopamine, in morning voided urine of 93 patients with childhood craniopharyngioma. The results were compared to age-matched HVA and VMA values in urine of 900 pediatric control patients representing a usual hospital pediatric population who were proven not to have a catecholamine secreting tumor. Furthermore, the self-estimated daily physical activity of participating craniopharyngioma patients has been recorded using a questionnaire (scores from -2: activity much less, to +2: activity much more, compared to healthy controls of same age).

In craniopharyngioma patients suffering from obesity (BMI > 2SDS) HVA and VMA values as well activity scores were significantly lower compared to craniopharyngioma patients with normal BMI. Patients with hypothalamic involvement of craniopharyngioma (CP) had higher BMI values, lower HVA, VMA and activity scores versus patients without hypothalamic involvement (BMI-SDS: 4.3±0.4 vs 1.4±0.3; ratio HVAc_p/HVAc_{control} 0.77±0.06 vs 1.06±0.07; ratio VMAc_p/VMAc_{control} 0.81±0.06 vs 0.88±0.05; activity score -1.11±0.1 vs -0.21±0.15; mean±SEM, p<0.01 for all comparisons).

These findings support the hypothesis of disturbed sympato-adrenergic regulation leading to reduced physical activity and severe obesity in patients with craniopharyngioma especially in those with hypothalamic tumor involvement. A disturbed sympathetic tone should be considered in further studies investigating treatment options for hypothalamic obesity.



ABSTRACTS
Cellular & Molecular



P1

The third intracellular loop of human SST5 is crucial for receptor internalization after SS28 stimulation

Giovanna Mantovani¹, Erika Peverelli¹, Andrea Lania¹, Davide Calebiro², Sara Bondioni¹, Paolo Beck-Peccoz¹, Anna Spada¹

¹Dpt. of Medical Sciences, University of Milan, Endocrine Unit, Fondazione Policlinico IRCCS, Milan, Italy, ²Istituto Auxologico Italiano IRCCS, Milan, Italy

Somatostatin (SS) is a widely distributed polypeptide that exerts inhibitory effects on hormone secretion and cell proliferation by interacting with five different receptors (SST1-SST5), that display important differences in tissue distribution, coupling to second messengers, affinity for SS and intracellular trafficking. SS analogues currently used in the treatment of acromegaly inhibit hormone secretion and cell proliferation by binding to SST2 and 5. Beta-arrestins have been implicated in regulating SST internalization but the structural domains mediating this effect are largely unknown. The aim of this study was to characterize the intracellular mechanisms responsible for internalization of human SST5 in the rat pituitary cell line GH3. To this purpose we evaluated by fluorescence microscopy SS28-mediated trafficking of receptor fused to DsRed2 and beta arrestin-1 or -2 fused to EGFP. To identify the SST5 structural domains involved in these processes, we evaluated progressive C-terminal truncated proteins, SST5 mutants in which serine, threonine or acidic residues within the third cytoplasmic domain were mutated (S242A, T247A and E243A) and a naturally occurring R240W mutant in the third intracellular loop previously found in one acromegalic patient resistant to somatostatin analogues. We tested the ability of these mutants to associate with beta arrestin and to internalize under agonist stimulation. The truncated mutants are comparable to the wild-type receptor with respect to beta arrestin recruitment and internalization, whereas third cytoplasmic loop mutants show a significantly reduced internalization and arrestin translocation upon SS28 stimulation. Our results indicate SST5 third intracellular loop as an important mediator of beta arrestin/receptor interaction and receptor internalization, while the role of the C-terminal tail would be to sterically prevent beta-arrestin/receptor interaction in basal conditions.

P2

Global Analysis of Genes Silenced through Epigenetic Mechanisms in Pituitary Tumours

Kevin Dudley, William E. Farrell

University of Keele, Keele, North-Staffordshire, United Kingdom

DNA methylation of promoter CpG islands is an epigenetic modification that is associated with inappropriate gene silencing in multiple tumour types, including those of pituitary origin. The aim of this study was to identify genes that are silenced through DNA methylation in pituitary tumour development. To this end, siRNA-mediated knockdown of the maintenance methyltransferase Dnmt1 was employed, in the murine pituitary adenoma cell line AtT-20, followed by differential expression analysis. 75% knockdown of Dnmt1 was confirmed at the mRNA level by qRT-PCR, and this corresponded with loss of protein expression as determined by western blotting. Sustained knockdown over an eight-day incubation period induced the expression of the imprinted gene Neuronatin. Importantly, this re-expression was associated with demethylation of the Neuronatin CpG island, thus implying that the effects of Dnmt1 knockdown are through repression of DNA methylation. Subsequent global microarray analysis, of three independent experiments, comparing expression in Dnmt1 knockdown cells to equivalent cells treated with a non-targeting control siRNA, identified ~300 transcripts that were significantly differentially expressed following Dnmt1 knockdown ($P < 0.05$; false discovery rate 20 %). Additional filtering for transcripts that (1), displayed significantly increased expression in at least 2/3 Dnmt1 knockdown replicates, (2), were not expressed in cells treated with the control siRNA, and (3), contained consensus CpG islands, provided a list of 92 silenced genes in AtT-20 cells. For selected genes these findings were confirmed in independent qRT-PCR experiments. Interestingly, genes belonging to apoptosis and developmental biological processes were over-represented in our list ($P < 0.05$), and these were, therefore, taken forward for analysis in primary human pituitary adenomas. Importantly, a number of these genes appear to display reduced expression in tumours compared to normal pituitary, suggesting that our approach might be useful for identifying novel genes that are silenced by epigenetic mechanisms during pituitary tumourigenesis.



P3

Acylated and Unacylated Ghrelin Inhibit Oxygen-Glucose Deprivation-Induced Apoptosis of Cortical Neuronal Cells

Hyunju Chung, Sanghee Seo, Minho Moon, Seungjoon Park

Kyunghee University School of Medicine, Seoul, United States

Ghrelin is an endogenous ligand for the GHS-R1a, produced and secreted mainly from the stomach. Ghrelin stimulates appetite, adiposity and GH release through the activation of GHS-R1a. Only acylated form of ghrelin (AG) binds to GHS-R1a and has central endocrine activities. In contrast, the unacylated form of ghrelin (UAG), which does not bind to GHS-R1a and has no GH stimulating activity, is the most abundant form of ghrelin in plasma. Recently we have reported that ghrelin acts as a survival factor for hypothalamic neuronal cells that inhibits apoptotic pathways and its neuroprotective effect is mediated via the activation of GHS-R1a. However, anti-apoptotic effect of UAG in neuronal cells is still unknown. Therefore, we investigated the role of AG and UAG in ischemic neuronal injury using primary cortical neurons exposed to oxygen-glucose deprivation (OGD). Here we report that treatment of cortical neurons with AG and UAG inhibited OGD-induced cell death and apoptosis. Exposure of cells to the receptor specific antagonist D-Lys-3-GHRH-6 abolished the protective effect of AG against OGD insult, whereas that of UAG was preserved in the presence of the GHS-R1a antagonist. Both AG and UAG caused rapid activation of ERK1/2. Anti-apoptotic effect and stimulatory effect on ERK1/2 of AG and UAG were blocked by inhibition of MAPK and PI3K pathways. Both AG and UAG attenuated OGD-induced activation of c-Jun NH2-terminal kinase and p-38. In addition, cells treated with both AG and UAG showed an increased Bcl-2/Bax ratio, prevention of cytochrome c release, and inhibition of caspase-3 activation. Our data indicate that, independent of its acylation, ghrelin can function as a neuroprotective agent that inhibits apoptotic pathways. A novel, yet to be identified receptor, which is distinct from GHS-R1a, is likely involved in the survival mechanisms of UAG.

P4

Vascular Endothelial Growth Factor mediates the effects of pasireotide on cell viability in non functioning pituitary adenomas.

Maria Chiara Zatelli¹, Daniela Piccin¹, Federico Tagliati¹, Maria Rosaria Ambrosio¹, Antonio Bianchi², Marta Bondanelli¹, Herbert A. Schmid³, Massimo Scanarini⁴, Laura De Marinis², Giulio Maira⁵, Ettore C degli Uberti¹

¹Section of Endocrinology, Department of Biomedical Sciences and Advanced Therapies - University of Ferrara, Ferrara, Italy, ²Institute of Endocrinology, Catholic University of Rome, Rome, Italy, ³Novartis Institutes for Biomedical Research, Oncology, Basel, Switzerland, ⁴Division of Neurosurgery - Hospital of Padova, Padova, Italy, ⁵Department of Neurosurgery, Catholic University of Rome, Rome, Italy

Somatostatin (SRIF) analogs have demonstrated contrasting results in medical therapy of non-functioning pituitary adenomas (NFA). SRIF has been demonstrated to exert antiproliferative effects also by reducing Vascular Endothelial Growth Factor (VEGF) secretion and action. Moreover, VEGF expression may be related to pituitary tumor growth. The aim of our study was to clarify the possible effects of a multireceptor SRIF ligand, pasireotide, on VEGF secretion and cell proliferation in human NFA primary cultures. SRIF receptors (SSTR1-5) expression and in vitro effects on VEGF secretion and on cell viability of SRIF and of the stable SRIF analogue pasireotide (SOM230) which activates SSTR1, 2, 3 and 5 have been investigated. Twenty-five NFA were examined by RT-PCR for expression of alpha-subunit, SSTR, VEGF, and VEGF receptors 1 (VEGF-R1) and 2 (VEGF-R2). Primary cultures were tested with SRIF and with pasireotide. All NFA samples expressed alpha-sub, VEGF and VEGFR-1 and 2, while SSTR expression pattern was highly variable. VEGF secretion inhibition by SRIF identified 2 different groups. VEGF secretion and cell viability were reduced by SRIF and pasireotide in the "responder" group, but not in the "non responder" group. SRIF and pasireotide completely blocked Forskolin-induced VEGF secretion. SRIF and pasireotide completely abrogated the promoting effects of VEGF on NFA cell viability. Our data demonstrate that pasireotide can inhibit NFA cell viability by inhibiting VEGF secretion, and suggest that the multireceptor-SSTR agonist pasireotide might be useful in medical therapy of selected NFA.

Pttg Is Required For Pituitary Tumor Development In Rb+/- Mice By Preventing p21-Dependent Senescence

Vera Chesnokova, Svetlana Zonis, Tami Rubinek, Run Yu, Anat Ben-Shlomo, Shlomo Melmed

Cedars-Sinai Medical Center-David Geffen School of Medicine, UCLA, Los Angeles, CA, United States

Pituitary tumor transforming gene (Pttg) initially isolated from pituitary tumor cells, is over-expressed in pituitary tumors. Pttg over-expression causes cell transformation, induces aneuploidy and promotes *in vivo* tumor formation. Rb+/- mice develop pituitary tumors with almost 100% penetrance, while only 30% of compound Rb+/- mice with deleted Pttg develop pituitary tumors. Here we examined mechanisms underlying the rescue of pituitary tumor development in Rb+/-Pttg-/- mice by *in vivo* activation of the Arf/p53/p21 senescence pathway. To confirm that p21 induction underlies pituitary tumor rescue in Rb+/-Pttg-/- mice, triply mutant Rb+/-Pttg-/-p21-/- animals were generated. Tumors developing in Rb+/- mice arise mostly from the intermediate lobe, and *in vivo* BrdU incorporation in the pre-tumorous pituitary intermediate lobe was higher in Rb+/-Pttg-/-p21-/- than in Rb+/-Pttg-/- animals ($6.8 \pm 1.4\%$ vs $2.05 \pm 0.8\%$, $p < 0.05$). To evaluate tumorigenic properties of Pttg-deficient cells we generated mouse embryonic fibroblasts (MEFs) derived from Rb+/- and Rb+/-Pttg-/- mice with and without p21 deletion. In p21-positive Pttg-/- and Rb+/-Pttg-/- MEFs, Pttg deletion resulted in p21 protein induction. Rb+/- MEFs developed colonies in soft agar, while Rb+/-Pttg-/- cells failed to produce colonies and also exhibited high levels of senescence-associated- β -gal. Aneuploidy was already evident in the 1st passage of Pttg-null MEFs likely provoking activation of the DNA damage signaling pathway and p53/p21 senescence observed in these cells. In contrast, deletion of p21 from the Rb+/-Pttg-/- background decreased senescence and enhanced anchorage-independent cell growth (3.25 ± 0.9 colonies in Rb+/-Pttg-/-p21+/+ vs 42.8 ± 8 colonies in Rb+/-Pttg-/-p21-/- MEFs, $p < 0.01$). Cell transformation after retroviral H-Ras/Myc transduction was also higher in Rb+/-Pttg-/-p21-/- MEFs, accompanied by up-regulated levels of phosphorylated RB and Cdk2, and markedly decreased numbers of senescent MEFs (23.4 ± 7.1 in Rb+/-Pttg-/-p21+/+ vs 8.25 ± 2.2 in Rb+/-Pttg-/-p21-/- cells, $p < 0.05$). Conclusions: Decreased tumorigenic properties of Pttg-deficient MEFs require p21. The results suggest that Pttg deletion rescues pituitary tumor development in Rb+/-Pttg-/- mice by evoking p21-induced senescence.

ABSTRACTS
Tumors



P6

History and Clinical Manifestations of Childhood Craniopharyngioma at Primary Diagnosis in 311 Patients

Hermann L. Muller¹, Ursel Gebhardt¹, Niels Soerensen²

¹Department of Pediatrics, Klinikum Oldenburg gGmbH, Oldenburg, Germany, ²Department of Pediatric Neurosurgery, University Hospital, Wurzburg, Germany

Introduction: Craniopharyngioma are congenital embryonic midline malformations of low grade malignancy developing from remnants of Rathkes pouch. 30-50% of all cases are diagnosed during childhood and adolescence with a peak of incidence at an age of 8 years. In spite of a high overall survival rate (92%) the long-term prognosis is severely impaired by late effects of tumour and treatment.

Methods: The records of 311 patients with childhood craniopharyngioma were evaluated in regard to clinical manifestations, symptoms and history before diagnosis of craniopharyngioma.

Results: The diagnosis of craniopharyngioma was incidental without any observed symptoms and complaints in the history of 3% of all patients. First symptoms and duration of history (months) until diagnosis of craniopharyngioma were headache (52%; median duration of history 24 mo [range: 0.5-96]), visual impairment (18%; 6 mo [1-48]), growth retardation (15%; 33 mo [12-96]), impairment of vigilance (8%; 2 mo [0.1-6]), polyuria / polydipsia as a symptom of diabetes insipidus neurohormonalis (5%; 26 mo [12-48]) and weight gain (5%; 24 mo [24-48]). The median duration of history in 311 patients was 12 months (range: 0.5 - 96 months). The long duration of history could be confirmed by analysis of anthropometric data collected in 90 patients before diagnosis of childhood craniopharyngioma in a nation wide health survey. Already at the time point U6 (10.-12. month of age) a significantly and persistently impaired growth rate was found especially for patients with hypothalamic involvement of craniopharyngioma. The correlation between the duration of history and functional capacity (ability scale Muenster-Heidelberg [FMH]) as a parameter of quality of life did not reach statistical significance.

Conclusions: A combination of the symptoms headache, visual impairment, polyuria / polydipsia and growth impairment should lead differential diagnostic efforts towards craniopharyngioma. Monitoring of growth is of essential importance in early diagnosis of childhood craniopharyngioma. A long history before diagnosis does not seem to have significant impact on long-term prognosis and functional capacity in patients with childhood craniopharyngioma.

P7

Prognostic Impact of Hypothalamic Involvement in Patients with Childhood Craniopharyngioma - Results of a Cross-Sectional Study in 306 Patients and Update on a Prospective Multicenter Surveillance Study in 118 Patients

Hermann L. Muller¹, Ursel Gebhardt¹, Andreas Faldum², Fabian Pohl³, Christian Roth⁴, Monika Warmuth-Metz⁵, Gabriele Calaminus⁶, Niels Soerensen⁷

¹Dep. of Pediatrics, Klinikum Oldenburg gGmbH, Oldenburg, Germany, ²Institute for Medical Biometry, Epidemiology and Informatics (IMBEI), University of Mainz, Mainz, Germany, ³Dep. of Radiooncology, University Hospital, Regensburg, Germany,

⁴Dep. of Pediatrics, University Hospital, Bonn, Germany, ⁵Dep. of Neuroradiology, University Hospital, Wurzburg, Germany, ⁶Dep. of Pediatrics, University Hospital, Dusseldorf, Germany, ⁷Dep. of Ped. Neurosurgery, University Hospital, Wurzburg, Germany

Whereas the overall survival rate in patients with childhood craniopharyngioma (CR) is high (92%), side effects such as severe obesity due to hypothalamic involvement (HI) have major impact on quality of life (QoL) in survivors of CR. We analyzed 212 patients with CR in regard to HI, growth, obesity (body mass index [BMI] SDS) and QoL (FMH scale, PEDQOL questionnaire).

In our cross-sectional study 59% of all patients with CR presented with HI at the time of diagnosis. Severe long-term obesity was correlated with HI and found to be a major risk factor for reduced QoL. Patients with CR and HI presented with higher BMI already at the time of diagnosis. Analyzing anthropometric data collected before diagnosis of CR we found reduced growth rates as early as at age of 12 months. Increases in BMI occurred at age of 5 years, shortly before diagnosis of CR. Analyzing pathogenic mechanisms for the development of severe obesity we found that patients with CR and HI showed reduced physical activity as measured by accelerometric movement analysis. Severe daytime sleepiness due to reduced nocturnal melatonin levels were found in patients with HI of CR. Differences in self-assessment of caloric intake



between patients with CR and age-, sex- and BMI-matched controls did not reach statistical significance. Aware of possible bias due to retrospective analysis in our cross-sectional study (n=290) we initiated a prospective multicenter study in 2001. Between September 2001 and October 2006 we were able to prospectively analyze 118 patients with CR.

We conclude that HI has major impact on BMI, long-term QoL and functional capacity in patients with CR. A major aim of our prospective study is to analyze the prognostic relevance of therapeutic strategies such as the degree of surgical resection, irradiation and rehabilitative efforts on outcome and QoL especially in patients with HI. In a multicenter trial the time point of irradiation (XRT) after incomplete resection (early versus XRT at progression of residual tumour) will be randomized (3 months after surgery) in patients ≥ 5 years of age. The endpoint of analysis will be quality of life (physical function as a domain of the PEDQOL questionnaire) 3 years after randomization.

P8

Frequent Early Relapses and Progressions of Residual Tumour in Patients with Childhood Craniopharyngioma - Interim Results of a Multicenter Prospective Study in 118 Patients and Design of a Randomized Multicenter Trial

Hermann L. Muller¹, Ursel Gebhardt¹, Fabian Pohl², Michael Flentje³, Rolf-Dieter Kortmann⁴, Angela Emser⁵, Monika Warmuth-Metz⁶, Thorsten Pietsch⁷, Niels Soerensen⁸

¹Dep. of Pediatrics, Klinikum Oldenburg gGmbH, Oldenburg, Germany, ²Dep. of Radiooncology, University Hospital, Regensburg, Germany, ³Dep. of Radiooncology, University Hospital, Wurzburg, Germany, ⁴Dep. of Radiooncology, University Hospital, Leipzig, Germany, ⁵Institute for Medical Biometry, Epidemiology, and Informatics (IMBEI), University of Mainz, Mainz, Germany, ⁶Dep. of Neuroradiology, University Hospital, Wurzburg, Germany, ⁷Dep. of Neuropathology, University Hospital, Bonn, Germany, ⁸Dep. of Ped. Neurosurgery, University Hospital, Wurzburg, Germany

Despite high overall survival rates (92%) in patients with childhood craniopharyngioma (CP), health-related quality of life (QoL) is frequently impaired due to sequelae resulting from hypothalamic involvement of CP such as severe obesity. Based on the results of an interim analysis of a multicenter prospective study radical surgery is no appropriate treatment strategy in patients with hypothalamic involvement of CP. Furthermore, tumour progression and relapses are frequent and early events in CP patients. The analysis of event-free survival rates (EFS) in 98 prospectively evaluated patients with CP showed a high rate of early events in terms of tumour progression after incomplete resection (EFS: 0.22 ± 0.087) and relapses after complete resection (EFS: 0.60 ± 0.098) during the first three years of follow-up. Therefore, innovative treatment strategies are warranted for patients with hypothalamic involvement of CP after incomplete resection.

Accordingly, in a new multicenter trial QoL, progression-free and overall survival rates in CP patients (age ≥ 5 years at diagnosis; after incomplete resection) will be analyzed after stratified randomization (according to postoperative QoL [PEDQOL domain: physical function]) of the time point of irradiation (XRT) after incomplete resection (immediate XRT versus XRT at progression of residual tumour). All patients with completely resected CP and patients of an age < 5 years, regardless of the degree of CP resection, will be recruited in a surveillance study. The schedule of prospective data collection and the set and definition of parameters is based on an international consensus achieved in the CP subgroup of the SIOP (International Society of Paediatric Oncology). Internationally standardized data sets on a rare disease such as CP will help to increase cohort sizes and facilitate common data evaluation.

In conclusion, this study represents the first randomized multicenter trial in patients with CP and the first study in children and adolescents with a CNS tumour analyzing health-related QoL as an endpoint. Aim of the study is to analyze the appropriate time point of XRT after incomplete resection in order to improve QoL and prevent hypothalamic obesity in patients with hypothalamic involvement.

Melatonin Treatment in Severely Obese Patients with Childhood Craniopharyngioma and Increased Daytime Sleepiness

Hermann L. Muller¹, Georg Handwerker², Ursel Gebhardt¹, Andreas Faldum³, Angela Emser³, Reinhardt Kolb¹, Niels Soerensen¹

¹Dep. of Pediatrics, Klinikum Oldenburg gGmbH, Oldenburg, Germany, ²Dep. of Pediatrics, University Hospital, Wurzburg, Germany, ³Institute for Medical Biometry, Epidemiology and Informatics, University of Mainz, Mainz, Germany, ⁴Dep. of Pediatric Neurosurgery, University Hospital, Wurzburg, Germany

Craniopharyngioma (CR) is a rare embryogenic tumor. Patients frequently suffer from endocrine deficiencies, sleep disturbances and obesity. A self-assessment daytime sleepiness questionnaire (German version of the Epworth Sleepiness Scale [ESS]) was used to evaluate 79 patients with childhood CR recruited in a prospective multicenter trial. Because hypothalamic lesions may explain daytime sleepiness in CR patients, salivary melatonin and cortisol concentrations were examined in severely obese (body mass index [BMI] ≥ 4 SD) and non severely obese (BMI < 4 SD) CR patients (n=79), patients with hypothalamic pilocytic astrocytoma (n=19), and control subjects (n=30). Therapeutic effects of a melatonin substitution in patients with severe daytime sleepiness and disturbed melatonin secretion were analyzed.

Analyzing the influence of BMI and tumour diagnosis on diurnal salivary melatonin in multivariate analysis we found that morning salivary melatonin levels were related to BMI (F test: p-value=0.004) and tumour diagnosis (F test: p-value=0.032). Also for nighttime salivary melatonin levels significant relations with BMI (p-value in F-test: < 0.001) and tumour diagnosis (p-value in F-test: 0.025) were detectable. Melatonin concentrations in saliva of CR patients collected at nighttime or in the morning showed a negative correlation (Spearman's rho: -0.42; p=0.001; Spearman's rho: -0.31; p=0.020) with the patient's ESS score. Severely obese CR patients and severely obese hypothalamic tumour patients had similar melatonin patterns. As decreased nocturnal melatonin levels were associated with increased daytime sleepiness, BMI and hypothalamic tumour diagnosis, we initiated an experimental melatonin substitution in 10 adult obese patients (5f / 5m) with childhood CR. In all treated patients daytime sleepiness significantly improved based on activity diaries, ESS, and actimetry.

We speculate that hypothalamic lesions might be responsible for both obesity and daytime sleepiness. As first experiences with experimental melatonin substitution were promising, further randomized double-blinded studies on the beneficial effects of a melatonin substitution are warranted.

P10

Quality of life in survivors of childhood craniopharyngioma - Current status of a prospective multicenter study and results of a retrospective study on 185 long-term survivors

Hermann L. Muller¹, Ursel Gebhardt¹, Andreas Wiener², Reinhard Kolb¹, Niels Soerensen³, Gabriele Calaminus²

¹Dep. of Pediatrics, Klinikum Oldenburg gGmbH, Oldenburg, Germany, ²Dep. of Pediatrics, University Hospital, Dusseldorf, Germany, ³Dep. of Pediatric Neurosurgery, University Hospital, Wurzburg, Germany

Patients with childhood craniopharyngioma, an intracranial tumour of low grade malignancy, frequently suffer from severe acute and long-term adverse effects, related to pituitary and hypothalamic alterations. The spectrum includes endocrine deficiencies, development of obesity and sleeping disorders. Such morbidities influence quality of life (QoL). We evaluated health related QoL within a multicenter prospective study (n=118) as well as in a retrospective investigation of 306 patients with childhood craniopharyngioma, to answer the question whether operation, treatment and appearance of endocrine deficiencies have impact on QoL.

We retrospectively analysed 185 patients with childhood craniopharyngioma at least two years off treatment. Questionnaires (KINDL, PEDQOL, EORTC-QIQ-30) were used and patients were grouped according to the degree of surgery, irradiation, and body mass index (BMI-SDS). 77 data sets were available. Generally compared to healthy controls, CP patients showed an impaired QoL in respect to cognition and social functioning with friends. In addition, patients with a BMI > 3 SD gave a more negative rating of body image, social functioning with friends and physical abilities. Over time, emotional functioning was rated



negatively. Within the prospective setting, 70 patients are so far registered, of whom 41 patients gave complete information on their QoL. After the operative intervention most patients valued their QoL positive. During follow-up, problems with friends, body image and physical functioning were constantly detectable. Parents rated their children's QoL more negatively. Over time both ratings (self/proxy) converged to each other.

We conclude that QoL in patients with childhood craniopharyngioma is influenced by endocrine deficiencies and severe obesity. QoL changes over time. Integration within peer groups, acceptance of body structures and emotional status are relevant problems. Patients with childhood craniopharyngioma need a good network structure of medical and psychosocial care to cope with the disease and its long-term effects.

P11

Secondary narcolepsy may be a causative factor of increased daytime sleepiness in obese patients with childhood craniopharyngioma

Hermann L. Muller¹, Sarah Muller-Stoeber², Ursel Gebhardt¹, Reinhard Kolb¹, Georg Handwerker², Niles Soerensen³

¹Dep. of Pediatrics, Klinikum Oldenburg gGmbH, Oldenburg, Germany, ²Dep. of Pediatrics, University Hospital, Wurzburg, Germany, ³Dep. of Pediatric Neurosurgery, University Hospital, Wurzburg, Germany

Prognosis in childhood craniopharyngioma survivors hinges upon late effects such as pituitary deficiency and obesity. Observations indicate that reduced physical activity and increased daytime sleepiness might be risk factors for obesity. We analyzed the degree of daytime sleepiness in 115 childhood craniopharyngioma patients (47% obese) using Epworth sleeping scale (ESS).

Thirty-five (30%) displayed increased daytime sleepiness (ESS score > 10) - 14 obese (26% of obese cohort). Polysomnography (PSG) and Multiple Sleep Latency Tests (MSLT) were conducted with 10 obese patients presenting increased daytime sleepiness, with only two craniopharyngioma patients revealing a sleep related breathing disorder. Four patients had repeated episodes of SOREM (sleep onset rapid eye movement), the classic PSG criterion for secondary narcolepsy. Three patients displayed hypersomnia. All but one patient qualified as acutely obese. Preliminary observations on the effect of central stimulating agents for treatment of secondary narcolepsy showed a significant beneficial effect. All four patients treated with Modafinil (daily dose 100-400 mg) or Methylphenidate (daily dose 10-30 mg) in terms of an individual experimental therapy showed a significant improvement of daytime sleepiness (median reduction on ESS: 3 points, ranging from 2 to 12 points) and an improved self-assessment of daily activity documented by activity diaries.

We speculate that secondary narcolepsy is an exacerbating condition of childhood craniopharyngioma obesity, supported by recent reports on orexin and narcolepsy which suggest hypothalamic failure in idiopathic narcolepsy.

P12

Intraoperative Acquisition of 3-D Imaging for Frameless Stereotactic Guidance During Transsphenoidal Pituitary Surgery Using the Siremobil Iso-C3D System

William Chandler, W. Christopher Fox

University of Michigan, Ann Arbor, Michigan, United States

Introduction: Intraoperative fluoroscopy has long been used for anatomic localization in transsphenoidal pituitary surgery. More recently, frameless stereotactic guidance with preoperative MR or CT studies has been used to supplement fluoroscopy with 3-dimensional multiplanar reconstructions. This provides the safety of 3-dimensional localization of sellar anatomy during surgery. The Siremobil Iso-C3D system allows both conventional fluoroscopic views and multiplanar reconstructions to be acquired intraoperatively, without the need for preoperative planning studies. This system has been used mainly on the spine and has never been used during pituitary surgery. We report our initial experience using the Iso-C3D system during transsphenoidal pituitary surgery.

Methods: We first placed a dehydrated human skull in the radiolucent head holder, and obtained standard 2-dimensional fluoroscopic images of the skull base and sella turcica. We then used the Iso-C3D system in combination with a frameless stereotactic system to acquire and register 3-dimensional multiplanar reconstructed images of the skull base anatomy. Since these experimental studies compared favorably to

intraoperative x-rays and frameless stereotactic CT studies from patients with pituitary tumors, we then utilized the Iso-C3D system during 25 transsphenoidal pituitary tumor operations.

Results: Iso-C3D 2-dimensional fluoroscopic x-rays matched or exceeded the quality of images acquired by standard C-arm machines. Iso-C3D multiplanar reconstructions provided excellent and comparable images of the bony anatomy of the skull base when compared to preoperative CT studies. Intraoperative frameless stereotactic navigation using Iso-C3D technology was highly accurate, and, in fact, more reliable than registering preoperative CT images. We never had to resort to standard preoperative CT imaging.

Discussion: Iso-C3D technology provides excellent quality 2- and 3-dimensional images during transsphenoidal pituitary surgery and intraoperative frameless navigation using these images is highly accurate. The Iso-C3D system is easy to use and image acquisition takes no longer than registration during a frameless stereotactic case. Based upon our experience, Iso-C3D technology has precluded the need for preoperative CT studies in patients with pituitary lesions requiring frameless stereotactic navigation.

P13

Matrix Metalloproteinase 9, a Potential Biological Marker in Invasive Pituitary Adenomas

Jian Gong², Yunge Zhao¹, Rana Abdel-Fattah¹, Samson Amos¹, Aizhen Xiao¹, M. Beatriz S. Lopes¹, Isa M. Hussaini¹, Edward R. Laws, Jr.¹

¹University of Virginia, Charlottesville, VA, United States, ²Beijing Neurosurgical Institute, Beijing, China

Introduction - Tumor invasion is a major factor determining the outcome of therapy and prognosis of pituitary tumors. Matrix metalloproteinase-9 (MMP-9) specially degrades type IV collagen, a major component of the sellar dura, and paves the way for tumor cells to migrate and invade surrounding tissues. Herein, we analyzed MMP-9 expression in invasive and non-invasive adenomas and explored the possibility that MMP-9 is a potential biological marker of pituitary adenoma invasiveness.

Methods - 73 pituitary tumor specimens were collected immediately after surgical resection. RT-PCR was used for analysis of MMP-9 mRNA transcripts; protein activity by gelatine zymography and validated by Western blot. Immunohistochemistry (IHC) was performed in FFPE tissues. Student's t-test or one way ANOVA were used for statistical analyses. $p < 0.05$ was considered significant. The study was compliant with the University of Virginia IRB.

Results - MMP-9 mRNA expression was significantly increased in all invasive adenomas ($p < 0.01$), both functioning (FA) and non-functioning (NFA). Similarly, MMP-9 activity was significantly increased in all invasive FA ($p < 0.05$) and NFA ($p < 0.01$). Invasive active and silent corticotroph adenomas ($p < 0.05$) and prolactinomas ($p < 0.05$) showed slightly higher MMP-9 mRNA expression and significantly higher protein levels than other adenoma subtypes. IHC demonstrated diffuse/intense cellular cytoplasmic MMP-9 reactivity in invasive adenomas but faint/focal staining in noninvasive tumors. MMP-9 expression in NFA correlated with tumor size. However, in FA, MMP-9 expression in microadenomas was higher than in macroadenomas. This may reflect the high level of invasiveness in certain microadenomas such as ACTH-adenomas. MMP-9 expression was significantly higher in recurrent NFA ($p < 0.01$).

Discussion - MMP-9 expression consistently discriminated invasive from noninvasive adenomas and correlated significantly with tumor subtype, size, tumor extension, and recurrence, even at early stages of invasiveness. MMP-9 may be considered a potential biomarker to determine and predict the invasive nature of pituitary tumors.

P14

Endoscopic Transsphenoidal Surgery For Non-Functioning Pituitary Macroadenomas: Early Experience With 100 Consecutive Patients

John Jane, Jr, Adam Kanter, Edward Laws, Jr

University of Virginia, Charlottesville, Virginia, United States

Introduction: Endoscopic pituitary surgery has recently emerged as an alternative to the more traditional microscopic approaches. This technique is purported to provide a less invasive and possibly more effective surgery. Since 2004, our service has gained experience with the technique and has performed more than 250 endoscopic transsphenoidal surgeries. We report our experience in patients with non-functioning adenomas.



Methods: A retrospective review was performed from a prospectively acquired database of our first 100 patients with non-functioning pituitary adenomas treated via a purely endoscopic transsphenoidal approach. All patients were evaluated by a neurosurgeon and endocrinologist with a minimum 2 month clinical and radiographic follow-up evaluation.

Results: Eleven percent of patients had previously been operated upon at outside institutions. Presenting symptoms included headache (43%), visual (39%) or endocrine (39%) disturbance. Ninety-six percent were macroadenomas, 62% extended into the suprasellar compartment and 29% invaded the cavernous sinus.

Eighty-five percent of patients received a gross total tumor resection. Seventy-eight percent of patients reported headache resolution, 88% reported improvement in visual function, and nearly 50% had resolution of a pre-existing endocrinopathy. Eleven percent of patients experienced iatrogenic pituitary dysfunction. Four percent of patients required a second operation for repair of a persistent CSF leak. Further complications included sinusitis (8%), SIADH (7%), and meningitis (1%). There were no operative deaths. At a minimum 2 month follow-up, only one patient was found to have radiographic tumor recurrence.

Discussion: Neuroendoscopy offers a safe and effective alternative approach for the resection of sellar and suprasellar non-functioning adenomas. The early outcomes require long-term follow up, but appear comparable to the microscopic technique.

P15

NcoI C/T Dopamine D2 receptor gene polymorphism is associated with resistance to Cabergoline therapy in patients with prolactin-secreting pituitary adenomas

Andrea Lania¹, Marcello Filopanti¹, Annamaria Barbieri¹, Annamaria Colao², Silvia Grottoli³, Alessandro Peri⁴, Marco Faustini-Fustini⁵, Stefano Mariotti⁶, Patrizia Del Monte⁷, Giorgio Borretta⁸, Marie Lise Jaffrain-Rea⁹, Bruno Ambrosi¹⁰, Maurizio Gasperi¹¹, Paolo Beck-Peccoz¹, Giovanna Mantovani¹, Anna Spada¹

¹Endocrine Unit, Dept. Medical Sciences, University of Milan, Fondazione Ospedale Maggiore IRCCS, Milano, Italy, ²Department of Molecular and Clinical Endocrinology and Oncology, University Federico II, Napoli, Italy, ³Division of Endocrinology and Metabolism, University of Turin, Torino, Italy, ⁴Endocrine Unit, Department of Clinical Physiopathology, University of Florence, Firenze, Italy, ⁵Division of Endocrinology, Bellaria Hospital, Bologna, Italy, ⁶Endocrinology Unit, Department of Medical Sciences "M. Aresu", University of Cagliari, Cagliari, Italy, ⁷Unit of Endocrinology, Galliera Hospital, Genova, Italy, ⁸Endocrinology Unit, Santa Croce Hospital, Cuneo, Italy, ⁹Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy, ¹⁰Dept. Medical and Surgical Sciences, University of Milan, San Donato, Italy, ¹¹Chair of Endocrinology, Univeristà del Molise, Campobasso, Italy

Dopamine agonist cabergoline (CB) is effective in reducing PRL secretion and tumour size in about 80% of patients by binding dopamine D2 receptor (DRD2). In the absence of reported DRD2 mutations, the mechanisms responsible for the different responsiveness to CB remain largely unknown.

In the present study we analyzed the possible association of DRD2 gene polymorphisms (i.e. and TaqI A1/A2, TaqI B1/B2, HphI G/T and NcoI C/T) with clinical parameters and sensitivity to CB in patients with prolactin-secreting adenoma (PRL-oma). A cross-sectional multicentric retrospective study was carried out including 203 patients with PRL-oma and 212 healthy subjects. Genotyping was carried out by RFLP on blood DNA.

In our series about 10% of patients failed to normalize PRL levels taking CB at > 3 mg/week and were considered resistant. DRD2 allele frequencies did not differ between patients and healthy subjects. Conversely, the proportion of patients homozygous or heterozygous for the NcoI T+ allele was higher among the resistant than the responsive patients, considering both PRL normalization (56.6% vs of 45.3%, Fisher's test P=0.038) and > 30% tumor size reduction (70.4% vs 41.4%, P=0.006). This association were further confirmed by the observation that the haplotype negative for NcoIT+ allele, i.e. [TaqI A1-/TaqI B1-/HphI T-/NcoI T-], was associated with a greater sensitivity to CB treatment in term of PRL normalization (Fisher's test P=0.021).

In conclusion, resistance to CB treatment was associated with NcoI T+ polymorphism of DRD2, consistent with evidence suggesting that this variant may affect DRD2 mRNA stability and receptor synthesis.

P16

Pituitary tumors in the elderly

Mirtha Guitelman, Natalia Elias, Josefina Bomarito, Pablo Constanzo, Natalia Garcia Basavilbaso

Endocrinology Division Durand Hospital, Buenos Aires, Argentina

Introduction: Pituitary adenomas (PAs) in the elderly account for less than 10-15% of all PAs, being non-functioning adenomas (NFPAs) the most frequently reported. Due to life expectancy increase and health care improvement, PAs in this age group are increasingly more common. **Objective:** To analyze the clinical presentation, diagnosis, therapeutic management and outcome of elderly patients with PAs. **Methods:** The records of 27 patients older than 60 years with PAs were reviewed retrospectively. Results were expressed as X \pm SD. **Results:** Twenty out of 27 patients were women, with a mean age of 70 \pm 7 years old (range: 60-84). Nineteen (70%) were macroadenomas and 8 (30%) were microadenomas. Acromegaly was diagnosed in 13 female (54% microadenomas), NFPAs in 6 women and 6 men (100% macroadenomas), and prolactinomas in a woman and a man with macro and microadenoma, respectively. The most frequent complaints were features of acromegaly and acral enlargement in acromegalic patients (69%), and incidental finding in NFPAs. Visual field disturbance was found in 11 patients (7 NFPAs, 3 acromegaly, 1 prolactinoma). Some degree of hypopituitarism was found in 70% of patients with NFAs, but only in one patient with acromegaly. Both patients with prolactinomas had hypogonadism as a unique sign of pituitary deficiency. Transsphenoidal surgery was performed in 9 patients, with tumor persistence in the majority. Somatostatin analogs were indicated in 6 acromegalic patients (as primary therapy in three), and cabergoline was successfully used in prolactinomas.

Conclusions: We found a high predominance of acromegaly in our series of elderly patients with PAs. The fact that microadenomas are the most commonly reported tumor, along with the better treatments nowadays available for diabetes and hypertension, could explain the greater than expected life expectancy in this population. The high incidence of incidentalomas in NFPAs, which in fact presented with symptoms related to pituitary dysfunction, may not only suggest a delayed diagnosis in this age group but also the low and not aggressive growth of these tumors.

P17

MEN-1 Presenting As Cushing's Disease

L Higashi, A Villela, MC Huayllas, KC Nogueira, B Liberman, A Cukiert

Hospital Brigadeiro, Sao Paulo SP, Brazil

Introduction: Signs or symptoms derived from pituitary adenoma represent 25% of the initial patterns of clinical presentation in patients with MEN-1. Prolactin-secreting adenoma is by far the most common of them (up to 60% of all MEN-1 related pituitary tumors) and ACTH-secreting are extremely rare, especially as the initial clinical presentation. We report a patient with MEN-1 who initially presented as Cushing's disease.

Case report: MRFR, a 34 years-old lady, presented with weigh gain and amenorrhea over the last year. Biochemical investigation disclosed ACTH-dependent hypercortisolemia. Plasma cortisol and ACTH levels were 21.4ug/dl (non-suppressive) and 37.7pg/ml, respectively. Free urinary cortisol level was 160 ug/24 hours. Both plasma cortisol and ACTH were responsive during a DDAVP activation test. MRI showed a 4-mm microadenoma. Low calcium (13,1 mg/dl), high phosphate (2,5 mg/dl) and parathormone (149 pg/ml) levels were noted during regular biochemical screening performed in all patients with pituitary tumor. Gastrin plasma level was 126 pg/ml. Scintilographic imaging (sestamibe) was suggestive of hyperparathyroidism. She was submitted to cervicotomy and transesphenoidal surgery. Parathormone plasma level normalized after cervicotomy and the patient developed adrenal insufficiency after transesphenoidal surgery. Pathological examination of the specimens obtained from the above mentioned procedures found parathyroid hyperplasia and ACTH-secreting pituitary adenoma.

Discussion: This patient presented with Cushing's syndrome and silent hyperparathyroidism. Cushing's disease is a very rare pattern of clinical presentation in patients with MEN-1 (less then 2%). Up to 80% of these patients might have entero-pancreatic tumors but there was no abdominal tumor documented so far in this patient. Routine screening for MEN-1 in patients with pituitary tumor might increase our ability to further diagnose this syndrome.



P18

Closure Of Intraoperative Low-Flow CSF Leaks Using Fibrin Glue Alone And No Lumbar Drainage During Transesphenoidal Surgery

A Cukiert, JA Burattini, PP Mariani, L Seda, MK Huayllas, KC Nogueira, B Liberman

Hospital Brigadeiro, Sao Paulo SP, Brazil

Introduction: The closure of CSF leaks during transesphenoidal surgery might represent a challenge. Persistent CSF leak might lead to meningitis and often needs reoperation. We recently published on the use of fibrin glue alone (without any grafting) and lumbar drainage as a technique for closing these leaks. In this paper, we present the results of closing low-flow CSF leaks with fibrin glue alone without lumbar drainage or any type of grafting.

Methods: Thirty consecutive patients submitted to transesphenoidal surgery in whom an intraoperative low-flow CSF leak was noted were studied. We considered a "low-flow" intraoperative leak those leaks which were visually sealed after the application of haemostatic material (surgical) and fibrin glue. A two-layer seal including haemostatic material/fibrin glue was used in all patients. No graft or lumbar drainage was used. Patient were ask to remain in bed for 2 days.

Results: No postoperative CSF leak was noted in these patients. There was no meningitis and no need for reoperation in this series.

Discussion: No grafting or lumbar drainage is needed for the treatment of low-flow intraoperative CSF leaks occurring during transesphenoidal surgery. Lumbar drainage might be needed in high-flow intraoperative CSF leaks.

P19

HoxD10 Suppresses Prolactinoma Tumorigenesis and Inhibits Expression of Angiogenic Factors

Mi-Ae Cho¹, Sun Ho Kim¹, Yumie Rhee¹, Sungkil Lim¹, Hyun Chul Lee¹, Eun Jig Lee², Taewoong Noh¹

¹*Yonsei University College of Medicine, Seoul, United States, ²Northwestern University, Feinberg School of Medicine, Chicago, United States*

Introduction: Prolactinomas are the most common pituitary adenomas. Angiogenesis has been described as an important mechanism of growth in these lesions. The authors describe a method of inhibiting adenoma formation in vivo by delivery of HoxD10 gene, an inhibitor of angiogenesis.

Methods: Adeno/AAV hybrid vectors were generated carrying CMV promoter followed by mouse HoxD10 gene (Hyb-HoxD10) or a control gene, β -galactosidase (Hyb-Gal). Rat GH4 cells were infected with either Hyb-HoxD10 or Hyb-Gal and cultured. To test in vivo adenoma growth, these GH4 cells were then transplanted into nude mice and tumor sizes were measured for 3 weeks.

Results: A proliferation assay was run with cells infected with varying concentrations of adenovirus (0 - 50 pfu/cell). Data demonstrated a significant inhibition in proliferation at 10 and 25pfu per cell. RT-PCR analysis of GH4 cells infected with Hyb-HoxD10 and Hyb-Gal demonstrated significant decrease in transcripts for FGF-2. Of 10 Hyb-Gal-infected GH4 cell masses transplanted into nude mice, 7 developed into tumors of significant size while in the Hyb-HoxD10 group, no adenomas developed within the study period in any of the 10 nude mice.

Conclusion: These results indicate that delivery of anti-angiogenic factors, such as HoxD10, has the potential to inhibit growth of PRL-secreting tumors. This approach may provide a useful tool for targeted therapy of prolactinomas and other angiogenic disorders

P20

Remarkable improvement of visual field perimetry without tumoral size reduction in a patient with NFPA after long term treatment with cabergoline

Soledad Lovazzano, Mariela Glerean, Patricia Fainstein Day

Hospital Italiano, Buenos Aires, Argentina

Although dopamine receptors are expressed in non clinically functioning pituitary adenomas (NFPA), medical treatment with dopamine agonists has been disappointing and visual field improvement has been

found in the minority of patients that showed tumoral size regression. The contribution of dopaminergic neurons in visual processing was recognized since visual impairment was found in patients with Parkinson disease.

We report a 50-year old male who presented sexual impotence and visual impairment. A NFPA, with extrasellar extension and hydrocephaly was diagnosed. The patient underwent two surgery procedures, transesphenoidal and then through the intracranial approach and a ventricular-peritoneal valve was inserted.

Preoperative and postoperative hormonal evaluation was consistent with panhypopituitarism.

Visual field: bitemporal hemianopia plus binasal fields contraction.

Histopathologic diagnosis: pituitary oncocytoma. Immunohistochemistry for FSH, LH, ACTH, GH, PRL, TSH was negative.

Postoperative MRI: sellar tumor of 28 mm maximal diameter.

Following surgery there was no improvement of visual field. Chronic medical treatment of hypopituitarism was indicated.

A negative octreotide-radiolabeled scan suggested absence of tumoral somatostatin receptors. A clinical-radiological follow-up was decided, given the low sensitivity to radiotherapy reported in oncocytomas. Treatment with cabergoline was initiated, on which the patient has been at a dose of 2 mg/week during eight years. The patient refers a great improvement of visual defects, confirmed by visual fields perimetry, the last of which shows small superior bitemporal fields cut (aprox. 70% off recovery). Annual control RMIs didn't show any reduction of tumoral size.

Remarkable visual field perimetry improvement achieved with long term cabergoline treatment in the absence reduction of tumoral size could be related to stimulation of retinal and/or thalamic relay dopaminergic neurons.

P21

Gender Differences In Macroprolactinomas (MAC)

Mariela Glerean, Soledad Lovazzano, Alejandra Rabadán, Antonio Carrizo, Marcelo Pietrani, Patricia Fainstein Day

Hospital Italiano, Buenos Aires, Argentina

Microprolactinomas are more frequently seen in women and have a benign evolution, even without treatment. On the other side, prolactinomas in men are usually MAC and treatment is always needed to control tumor growth. It has been hypothesized that prolactinomas have gender differences regarding biologic behavior, been more aggressive in men.

We conducted a retrospective chart review to assess gender differences regarding clinical characteristics and response to medical treatment focused in MAC. Diagnostic criteria for MAC were serum prolactin levels of 200 ug/ liter or more and evidence on MRI of a pituitary tumor that was more than 10 mm in diameter. Sixty-one MAC out of 129 patients patients with prolactinoma, diagnosed between 1996 to 2005 in our Neuroendocrine Section were included. Prolactin levels were measured by RIA (normal range: 5-25 and 2-20 ug/L, for male and female respectively). Tumoral diameters were measured in MRI by the same radiologist.

	WOMEN	MEN	p
Patients % (n)	44,2 (27)	55,7 (34)	ns
Age (X +/- SD) (r: 15-81 years)	34,2 ±10,1	41,7 ± 14,6	< 0.03
Basal Prolactin (ng/mL)	570.8 ± 828	5001 ± 800.8	<0.007
Basal Maximal Diameter (mm)	20.6 ± 10.5	36.5 ± 21.4	<0.01
Prolactin 24 months	60.1 ± 85.2	51.4 ± 80.1	ns
Max diameter 24 months	4.1 ± 6.3	10.4 ± 16.5	ns
% Tumor Reduction	70,9 ± 27,4	63,8 ± 32	ns
Cabergoline dose	1.2 ± 1.3	1.9 ± 1.4	ns

There was no difference in gender distribution of MAC. Male patients were older at presentation, had greater tumors, higher prolactin levels and more frequent visual defects. Nevertheless, the response to cabergoline treatment was similar in both genders.



P22

Expression of Pituitary Tumor-Derived, N-Terminally Truncated Isoform of Fibroblast Growth Factor Receptor 4 (Ptd-FGFR4) in Human GH-Secreting Pituitary Adenomas Correlates with Tumor Invasiveness, But Not with Gsp Mutation.

Junko Yasufuku-Takano¹, Koji Morita¹, Koji Takano¹, Shozo Yamada², Akira Teramoto³, Toshiro Fujita¹

¹Department of Nephrology and Endocrinology, University of Tokyo Faculty of Medicine, Bunkyo-ku, Tokyo, Japan, ²Department of Hypothalamic & Pituitary Surgery, Toranomon Hospital, Minato-ku, Tokyo, Japan, ³Department of Neurosurgery, Nippon Medical School, Bunkyo-ku, Tokyo, Japan

Introduction: Apart from the constitutively activating mutation of Gs alpha (gsp mutation), factors involved in tumorigenesis or those in tumor behavior remains elusive in sporadic GH-secreting pituitary adenomas. Recently, ptd-FGFR4 (N-terminally-truncated form of FGFR4 (fibroblast growth factor receptor-4)) was identified in pituitary adenomas. This aberrant receptor has transforming activity, and causes pituitary adenomas in transgenic mice. Our objectives were two fold: First, to examine how the expression of ptd-FGFR4 relates to gsp mutations in acromegalic patients. Second, to see whether patients with this receptor have peculiar clinical characteristics.

Methods: mRNA were extracted from excised adenomas of 45 Japanese acromegalic patients. Ptd-FGFR4 expression and gsp mutations were determined by RT-PCR and direct sequencing. Preoperative clinical data were collected by reviewing medical charts and MRI. The use and analysis of surgically resected human pituitary tissues as experimental materials was permitted by the Ethical Committee of our institution.

Results: Ptd-FGFR4 mRNA expression was detected in 19 out of 45 tumors (42.2%) while the gsp mutations were detected in 25 out of 45 tumors (55.6%). The prevalence of ptd-FGFR4 expression did not differ between gsp-positive (44.0%) and gsp-negative (40.0%) tumors (P=1.00). Ptd-FGFR4-positive tumors invaded the cavernous sinus more frequently (P=0.0098) than did the ptd-FGFR4-negative tumors. Ptd-FGFR4-positive tumors tended to be larger than ptd-FGFR4-negative tumors, although without statistical significance (P=0.099) The presence of ptd-FGFR4 did not correlate with age at operation, sex ratio, preoperative serum GH and IGF-1 levels.

Discussion: We found that ptd-FGFR4 expression and gsp mutation occur independently of each other, and that ptd-FGFR4 expression is associated with more invasive tumor in patients with GH-secreting pituitary adenoma.

P23

Wnt Inhibitory Factor-1 is strongly down-regulated and methylated in pituitary tumors

Marianne Elston¹, John Conaglen², Anthony Gill³, Adele Clarkson³, Janet Shaw¹, Roderick Clifton-Bligh¹, Bruce Robinson⁴, Kerrie McDonald¹

¹Cancer Genetics Unit, Kolling Institute of Medical Research, Royal North Shore Hospital, Sydney, NSW, Australia, ²Department of Endocrinology, Waikato Hospital, Hamilton, New Zealand, ³Department of Anatomical Pathology, Royal North Shore Hospital, Sydney, NSW, Australia, ⁴Faculty of Medicine, University of Sydney, Sydney, NSW, Australia

The aetiology of sporadic pituitary tumors is currently unknown. The Wnt pathways have been implicated in the pathogenesis of a variety of human tumors but the role of these pathways in pituitary tumors is currently unclear.

The objective of the study was to identify genes that may be involved in pituitary tumorigenesis.

Human pituitary tumors were collected at the time of surgery, snap-frozen in liquid nitrogen and stored at -80°C until extracted. The study was undertaken with permission, and in accordance with, local ethics committee guidelines. Microarray analysis, real-time quantitative RT-PCR (qPCR), bisulfite-treated DNA sequencing and immunohistochemistry were performed on the tumors.

Microarray analysis using the Affymetrix HG U133 plus 2.0 GeneChips (analysis performed using AffymGUI analysis suite of Bioconductor) identified Wnt inhibitory factor 1 (Wif1) as being differentially expressed in pituitary tumors (n=20) compared with normal pituitary controls (n=3). Validation using qPCR confirmed reduced Wif1 mRNA expression in the tumors (n=42) compared with normal pituitary (n=5) (p < 0.001). Sequencing of the Wif1 promoter demonstrated hypermethylation of tumors (n=41) compared with normal pituitary controls (n=6) (p=0.001). Seventy-six percent of pituitary tumors (n=41)

demonstrated absent or weak cytoplasmic Wif1 staining using immunohistochemistry compared with strong staining in 92% of the normal pituitary controls (n=13) (p<0.001). Nuclear accumulation of beta-catenin however, was not observed in the pituitary tumors (n=70) using immunohistochemistry, and cytoplasmic beta-catenin staining was only weakly correlated with Wif1 mRNA expression (p=0.058). Wif1 was down-regulated in all three pituitary tumor subtypes examined suggesting that the Wnt pathways are important in tumorigenesis. Our data supports that loss of Wif1 in pituitary tumors occurs as an early event, since it is common to all subtypes, and that further genetic events may explain the differences in tumor behaviour between the tumor subtypes.

P24

MAPK 1/2 signalling inhibition by Forskolin in a human tumor pituitary cell line

Tomas Lucas¹, Alfonso Martinez-Conde², Pilar Mayor², Ariel F. Castro³, Jose Garcia-Uria⁴

¹Dep. Endocrinology Hospital Universitario Puerta de Hierro, Madrid, Spain, ²Dep. Biochemistry and Molecular Biology Fac. Medicina UCM, Madrid, Spain, ³UCSF Department of Surgery, San Francisco, CA, United States, ⁴Dep. Neurosurgery Hospital Universitario Puerta de Hierro, Madrid, Spain

We study the effect of cAMP analogs 8-CPT-2Me-cAMP and 8Br-cAMP, and Adenylate Cyclase activator Forskolin on proliferation of a cell line obtained from a human GH secreting pituitary tumour. Effects on MAPK1/2 signalling pathway are analyzed.

A fragment of a GH secreting pituitary adenoma was isolated and cultured in appropriated medium. Cell line 40 was used between passes 30 and 50. Table shows results obtained by using the BrdU proliferation assay with EPAC allosteric effector 8-CPT-2Me-cAMP (40 uM), EPAC and PKA allosteric effector 8Br-cAMP (40 uM) or Adenylate Cyclase activator Forskolin (40 uM).

	Control	8CPT-2Me-cAMP	8Br-cAMP	FORSKOLIN
line 40 +5%FCS	100 ± 0.88	159.4 ± 5.89	115.9 ± 4.40	75.1 ± 2.74

Results are expressed as % of Control value. Values are mean±SE of four separate experiments made in triplicate.

Measurement of ppMEK1/2 and truncated form of ppMEK1/2 was carried out by western blot. After 24h starvation cells were treated for 1h with 40 uM of different chemicals. Lysates were western-blotting with a specific antibody against doubly phosphorylated ppMEK1/2 at Ser218/Ser222.

Results evidence that as much 8-CPT-2Me-cAMP as 8Br-cAMP increase proliferation while Forskolin decreases it. Westerns-blot show that treatment of cultures with 8-CPT-2Me-cAMP or 8Br-cAMP does not alter the ppMEK1/2 band pattern of controls. Treatment with Forskolin causes a new band which is a 37 kD form of ppMEK1/2 (truncated form or tMEK). tMEK has been described as an inactive form of MEK produced either by proteolytic CyclinB/Cdk1-dependent or Cdk5/p35-dependent ppMEK cleavage. Forskolin would activate one of both cleavage systems, causing the feed-back down-regulation of MAPK pathway by 45 kD ppMEK cleavage to 37 kD ppMEK.

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P25

Constitutive active RapGTP in human tumor pituitary cell lines

Tomas Lucas¹, Alfonso Martinez-Conde², Pilar Mayor², Ariel F. Castro³, Jose Garcia-Uria⁴

¹Dep. Endocrinology Hospital Universitario Puerta de Hierro, Madrid, Spain, ²Dep. Biochemistry and Molecular Biology UCM, Madrid, Spain, ³UCSF Department of Surgery, San Francisco, CA, United States, ⁴Dep. Neurosurgery Hospital Universitario Puerta de Hierro, Madrid, Spain

We have assayed the RapGTP level in five pituitary tumor cell lines. We checked the effect of Adenylate Cyclase inhibition and also compared RapGTP levels in Pituitary cells to level in a Rap constitutively active transformed human cell line.

Fragments collected after transsphenoidal surgery of GH secreting pituitary adenomas, and then cells were isolated and cultured in appropriated medium. Cultures for this experiment were used between passes 15 and 22. As negative control for RapGTP we used two different human cell lines : MCF7 (human breast cancer cell line) and HEK293T (human embryonic kidney transformed with SV40 large T antigen). As positive control for RapGTP constitutive active we have transfected MCF7 cells with a mutant form of Rap (Rap63E). MCF-7 breast cancer cells were transfected with pCGN-HARap63E using FuGENE 6. Cells stably expressing Rap63E were selected on 100 ug/ml Hygromycin B.



After 24h of starvation, dishes for Pituitary basal, MCF7, MCF7Rap63E and HEK293T were lysated. Dishes treated with 480 uM 9-Cyclopentiladenine were lysated after 24h of starvation and 1h of treatment. Rap1 activation assay was carried out by pull down with GST-RalGDS(RBD) fusion protein bound to glutathione-Agarose beads. Lysates were subject to western blot analysis by using pan-Ras as Primary antibody.

Pituitary Basal	Pituitary+ 9CYP	MCF7	MCF7Rap63E	HEK293T
20050	5838	1373	25849	2451

The table shows the Densitometry results (Arbitrary Units). Similar results were obtained in five experiments using different Pituitary cell lines (lines 1, 6, 10, 15 and 40).

Results show that Rap1GTP (active Rap) levels in Pituitary cells are similar to those found in MCF7 cells transfected with a Rap1GTP constitutively active mutant. Inhibitory effect of 9-Cyclopentiladenine on Rap activation shows the dependence of that activation on Adenylate Cyclase catalytic activity.

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P26

Delayed visual loss is not an uncommon complication of Cabergoline treatment for macroprolactinoma.

Gerald Raverot, Maud Jacob, Emmanuel Jouanneau, Alain Vighetto, Françoise Borson-Chazot

¹Hopital Neurologique, Hospices Civils de Lyon, Lyon, France, ²Inserm U842, Lyon, France, ³Universite Lyon1, Lyon, France

Context: Among side-effect of medical treatment of macroprolactinoma, secondary deterioration in visual fields is rare and described only one time with cabergoline.

Objective: The aims of our study were to evaluate: i) the frequency of delayed visual field defect due to chiasm herniation in patient with macroprolactinomas treated by cabergoline, ii) our medical management of this side-effect.

Design: Retrospective cases series.

Patients: The study included 28 patients (11 women and 17 men) aged 14-85 years treated for macroprolactinoma with cabergoline at our center from 1997 to 2006. Inclusion criteria were: serum prolactin levels > 200 mg/l and a pituitary tumor >1 cm in diameter on pituitary magnetic resonance imaging (MRI).

Results: Delayed visual field defect due to chiasm herniation was documented in 3 men with macroprolactinoma which presented initially with elevated prolactin levels and visual field defects. In 2 out of the 3 men the visual fields worsen 6 to 8 month after normalization of the initial visual field defect. For one patient initial visual defect was not improved by cabergoline treatment because of the rapid tumor shrinkage and chiasm herniation. In all 3 cases, visual field was restored after cabergoline withdraw.

Conclusions: These data demonstrate that delayed visual field defect by chiasm herniation could be associated with cabergoline treatment of macroprolactinomas, and could be corrected by medical management only without surgery (chiasmopexia).

P27

Tumor Volume Analysis of Non-functioning Pituitary Adenoma Growth

John Carmichael, Vivien Bonert, Barry Pressman, James Tourje, Adam Mamelak, Shlomo Melmed

Cedars-Sinai Medical Center, Los Angeles, CA, United States

INTRODUCTION: To evaluate growth of non-functioning adenomas (NFA), we retrospectively reviewed records of patients harboring NFAs prior to surgical intervention.

METHODS: Subjects harboring NFA with 2 or more serial MRIs performed a minimum of 3 months apart were included. Exclusion criteria included prior radiation therapy and prolactin levels >100ng/ml. Subjects receiving dopamine agonists or somatostatin analogues were analyzed separately. Pituitary MRI studies were de-identified and read independently by 2 neuroradiologists. Tumor volume was estimated using the Di Chiro formula ($4\pi/3 \times \text{height} \times \text{width} \times \text{length}$). First and last scans were used for tumor size comparison. Growth was defined as an increase in greatest diameter >1mm and >10% increase in volume. Comparison of groups was performed using the Student's t-test or Mann-Whitney U test. This study was approved by the Cedars-Sinai Medical Center IRB.

RESULTS: Twenty-six subjects were analyzed (10M/16F; 13NFMicros/13NFMacros). Median follow-up for



NFMacros was 6 months (range 3-58 months) and for NFMicros 15 months (range 7-45 months). There was a significant difference in age at diagnosis between NFMacros ($57.1y \pm 16.0$) and NFMicros ($34.1y \pm 9.9$) ($p < 0.001$). Change in tumor volume (ΔTV) and growth rate by volume (GRV) was higher in NFMacros compared to NFMicros (ΔTV : 368 ± 566 vs. 13 ± 59 mm³; GRV: 63 ± 138 vs. 0.7 ± 2.8 mm³/month; $p < 0.05$ for both). No significant difference in growth as assessed by greatest diameter was detected between NFMacros and NFMicros. When subjects receiving dopamine agonists or somatostatin analogues were excluded from analysis, the NFMacros ($n=8$) had a higher mean change in TV and higher GRV than NFMicros ($n=9$) (ΔTV : 466 ± 422 vs. 13 ± 49 mm³; GRV: 98 ± 146 vs. 0.5 ± 2.5 mm³/month; $p < 0.001$ for both). CONCLUSIONS: NFMacros demonstrate a significantly higher pre-operative growth rate compared to NFMicros when assessed by volume changes. This increase in growth is not reflected by changes in greatest diameter.

P28

Case report: prolactinoma in the clivus

Alessandra Fusco¹, Antonio Bianchi¹, Eugenia Sacco¹, Francesco Doglietto², Vincenzo Cimino¹, Alessandro Ciampini², Antonella Giampietro¹, Liverana Lauretti², Giulio Maira², Laura De Marinis¹

¹Department of Endocrinology, Catholic University School of Medicine, Rome, Italy, ²Department of Neurosurgery, Catholic University School of Medicine, Rome, Italy

A 68 year-old man came to our attention for the sudden onset of diplopia due to a left sixth cranial nerve palsy. Neuroradiological examination documented a space occupying lesion of the left superior third of the clivus, which was interpreted as a chordoma. Routine pre-operative tests were normal, with a mild hyperprolactinemia. The patient underwent transsphenoidal surgery and a partial excision of the lesion was possible. Histological examination ruled out a bone tumour and showed pituitary cells that were immunohistochemically positive for PRL. The patient underwent a thorough post-operative hormonal screening, which documented high PRL levels. At one year follow-up he is undergoing medical therapy with cabergoline and the sixth cranial nerve deficit has resolved completely. Though rarely, pituitary adenomas can involve the clivus, either as ectopic lesions or as extensions of small intrasellar tumours.

P29

A dopamine-agonists resistant macroprolactinoma responsive to combined cabergoline and octreotide treatment

Alessandra Fusco¹, Francesca Lugli¹, Antonio Bianchi¹, Laura Tilaro¹, Vincenzo Cimino¹, Teresa Porcelli¹, Domenico Milardi¹, Marilda Mormando¹, Francesco Doglietto², Giulio Maira², Alfredo Pontecorvi¹, Laura De Marinis¹

¹Department of Endocrinology, Catholic University School of Medicine, Rome, Italy, ²Department of Neurosurgery, Catholic University School of Medicine, Rome, Italy

We report the history of a 20 years old affected by macroprolactinoma diagnosed 5 years ago. He presented with severe bitemporal hemianopsia and anterior and posterior pituitary insufficiency. MRI showed an invasive pituitary mass, extending into both cavernous sinuses and the optic chiasm. Prolactin levels at diagnosis were higher than 14.000 ng/ml. Medical therapy with cabergoline was started, which resulted in decrease of prolactin levels without complete normalization. However, the visual field defect worsened with amaurosis in left eye. The patient was therefore operated through transsphenoidal approach. Post-surgical MRI showed the persistence of a voluminous residual adenoma.

Due to further worsening of visual defect, the patient underwent one other neurosurgical intervention and a significant reduction of tumour mass was obtained. Serum prolactin levels decreased without normalization even at high doses of cabergoline. Tumor tissue showed positive immunostaining for prolactin and p53 and presented with a Ki67 value of 5%. The patient underwent scintigraphy with ¹¹¹In-pentetreotide which revealed a very intense tracer uptake in the sellar region. Administration of long-acting octreotide was therefore started. After six months of therapy, prolactin levels normalized and MRI did not reveal any tumour progression. This is a case of a macroprolactinoma successfully controlled, after neurosurgery, by combined approach with neurosurgery, cabergoline and octreotide.



P30

Comparison of Fully Endoscopic Endonasal and Endonasal Microscopic Transsphenoidal Surgery for Pituitary Adenoma

Adam Mamelak, Vivien Bonert, John Carmichael, Barry Pressman, Shlomo Melmed

Cedars-Sinai Medical Center, Los Angeles, CA, United States

Rationale: Controversy exists as to whether fully endoscopic pituitary surgery has advantages over endonasal microscopic surgery, and this controversy causes confusion to both patients and treating physicians. A review of 64 consecutive microscopic and endoscopic cases was performed to determine if either method yields superior results.

Methods: Results of endonasal microscopic (EM) transsphenoidal pituitary tumor surgery were compared to fully endoscopic endonasal (EE) surgery performed over a 15 month period. Outcomes measured were MRI-determined extent of resection, endocrine response, and surgical complications. In addition, pre-operative surgeon assessment of extent of resection was compared to actual result achieved.

Results: 64 consecutive endonasal transsphenoidal procedures were reviewed, including 30 EM and 34 EE procedures. Tumor pathologies were non-functioning in 37(20 EM, 17 EE) ACTH secreting in 1 (0 EM, 1 EE), 18 GH secreting (7 EM, 11 EE), 2 prolactinoma (1 EM, 1 EE), and 6 Rathke's Cleft cyst (3 EM, 3 EE). A gross total resection was planned in 28 EE and 24 EM cases based on pre-operative imaging. Extent of resection based on MRI evaluation at 3 months to 1 year after surgery revealed a gross or near gross total removal in 31 EE and 20 EM procedures. Evidence of hormonal control in patients with ACTH or GH secreting adenomas at 3 months after surgery was 3/7 (43%) in EM cases, and 8/12 (67%) in EE cases. Both patients with PRL secreting tumors underwent planned debulking due to known cavernous sinus invasion. Two patients with GH secreting tumors had subtotal resections which the surgeon attributed to more limited visualization with the EM method. Complications were similar across the EM and EE groups. There were no deaths, vascular injuries or persistent CSF leaks. Overall patient satisfaction seemed higher in the EE group with less complaints of prolonged sinus congestion. The EE method provided wider surgical exposure, greater ease of instrument manipulation and better visualization of the cavernous sinuses and suprasellar cistern compared with the EM method.

Conclusions: Both EM and EE approaches resulted in good surgical outcomes but the EE method achieved more total removals and higher remission of endocrine active tumor in this limited series. In our opinion EE provides superior visualization and is the preferred method of transsphenoidal surgery for most pituitary adenomas

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Differential expression of GPR54 in pituitary adenomas

Maria Chiara Zatelli¹, Andrea Luchin¹, Federico Tagliati¹, Daniela Piccin¹, Marta Bondanelli¹, Massimo Scanarini², Angelo Margutti¹, Ettore C degli Uberti¹

¹Section of Endocrinology, Dept of Biomedical Sciences and Advanced Therapies, University of Ferrara, Ferrara, Italy, ²Division of Neurosurgery - Hospital of Padova, Padova, Italy

The KiSS-1 gene encodes kisspeptins, the endogenous agonists for the GPR54 receptor. The kisspeptin/GPR54 system is a key regulator of the reproductive system, but KiSS-1 was initially discovered as a metastasis suppressor gene. The role of the kisspeptin/GPR54 system in pituitary adenoma development has never been investigated, so far. We therefore aimed at evaluating the expression of the KiSS-1/GPR54 system in normal pituitary and in a series of pituitary adenomas. The samples underwent total RNA isolation and then RT-PCR analysis was performed. Our results confirmed that GPR54, but not KiSS-1, is expressed in normal pituitary. GPR54 expression was found in 30% of the 57 examined pituitary adenomas. Gene expression analysis was further confirmed by immunofluorescence, that demonstrated GPR54 protein on the membrane of GPR54 expressing pituitary adenoma cells in primary culture. Co-immunofluorescence with alpha-subunit, PRL or ACTH confirmed the pituitary origin of the cultured cells. No correlation with patients characteristics (sex, age, disease duration) or tumor diameter was evidenced. However, GPR54 expression was not evident in recurrent and/or aggressive pituitary adenomas. In addition, GPR54 expression was different among the various histotypes, since it was found in 57% of 14 ACTH-secreting, in 36% of 14 PRL-secreting, in 30% of 14 non functioning pituitary adenomas (NFA), but in none of 15 GH-secreting pituitary adenomas.

These data indicate that GPR54 is variably expressed in pituitary adenomas, suggesting a possible role for the kisspeptin/GPR54 system in the development of pituitary adenomas.

EGF receptor - mediated control of pituitary tumor growth and hormone secretion.

George Vlotides, Ines Donangelo, Shiri Gutman, Song-Guang Ren, Shlomo Melmed

Division of Endocrinology, Cedars-Sinai Research Institute, University of California School of Medicine, California, United States

Epidermal growth factor (EGF) and its receptor (EGFR) are implicated in pituitary development, hormonal regulation and cell proliferation. We examined EGFR-mediated pituitary signaling to characterize effects of EGFR blockade on cell proliferation and hormonal regulation in vitro and in vivo.

EGF modestly inhibited GH3 cell proliferation and Pttg1 expression, but attenuated GH and potently enhanced both baseline and serum-induced PRL. EGF had no effects on AtT20 cell proliferation, Pttg1 and POMC gene expression.

Gefitinib, an EGFR antagonist, suppressed serum-induced cell proliferation and Pttg1 expression in GH3 cells, increased baseline / serum-induced GH and decreased PRL, and reversed EGF-mediated lactotroph phenotype switching.

Specific inhibitors for EGFR (gefitinib), ERK (U0126), PI3K (LY294002), PKA (H89) and PKC (GF109203X) but not Rho- (Y-27632), Src- (PP2) and JAK tyrosine kinase (JAK inhibitor I) indicated involvement of ERK and PI3K in the gefitinib-mediated response. Despite the potent effect of EGF on ERK phosphorylation, it failed to activate PI3K. In contrast, serum activated both pathways, suggesting a role of ERK and PI3K signaling for GH3 cell growth. Gefitinib dose-dependently suppressed both EGF and serum-induced ERK activation.

GH3 cells were implanted sc into female NCRNU athymic nude mice and oral gavage was performed in unanaesthetized animals. Gefitinib (125 mg/kg; 5 day per week regimen; 3 weeks) vs. vehicle (0.5% Tween 80)-treated animals resulted in ~ 30% decrease in tumor volume and impaired weight gain (~ 16%; $p < 0.001$), associated with a ~ 26% decrease in serum IGF-I levels ($P < 0.01$).

These results indicate gefitinib-mediated inhibition of EGF-induced lactotroph phenotype and serum-induced GH3 cell proliferation, mediated primarily through blockade of EGFR / ERK signaling. Pharmacological tyrosine kinase inhibition with gefitinib in a subset of lacto-somatotroph adenomas expressing EGFR could provide a useful tool for clinical control of pituitary hormone secretion and tumor load.

ABSTRACTS

Acromegaly

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Long-term (up to 18 years) efficacy on GH/IGF-1 hypersecretion and tumor size of primary somatostatin analog therapy in patients with growth-hormone secreting pituitary adenoma responsive to somatostatin analogs

Jean-Christophe Maiza¹, Delphine Vezzosi¹, Maria Matta¹, Florence Donadille¹, Florence Loubes², Maxime Cournot³, Antoine Bennet¹, Philippe Caron¹

¹Department of endocrinology, CHU Rangueil, Toulouse, France, ²Department of Neuroradiology, CHU Rangueil, Toulouse, France,

³Department of Epidemiology, CHU Rangueil, Toulouse, France

The place of somatostatin analogs (SSTa) is debated in the therapeutic armamentarium of acromegaly. During this clinical study, we evaluate prospectively the anti-hormonal and anti-tumor efficacy of long-term primary treatment with SSTa in patients with GH-secreting pituitary adenoma responsive to SSTa. Thirty six (17 men, 19 women) acromegalic patients (aged 17-75), (GH post oral GTT > 1 μ g/L, increased IGF-1 for age and sex), were monitored in a single centre and treated with SSTa as first-line therapy. The mean pre-treatment GH level was 13.5 +/- 3.1 μ g/L, and IGF-1 (as a % of the value over the normal range) was 302 +/- 26 %. The patients had macroadenoma (n = 25), microadenoma (n = 8) or empty sella turcica (n = 3). The mean duration of treatment was 8 years (3-18 years). Hormonal and morphological monitoring was undertaken after 6 months, and then the patients were followed annually. After 1 year, the mean GH and IGF-1 levels had considerably reduced (GH = 2.4 +/- 0.3 μ g/L, IGF-1 = 174 +/- 14%, p < 0.01), and they continued to decrease over 10 years with a mean GH level of 1.6 +/- 0.1 μ g/L and IGF-1 of 123 \pm 18% (p = 0.02). GH < 2 μ g/L, normal IGF-1, or both were observed in 25 (70 %), 24 (67 %) and 21 (58 %) patients, respectively. The mean reduction in tumor volume was 43 % (13 - 97 %) and shrinkage > 20 % was obtained in 21 patients (72 %). SSTa treatment was well tolerated with few digestive or metabolic side effects. In conclusion, this clinical study confirms that long-term (up to 18 years) treatment with SSTa used as first line therapy is effective both from an anti-hormonal and anti-tumor perspective, and it is well tolerated in acromegalic patients.

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Gamma-Knife Surgery (GKS) in patients with acromegaly: safety and efficacy

Debora Katz¹, Karina Miragaya¹, Eduardo Tenca², Alejandro Margni², Carlos Artes², Julio Antico²

¹Endocrinology-FLENI, Buenos Aires, Argentina, ²Gamma Knife Unit-FLENI, Buenos Aires, Argentina

Introduction: Acromegaly is associated with significant morbidities and a 2 to 3-fold increase in mortality that of the general population. Because surgical and medical therapies for acromegaly all have specific limitations, stereotactic radiosurgery by Gamma-Knife has been used as a therapeutic option.

Objectives: We wanted to evaluate the efficacy and safety of GKS in patients with acromegaly.

Methods: 34 patients with active acromegaly (14 men and 20 women, with a mean age of 48 \pm 23 years) were prospectively studied over a six year period. 29 patients had macroadenoma, 27 had previous transphenoidal surgery whereas in 3 GKS was administered after tumor shrinkage with cabergoline. Two also previously received conventional radiotherapy. All patients were treated with multiple isocenters (range 10-15), using 4 mm collimators. The 55% isodose was used in all patients. The mean margin dose was 30 \pm 5.2 Gy (range 18-35), and the dose to the visual pathway was always less than 7 Gy. Baseline and follow-up studies involved magnetic resonance imaging, hormone evaluation and neuroophthalmologic examination every 3 months after GKS.

Results: The median follow-up was 21.2 months (range 3-67). Only 10 patients could have been followed for at least 3 years after the GKS. Five of these 10 patients were in remission by the 3rd year. After the first year of follow-up, only one patient was in remission. After the 2nd year two more patients fulfilled these criteria and at the end of the 3rd two more did so. No patient complained of side-effects. No patient developed visual deficit. Anterior pituitary failure was not detected.

Conclusions: Our data suggest that GKS could be an effective and safe adjuvant therapy for acromegalic patients that have displayed suboptimal response to conventional therapy. Longer follow-up is needed for a more complete assessment of late toxicity and treatment efficacy.



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Radiotherapy cures severe headaches initially responsive to octreotide in acromegaly

Rebecca Cox, Niki Karavitaki, John AH Wass

Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, United Kingdom

Headaches are found at presentation in approximately 55% of acromegalics; they have no clear definitive cause and their mechanism may be multifactorial. In a small number of cases, they can be debilitating and can be relieved only by regular subcutaneous injections of octreotide. We present three patients with acromegaly suffering from severe headaches alleviated with somatostatin analogues and subsequently improved significantly or cured after radiotherapy.

Case 1: A 19-year old female with acromegaly treated by surgery and external irradiation had a history of long-standing headaches, non responsive to a number of medications. Subcutaneous injections of octreotide provided significant relief (dose up to 100 mg up to 12/day). Over the next 10 years, the patient achieved safe GH levels and the headaches remitted no longer requiring octreotide.

Case 2: A 58-year old female with acromegaly treated by surgery and radiotherapy suffered from severe headaches improving with subcutaneous injections of octreotide (dose up to 100 mg up to 6/day). Over the next 4 years, the patient achieved safe GH levels and the headaches improved allowing reduction of the dose of octreotide.

Case 3: A 32-year-old female with acromegaly treated by surgery and radiotherapy had severe headaches finally controlled by subcutaneous injections of octreotide (dose up to 200 mg up to 4/day). Over the next 10 years, the patient achieved safe GH levels and the headaches remitted no longer needing somatostatin analogue administration.

Headaches in acromegaly are not clearly understood. The amelioration or cure of severe headaches responding only to regular octreotide injections can be achieved long-term by external beam irradiation. This suggests that the tumour and/or its hormonal activity are implicated in the pathogenesis of the headaches in subjects with acromegaly.

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Long-term treatment of acromegaly with pasireotide (SOM230): preliminary results from a Phase II extension study

Stephan Petersenn¹, Joan E Glusman², Roger Abs³, Ariel Barkan⁴, Jochen Schopohl⁵, David Kleinberg⁶, Phillipe Caron⁷, Rolf Gaillard⁸, Ross Cuneo⁹, Annamaria Colao¹⁰, Bo Gao², Shlomo Melmed¹¹

¹University of Essen, Essen, Germany, ²Novartis Pharmaceuticals Corporation, East Hanover, United States, ³Universitair Ziekenhuis Antwerpen, Edegem, Belgium, ⁴University of Michigan Medical Center, Ann Arbor, United States, ⁵Medizinische Klinik Innenstadt, Munich, Germany, ⁶New York University Medical Center, New York, United States, ⁷CHU Rangueil-Larrey, Service d'Endocrinologie, Toulouse, France, ⁸CHUV Vaudois Service d'Endocrinologie/Diabetologie, Lausanne, Switzerland, ⁹Princess Alexandra Hospital, Department of Diabetes and Endocrinology, Brisbane, Australia, ¹⁰Dipartimento di Endocrinologia ed Oncologia Molecolare e Clinica, Università degli Studi di Napoli Federico II, Napoli, Italy, ¹¹Cedars-Sinai Medical Center, Los Angeles, United States

Introduction: Pasireotide (SOM230), the novel multi-receptor ligand somatostatin analogue, was evaluated in a 16-week Phase II study in patients with de novo, persistent or recurrent acromegaly. Preliminary results from the long-term extension to this study are presented.

Methods: This extension study enrolled patients who achieved biochemical control (GH \leq 2.5 μ g/L and normalized IGF-I), or derived clinical benefit, during 12 weeks' pasireotide treatment in the original core study. Patients received pasireotide at the dose (200, 400 or 600 μ g sc bid) at which biochemical control was achieved in the original core study or were permitted to dose escalate to 900 μ g sc bid, if required. Patients were continued on treatment for as long as benefit was derived. Efficacy and safety were assessed every 4 weeks. Efficacy was defined as GH levels \leq 2.5 μ g/L and/or normalization of IGF-I and was evaluated at 6 months.

Results: In the original short-term analysis of 59 patients, 64% of patients (n=38) achieved GH \leq 2.5 μ g/L or normalized IGF-I during at least one pasireotide treatment period. 33 patients (56%) had normalized GH and 29 patients (49%) had normal IGF-I levels. Gastrointestinal disturbances, headache and generally transient increases in blood glucose were the most common adverse events. HbA1C (normal range 4-6%)



was 6.01% \pm 0.57 SD at baseline (n=58) and 6.45% \pm 0.84 SD after 12 weeks' pasireotide treatment. Thirty patients entered the extension study. Of 25 patients who received pasireotide for at least 6 months, 72% (n=18) achieved GH \leq 2.5 μ g/L and/or normalized IGF-I. Adverse events were consistent with those observed in the core study.

Conclusions: These results indicate that in previously uncontrolled patients, pasireotide effectively controls acromegaly at 6 months, including patients resistant to prior surgery or medical therapy.

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The Majority of Recently Diagnosed Acromegalic Patients Receive Primary Medical Treatment: Interim Results from the International Study OASIS

Susan Webb¹, Lesly Portocarrero Ortiz², Güngör Akçay³, Hakim Bouterfa⁴, Michael Droste⁵, Yona Greenman⁶, Minseon Kim⁷, Jan Podoba⁸, Antonio P. Silva⁴, Alexander Tarasov⁹, Nicolás MJ Vitale¹⁰, Moises Mercado¹¹

¹Hospital Sant Pau, Barcelona, Spain, ²National Institute of Neurology and Neurosurgery, Department of Endocrinology, Mexico City, Mexico, ³Ataturk University Hospital, Erzurum, Turkey, ⁴Novartis Pharma AG, Basel, Switzerland, ⁵Oldenburg, Germany, ⁶Tel Aviv Medical Center, Tel Aviv, Israel, ⁷Asian Medical Center, Seoul, Korea, Republic of, ⁸First Internal Clinic University Hospital, Comenius University, Bratislava, Slovakia, ⁹Regional Endocrinology Center, Ekaterinburg, Russian Federation, ¹⁰Hospital Oftalmologico Santa Lucia, Buenos Aires, Argentina, ¹¹Endocrinology Service, Hospital de Especialidades, Centro Medico Nacional Siglo XXI, IMSS, Mexico City, Mexico

There is significant interest in how different treatment regimens impact the clinical course of acromegaly. This study has been designed to better understand the clinical decision making and outcomes in the context of various real-world treatment options.

The Observational Acromegaly Study on Impact of Sandostatin LAR (OASIS) is an international, observational study in recently diagnosed acromegalic patients. Observations include biochemical parameters, acromegaly symptoms, tumor volume, safety and tolerability. Patient data are collected under normal practice conditions over 12 months. It is planned to enroll more than 700 patients from approximately 160 centers worldwide. Ethical committee approval was obtained where applicable.

As of March 2007, 560 patients have been enrolled from 123 centers in 22 countries, representing one of the largest international, prospective acromegaly registries to date. Baseline characteristics of the first 129 patients with available data are reported here. More than half of the patients are female (61%), the majority (64%) are Caucasian, the mean age is 48 years. Most patients (69%) have a diagnosed macroadenoma. Available baseline mean levels were GH 23.3 ng/mL in 118 patients and IGF-1 731 ng/mL in 101 patients. At first quarter follow-up data were available for 53 patients with mean GH 15.4 ng/mL and for 41 patients with mean IGF-1 486 ng/mL.

Fifty four percent of patients (n=70) were initially treated with Octreotide LAR; 5% (n=7) with surgery + Octreotide LAR; 32% (n=42) with surgery alone; 1 with radiotherapy; 1 with radiotherapy + surgery; and 1 with other. The most common Octreotide LAR starting dose was 20 mg (75% of patients) followed by 10 mg (19%) and 30 mg (4%).

The majority of patients received medical treatment with Octreotide LAR as part of their initial management, in contrast to the current treatment algorithm. Follow-up data will clarify whether this represents primary medical therapy or pre-surgical treatment with Octreotide LAR.



Infrequent Use of Octreotide Test Dose in the Medical Treatment of Recently Diagnosed Acromegalic Patients: Interim Results from the International Study OASIS

Pinar Kadioglu¹, Lesly Portocarrero Ortiz², Christina Alvarez³, Feng Gu⁴, Hakim Bouterfa⁵, Mirtha Guitelman⁶, Sungwoon Kim⁷, José Fernando Botero⁸, Josef Marek⁹, Chiung-Chyi Shen¹⁰, Antonio P. Silva⁵, César Varela¹¹, Henri Wallaschofski¹², Moises Mercado¹³

¹Istanbul University Cerrahpaşa#351; a School of Medicine Endocrinology Department, Istanbul, Turkey, ²National Institute of Neurology and Neurosurgery, Department of Endocrinology, Mexico City, Mexico, ³Hospital La Paz, Madrid, Spain, ⁴Peking Union Medical College Hospital, Beijing, China, ⁵Novartis Pharma AG, Basel, Switzerland, ⁶INUBA Neurosciences Institute School of Medicine, Buenos Aires University, Buenos Aires, Argentina, ⁷Kyung Hee University, Seoul, Korea, Republic of, ⁸Endocrino S.A. Centro de Estudios Hormonales, Medellín, Colombia, ⁹Third Department of Internal Medicine, 1st Medical Faculty, Charles University, Prague, Czech Republic, ¹⁰Taichung Veterans General Hospital, Taichung, Taiwan, Province of China, ¹¹Hospital Ramón y Cajal, Madrid, Spain, ¹²Dept. of Gastroenterology, Endocrinology and Nutrition, University of Greifswald, Greifswald, Germany, ¹³Endocrinology Service, Hospital de Especialidades, Centro Medico Nacional Siglo XXI, IMSS, Mexico City, Mexico

Test dose of octreotide provides information on drug tolerability and is recommended prior to Sandostatin LAR therapy. There are discrepancies as to whether test dose is predictive of response to treatment. This study provides information on world wide test dose practices and association between test dosing and clinical efficacy and safety.

The Observational Acromegaly Study on Impact of Sandostatin LAR (OASIS) is an international, observational study in recently diagnosed acromegalic patients. Data collected include GH, IGF-1, acromegaly symptoms, tumor volume, safety and tolerability. Patient data are collected under normal practice conditions over 12 months. It is planned to enroll >700 patients from ~160 centers worldwide. Ethical committee approval was obtained where applicable.

As of March 2007, 560 patients are enrolled from 123 centers in 22 countries. Baseline characteristics and test dose information from the first 155 patients with available data are reported. Most patients are Caucasian (64%), female (61%), harbor macroadenomas (69%), with a mean age is 48 years. Of 149 patients with test dose information, only 32% had a test dose. Greatest proportion of patients with test dose is observed in Asia (54%), followed by Western Europe (39%), Eastern Europe (25%), Latin America (11%) and Middle East (0%). Test dose was not associated with incidence of adverse events (AEs) after treatment start. In patients with both data points available, baseline and 3 month GH values were 33.8 ng/mL and 9.9 ng/mL, respectively (n=40); IGF-1 values were 689 ng/mL and 453 ng/mL, respectively (n=32). Linear regression, adjusted for age, sex and treatment type demonstrated that test dose was not predictive of change in GH (p=0.69) or IGF-1 at 3 month post-treatment (p=0.49).

Test dosing with octreotide is infrequent in the real-world setting and varies by world region. Test dose is apparently not associated with AEs or with change in GH or IGF-1 at 3 month post-treatment.

Multiorgan Dysfunction Syndrome following Sandostatin-LAR® injection

Harald Etzrodt, Athanasios Alexopoulos, Gwendolin Etzrodt-Walter

Gemeinschaftspraxis Dr. H. Etzrodt Dr. A. Alexopoulos, Ulm, Germany

A 61-year-old man suffering from acromegaly, was stable under therapy with octreotide-LAR, since april 1998 10 mg every month, since july 1999 20 mg, and since november 2000 30 mg. Injections were well tolerated, acromegaly was controlled: growth hormone of 1.6 mg/ml and IGF1 of 168 ng/ml.

In august 2006, 3 days after Sandostatin-LAR® was injected into the left gluteal muscle, the patient presented with fever and pain in the left gluteal area, diarrhea and nausea, the gluteal region unsuspecting on inspection, slightly painful at palpation, and fever of 40°C. Initial laboratory analyses and ultrasound of the gluteal muscle were unremarkable. The following night, the patient became instable, cardiovascular shock symptoms required catecholamines. A tricytopenia and consumption-coagulopathy developed with need for substitution. Creatinkinase reached 13.000U/l. Ultrasound of the gluteal region now showed pinnation of the muscle.

Large debridement and resection of the gluteus muscle was performed. The patient needed long-term artificial ventilation and hemodialysis. The pathologist saw necrotic muscle with no indication of any cause.

The patient recovered. Secondary healing was disturbed by several infections. Actually the patient is back from rehabilitation and back to normal life. Again his acromegaly needs treatment.

The reason of this dangerous complication remains unclear. Any abscess or collection of pus was seen neither by the surgeon nor by the pathologist. One explanation may be the sportive activity of the patient. 3 days prior to injection of Sandostatin-LAR® the patient participated in carriage-riding competition. We therefore assume that the muscle has been harmed by intensive strain and therefore injection of Sandostatin-LAR® caused large necrosis of muscular tissue. Consequently before treatment with sandostatin-LAR®, patients should be discouraged from sportive activity which may harm the gluteal region.

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Evaluation of ApneaLink as a screening device for sleep apnoea syndrome in patients with acromegaly

Christian Berg¹, TE Wessendorf², Harald Lahner¹, H Teschler², Klaus Mann¹, Stephan Petersenn¹

¹Clinic of Endocrinology, University of Duisburg-Essen, Essen, Germany, ²Ruhrlandklinik, University of Duisburg-Essen, Essen, Germany

Obstructive sleep apnea syndrome (OSAS) is an important comorbidity in patients with acromegaly. Polysomnography (PSG) is considered the gold standard in the diagnosis of sleep apnea syndrome (SAS). However, in clinical practice because of costs and labor-intensity it is not performed routinely. ApneaLink, a newly developed screening device is based on respiratory pressure measurements via nasal canula as an alternative sensitive method for SAS detection in combination with measurement of oxygen desaturation. This method allows analysis of apnoeas, hypopnoeas, snoring and oxygen desaturation. Aim of this study was to evaluate the reliability of ApneaLink in diagnosing OSAS in patients with acromegaly, simultaneously studied by PSG in a sleep laboratory and by the ApneaLink method.

In 11 patients with confirmed acromegaly suspected of having OSAS (5 men, mean age 42 [28-57], mean IGF-I SDS 1.54 [1.06-3.97]), we compared ApneaLink-generated analysis with results of simultaneously collected PSG. All patients were treated for acromegaly and on stable doses of sandostatin (n=6), pegvisomant (n=3) or cabergoline+sandostatin (n=2). 3/10 patients had active acromegaly with elevated IGF-I SDS. Apnea hypopnea index (AHI) was defined as number of apnoeas and hypopnoeas /h of sleep. The polysomnographic criterion for OSAS was a more than 10 apnea-hypopnea episodes plus micro-awakenings related to respiratory efforts /h of sleep.

ApneaLink- and PSG-generated numbers of apnoeas ($r = 0.91$) and hypopnoeas ($r = 0.81$) as well as AHI ($r = 0.92$) correlated highly, displaying mean difference in AHI of 3.5/h. Sensitivity and specificity for SAS was 100% and 86.8%, at SAS-defining AHI of 10. Mean (94%, $r=0.90$) and minimal (86%, $r=0.90$) oxygen concentration correlated highly.

There is high concordance of AHI and ODI between the two methods. ApneaLink-generated AHIs are highly sensitive in detecting SAS in patients with acromegaly. ApneaLink, therefore, is a simple screening device for SAS in patients with acromegaly.

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A CASE OF ACROMEGALY WITH SEVERE IMPAIRMENT OF GLUCOSE HOMEOSTASIS

Antonio Bianchi¹, Laura Tilaro¹, Eugenia Sacco¹, Teresa Porcelli¹, Flora Veltri¹, Antonella Giampietro¹, Domenico Milardi¹, Francesco Doglietto², Annunziato Mangiola², Carmelo Anile², Giulio Maira², Laura De Marinis¹

¹Department of Endocrinology, Catholic University School of Medicine, Rome, Italy, ²Department of Endocrinology, Catholic University School of Medicine, Rome, Italy

A 48-year-old woman affected by acromegaly, diagnosed on 1996 for amenorrhea and visual field defects and not controlled by medical treatment (Somatostatin Analogs, SSA), underwent four folds (1996-2004) to neurosurgical treatment for an invasive GH-secreting pituitary adenoma with high proliferative index. On February 2005 patient showed symptoms characterized by intense asthenia, nausea, unexpected hunger, hyperhidrosis and hypoglycemia for which she was admitted to our Center for a clinical reevaluation. Biochemical evaluation showed severe hypoglycaemia with very high levels of insulin (13000 μ UI/ml). In the suspicion of an insulinoma a total body computed tomography and a selective intra-arterial calcium injection with hepatic venous sampling were performed but no evidence of pancreatic localization of an



insulinoma was found. An octreotide scan was negative. No other multiple endocrine disorders were diagnosed. For recurrence of hypoglycaemic episodes diazoxide treatment was started. On may 2005 for the persistent inadequately controlled GH (9,7 ng/ml) and IGF-I (738 ng/ml) levels, after neurosurgery and SSA, treatment with GH receptor antagonist pegvisomant was started. In few months a normalization of IGF-I level was achieved (298 ng/ml) and a significant reduction of insulin level was obtained (275 μ UI/ml) with withdrawn of diazoxide and disappearance of hypoglycaemic episodes. This case report support the important direct effects of GH on glucose metabolism and shows that pegvisomant could improve insulin sensitivity in acromegaly.

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FAMILIAL ACROMEGALY

Gonca Oruk, Orhan Kucuksahin, Dilek Soysal, Erhan Tatar, Cigdem Ozdemir

Ataturk Training and Research Hospital, Izmir, Turkey

Acromegaly is a syndrome caused by oversecretion of growth hormone (GH) due to a GH producing pituitary adenoma. Acromegaly is usually regarded as a disease which occurs sporadically. Familial occurrence of acromegaly due to pituitary adenoma without any other endocrinopathy is extremely rare. These patients should also be evaluated for multiple endocrine neoplasia type 1 (MEN 1) and Carney complex. Because of the low incidence of acromegaly in MEN 1 and in Carney complex and high penetrance of tumors in other endocrine glands, the presence of two or more cases of acromegaly in a family without other manifestations strongly suggests an inherited pituitary syndrome distinct from these syndromes. Because of the rare occurrence we report here a Turkish family with two members, a son and his mother with acromegaly and without any other manifestations.

Subject 1 is a 50-year-old woman and subject 2 is her 23-year-old son with elevated (GH) levels during OGTT and pituitary macroadenomas in Magnetic Resonance Imaging (MRI). Subject 1 was diagnosed nine years ago and had underwent transsphenoidal extirpation. But she was lost to follow up after the operation and she had not received any treatment. During her evaluation at the surgery unit for goitre it was noticed that her acromegaly symptoms were persisting, and she had recurrent pituitary macroadenoma in MRI. Transsphenoidal reoperation was not successful and medical treatment was applied. Subject 2 was diagnosed as acromegaly coincidentally when he was visiting his mother. He had high GH levels during OGTT and MRI revealed a macroadenoma of 10x15 mm. He was also treated with transsphenoidal surgery. We conclude that isolated familial acromegaly is an exceptional clinical entity, it may be more common than it has been realised. Genetic studies are needed to identify the responsible genes for this distinct syndrome.

P43 - WITHDRAWN

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Effects of Pegvisomant on Sleep Apnoea and Tongue Volume (MRI) in Patients with Acromegaly

Christian Berg¹, TE Wessendorf², J Goericke⁴, Burkhard Herrmann³, Klaus Mann¹

¹Clinic of Endocrinology, University of Duisburg-Essen, Essen, Germany, ²Ruhrlandklinik, University of Duisburg-Essen, Essen, Germany, ³Institute of Cardio-Diabetes, PPZ, Technology Centre, Bochum, Germany, ⁴Clinic of Radiology, University of Duisburg-Essen, Essen, Germany

Introduction: Sleep apnoea has been consistently reported to occur in acromegaly. In poorly controlled patients, the severity of sleep apnoea may influence physical activity in the daytime. To address this issue, we investigated the influence of disease activity on tongue volume and sleep apnoea in patients with acromegaly treated with the GH-receptor antagonist pegvisomant .

Patients and Methods: 14 patients with active acromegaly poorly controlled under octreotide (9 females; 5 males; mean age 55 \pm 14 yr; BMI 28.0 \pm 4.9 kg/m²; mean \pm SD) were switched to pegvisomant monotherapy (13.5 \pm 5.0 mg/die) over 6 months. Tongue volume was examined by magnet resonance imaging and sleep apnoea was characterized by polysomnography before and after 6 months treatment with pegvisomant. The initial tongue volume was significantly higher in patients with acromegaly (142 \pm 48 ml) in comparison to the BMI and age-matched healthy control group (97 \pm 5 ml, p=0.02).

Results: Interimresults of 7/14 were available. IGF-1 levels reduced after 6 months (361 \pm 161 to 212 \pm 71

µg/L; p=0.04). The tongue volume decreased significantly (142±48 to 105±25 ml; p=0.017) whereas the apnoea-hypnoea-index (AHI) only decreased as a tendency (26±26 to 22±26 /h; p=0.14).

Summary and Conclusion: In conclusion, successful treatment with pegvisomant can decrease tongue volume, which may have benefits for coexisting sleep disordered breathing.

P45

Assessment of the awareness and management of cardiovascular complications of acromegaly in endocrine referral centers in Italy - COM.E.T.A. (COMorbidities Evaluation and Treatment in Acromegaly) Italian Study Group

Andrea Giustina¹, Ernesto De Menis², Ettore degli Uberti³, Ezio Ghigo⁴, Enio Martino⁵, Francesco Minuto⁶, Paolo Boscani⁷, Annamaria Colao⁸

¹University of Brescia, Brescia, Italy, ²Ca' Foncello Hospital, Treviso, Italy, ³University of Ferrara, Ferrara, Italy, ⁴University of Turin, Turin, Italy, ⁵University of Pisa, Pisa, Italy, ⁶University of Genova, Genova, Italy, ⁷Ipsen S.p.A., Milan, Italy, ⁸University of Naples, Naples, Italy

The Italian COM.E.T.A. study group in order to assess the application in the clinical setting of the Versailles criteria for management of acromegaly complications prepared an ad hoc questionnaire on cardiovascular co-morbidities which was completed by 130 endocrine centers in Italy. According to answers received mild to severe hypertension is detected in 36-50% of acromegalics and diagnosis made by random BP assessment or ambulatory blood pressure monitoring (ABPM) (54% of the answers). Echocardiography (96%) and blood lipids (83.3%) are most frequently assessed. Antihypertensive treatment is mainly prescribed by endocrinologist (71.4%). Diabetes (79.4%), cardiac hypertrophy (62.7%) and arrhythmias (53.2%) are the co-morbidities which mainly influence antihypertensive prescriptions. Hypertension is successfully controlled by normalisation of GH/IGF-1 with somatostatin analogues (SSA) in only 20-60% of acromegalics. ACE inhibitors and ARBs are largely the first choice antihypertensive drugs (61%). Random BP every 3 months (61%) and yearly ABPM (23%) are mostly used in the follow-up. Annual echocardiography is recommended by most specialists (81.7%) as well as standard ECG (69.8%). The concomitant condition most frequently reported is left ventricular hypertrophy (LVH) (82.5%) which is related to duration of disease (73%) or poor control of GH/IGF-1 (53.2%). Congestive heart failure (CHF) has the greatest prognostic influence (74.6%) followed by cardiac ischemia (20.6%), arrhythmias (15.8%) and valvular diseases (8.7%). Finally for 68.2% of specialists biochemical control is key for planning modality of cardiovascular follow-up. In conclusion: 1. ABPM, echocardiography and blood lipids are investigated in most hypertensive acromegalic patients; 2. hypertension in acromegaly is prevalently managed by endocrinologists; 3. ACE inhibitors and ARBs are first choice antihypertensive treatment; 4. awareness of LVH and CHF in acromegaly is high whereas that of other cardiac complications is low; 5. SSA relevantly impact on LVH; 6. Echocardiography is mainstay for the diagnosis and follow-up of cardiovascular complications of acromegaly

P46

Ectopic Expression Of Glucose-Dependent Insulinotropic Polypeptide Receptor (GIP-R) In Pituitary Somatotropinomas: Relation To The Paradoxical Secretion Of GH?

G Occhi¹, M Losa³, N Albiger¹, M Olivieri¹, G Trivellini¹, M Scanarini², F Mantero¹, C Scaroni¹

¹Division of Endocrinology, Department of Medical and Surgical Sciences, Padova, Italy, ²Division of Neurosurgery, Hospital/University of Padova, Padova, Italy, ³Pituitary Unit, Department of Neurosurgery, Istituto Scientifico San Raffaele, Università Vita-Salute, Milan, Italy

Patients with active acromegaly show a lack of GH suppression (<1 µg/l) during an oral glucose-tolerance test (OGTT). Moreover, in about 20% of cases a paradoxical rise of GH (PRGH) during OGTT is observed. The mechanism of such paradoxical rise has recently hypothesized to be mediated by the glucose-dependent insulinotropic polypeptide (GIP) receptor, a G protein-coupled receptor. Similarly to food-dependent Cushing's syndrome, in which the ectopical expression of GIPR is sufficient to induce the symptoms of hypercortisolemia and the formation of a benign adrenocortical tumor, it was hypothesized that in somatotropinomas either the aberrant expression of GIPR or an altered regulated pathway of GIP



could stimulate GH secretion.

In present work we studied GIPR expression in somatotrinomas and correlated it with GH levels during OGTT.

An 2h OGTT with 75 g of glucose was performed in twelve patients with active acromegaly as part of the diagnostic process. After transphenoidal surgery GIPR expression was investigated by RT-PCR in tumor specimens. Three normal pituitary glands were collected at autopsy and used as controls.

Five out of twelve patients showed PRGH after OGTT with a mean increase of $50\% \pm 17\%$. In four cases the highest peak was observed within 60' (early), while in the fifth one only after 120'. In three "early" responders GIPR was detected by RT-PCR, while it was never found in patients without PRGH in normal pituitary glands and in the case with late response of GH to OGTT.

Present data support the hypothesis that GIPR, at least in some cases, might mediate the PRGH in acromegalic patients.

P47

Determination of precision and accuracy of two different methods for IGF-I compared to GH nadir on oGTT in Acromegalic Patients

LA Naves, L Vilar, PP Nascimento, LA Giacomini, LA Casulari

Endocrine Unit University of Brasilia, Brazil, Brazil

The evaluation of insulin-like growth factor 1 in serum (IGF-1) is a very important tool on diagnosis and follow up of acromegalic patients. Determination of IGF-I levels is useful as it correlates with clinical features of acromegaly and with the 24-hour mean GH levels. According to the most recent consensus, a random GH < 0.4 microg/l and IGF-I in the age- and gender-matched normal range exclude the diagnosis of acromegaly. If either of these criteria are not achieved, an OGTT should be performed, and then GHn < 1 microg/l during OGTT excludes acromegaly. Discrepancies between IGF-1 and GH levels have been reported in 15% of acromegalic patients, and impose some pitfalls on clinical evaluation. The aim of this study was to evaluate reproducibility and interassay variation coefficients of these two different methods used for IGF-1 determination and to compare the results to GH nadir on GTT in acromegalic patients. Methods: We studied 35 acromegalic patients recruited to collect fast blood samples to IGF-1 analysis and to perform oGTT with GH determination. All patients signed the informed consent. IGF-1 analysis was performed in triplicate by automated IMMULITE assay system (DPC, Los Angeles) and radioimmunoassay (RIA) with extraction. The precision and the interassay coefficient of variation were calculated for each method. These results were compared and correlated to GHn (IMMULITE 2000) on oGTT. Results: The patients were stratified by clinical status, as controlled (13 patients) and active (22 patients). The results have shown a very high correlation between the IGF-1 values obtained in the two assays ($r=0.94$, $p<0.001$). The mean coefficient of variation intraassay of IGF-1 for chemiluminescence was 4.94%, while for RIA, the CV was 9.67%. The correlation between GHn on oGTT and chemiluminescence was $r=0.71$ and the RIA was $r=0.66$. Conclusion: IGF-1 determination by RIA had a significantly higher variation interassay than chemiluminescence, that seems to have a better correlation to GH nadir on oGTT in acromegalic patients.



ABSTRACTS
CRH/ACTH/Cushing's



P48

EFFECT OF SHORT-TERM, HIGH DOSE CABERGOLINE IN PATIENTS WITH CUSHING'S DISEASE

Massimiliano Andrioli, Francesca Pecori Giraldi, Martina De Martin, Francesco Cavagnini

Chair of Endocrinology, University of Milan, Ospedale San Luca Istituto Auxologico Italiano, Milan, Italy

Introduction: Dopamine agonists, the treatment of choice for prolactinomas, have been employed also in ACTH-secreting pituitary tumors in view of the presence of D2 receptors in tumoral corticotrophs. In fact, bromocriptine and, most recently, cabergoline yielded variable results (Lamberts.1982; Invitti 1995, Casulari 2004, Pivonello 2004). A single study evaluated the effects of short term, standard dose, cabergoline treatment in pituitary corticotroph tumors and observed a significant inhibition of cortisol secretion in a subset of patients (Pivonello 2004). Aim of the present study is to assess the effects of short term, high dose cabergoline treatment in Cushing's disease.

Patients: Six patients with Cushing's disease (4 women, 2 men, age 40.7 ± 3.11 years, 4 relapses and 2 de novo diagnoses) were treated with cabergoline at progressively greater doses, starting at 0.75 mg/weekly and increasing up to 7 mg/weekly in 42 days, then maintained at this dosage for approx. 2 months. Urinary free cortisol (UFC), ACTH, serum cortisol, prolactin, GH and IGF-I were assessed before and at regular intervals during cabergoline treatment.

Results: treatment was well-tolerated in all patients and none reported side effects. Three patients exhibited a clear-cut reduction in UFC, reaching 44%, 45% and 68% of pretreatment values, respectively, although UFC did not normalize in any. No dampening of UFC secretion was observed in the other three patients. Prolactin levels became undetectable in all patients while no evident changes in other hormones were registered.

Conclusions: our results demonstrate that short-term, high dose, cabergoline treatment, blunts excess cortisol secretion in a subset of patients. However, in view of the recently reported increased risk of valvular heart disease during ergot derivative administration, treatment with cabergoline in patients with Cushing's disease should be considered carefully.

P49

Early Outcomes Of Endoscopic Transsphenoidal Surgery for Hormonally Active Pituitary Adenomas

John Jane, Jr, Adam Kanter, Edward Laws, Jr

University of Virginia, Charlottesville, Virginia, United States

Introduction: Endoscopic transsphenoidal surgery has gained increasing popularity for the treatment of pituitary adenomas. Although early reports on the surgical results of endoscopic transsphenoidal surgery for non-functioning pituitary macroadenomas are promising, few have documented the efficacy of the technique in treating hormonally active tumors. This paper examines our three-year experience utilizing a purely endoscopic transsphenoidal approach for the treatment of Cushing's disease and acromegaly.

Methods: We retrospectively reviewed the charts and radiographic studies from a prospectively acquired database of more than 200 patients who underwent purely endoscopic transsphenoidal surgery. Only patients with biochemically and pathologically confirmed ACTH and GH adenomas were included. All patients were evaluated pre- and post-operatively by both a neurosurgeon and an endocrinologist.

Results: A total of 37 patients underwent a purely endoscopic transsphenoidal surgery for the treatment of Cushing's Disease (20) or acromegaly (17). Eighteen patients had macroadenomas (50%) and 3 patients (all with Cushing's Disease) had negative magnetic resonance imaging. Nineteen Cushing's patients (95%) achieved immediate clinical remission and laboratory confirmed hypocortisolemia (serum cortisol of $<2\text{mg/dl}$) during their hospital course. Normalization of Insulin-like Growth Factor-1 (IGF-1) levels were achieved in 15 of 17 (88%) patients with acromegaly. A minimum of a 2 month post-operative follow-up period was obtained on all patients (mean: 3.6 mo). To date, no patient has acquired a new anterior pituitary deficiency.

Discussion: Our early results indicate that endoscopic transsphenoidal surgery is a safe and effective treatment for hormonally active pituitary tumors. The rate of endocrine remission is high (92%) and the rate of major post-operative complications is low. Patients require long-term follow-up for accurate assessment of disease recurrence and new endocrinopathies.



P50

ACTH-Secreting Adenoma Coexisting With Rathke's Cyst In A Patient Presenting With Cushing's Disease

CRL Scaglioni, VS Costa, MK Huayllas, KC Nogueira, B Liberman, A Cukiert

Hospital Brigadeiro, Sao Paulo SP, Brazil

Rationale: Rathke's cyst and ACTH-secreting adenoma are very rare pituitary lesions. We describe a patient presenting as Cushing's disease in whom both lesions were documented.

Case Report: RCS, a 23 years-old lady, presented with weight gain over the last 4 years, associated to acne, hypertrichosis and muscle weakness. She had a typical Cushing's phenotype, diabetes mellitus and hypertension. Plasma cortisol and ACTH levels were 17 mg/dL (non-suppressive) and 79.6 ng/ml, respectively, and free urinary cortisol level was 452 ug/24 hours. MRI showed a hypointense posterior sella cyst both on T1 and T2 sequences, and an otherwise normal adenohypophysis. She was submitted to transesphenoidal surgery and an intrasellar cyst containing mucoid material was evacuated. Pathological examination confirmed the presence of a Rathke's cyst. There was no modification in her hypercortisolemia. A post-operative MRI showed that the intrasellar cyst had been completely removed. The adenohypophysis remained normal-looking on MRI. She was submitted to bilateral petrosal sini catheterism and sampling which showed a central ACTH-secreting dynamics. She was submitted to a second transesphenoidal surgery, during which a microadenoma was found within the right portion of the pituitary gland. She developed adrenal insufficiency in the immediate postoperative period. Pathological examination found an ACTH-secreting adenoma.

Discussion: The association of ACTH-secreting adenoma and Rathke's cyst is extremely rare. Although Rathke's cysts might remain unchanged for long periods, many of them are quite big or can show increase in size during follow-up and need surgical removal. When this dual pathology is present, we believe that both lesions should be resected.

P51

Effect Of Preoperative Ketoconazole (KTZ) Treatment In The Timing For Adrenal Insufficiency Development After Successful Surgery In Patients With ACTH-Secreting Pituitary Adenoma

CG Chaves, LS Sales, TC Mattana, MK Huayllas, KC Nogueira, B Liberman, A Cukiert

Hospital Brigadeiro, Sao Paulo SP, Brazil

Rationale: Ketoconazole (KTZ) is an imidazole derivative which inhibits adrenal steroidogenesis and has successfully been used in the control of Cushing's disease. It could be used in an attempt to reduce hypercortisolemia and improve clinical condition prior to transesphenoidal surgery (TS) or after unsuccessful surgery while waiting for the effects from radiotherapy. There are no data in the literature concerning the effects of ketoconazole on the immediate postoperative cortisol dynamics and on the timing for the development of adrenal insufficiency (AI) after successful surgery. In this paper, we studied whether there was any influence of the preoperative use of KTZ on the timing for the development of adrenal insufficiency after successful transesphenoidal surgery.

Methods: We studied 7 patients with Cushing's disease submitted to TS who were treated with KTZ for at least XXXX months prior to surgery (mean dose= XXXX) (Group A) and compared them with 15 patients who did not received KTZ (Group B). All patients had successful surgery and developed adrenal insufficiency at the immediate postoperative period. No patient received glucocorticoid replacement therapy during/after surgery. Cortisol level during the first postoperative hours and the timing for adrenal insufficiency development were studied. Adrenal insufficiency was considered when cortisol plasma level was less than 5.0 mcg/dl associated to the classical AI symptoms or when cortisol plasma level was less than 2,0 mcg/dl in asymptomatic patients.

Results: There was no significant difference on the preoperative hormone levels between Group A and B patients. AI occurred $16,86 \pm 2,4$ hours postoperatively in Group A and after $27,8 \pm 3,0$ hours in group B ($p < 0,05$).

Discussion: AI developed significantly earlier in patients with Cushing's disease who had successful adenoma removal and received KTZ preoperatively, when compared to those who did not receive KTZ. Since AI might represent a life-threatening event, special attention should be drawn to those patients receiving KTZ preoperatively for earlier postoperative detection of AI signs and symptoms.

Free cortisol Index is useful in evaluating HPA axis and predicting 30-day mortality in patients with critical illness

Kyung-Won Kim¹, Seong-Jae Park², Dong-Hoon Shin¹, Sung-Hoon Yu¹, Eun-Jeong Lee¹, Sun-Wook cho¹, Yu-Jin Lee¹, Sang-Wan Kim³, Chan-Soo Shin¹, Seong-Yeon Kim¹

¹Seoul National University, Seoul, United States, ²Seoul National University Hospital Clinical Research Institute, Seoul, United States,

³Seoul National University Boramae Hospital, Seoul, United States

Inappropriate response of cortisol is related to mortality in critical ill patients. Those patients show low levels in albumin and cortisol binding globulin (CBG), and this suggests that serum total cortisol may not reflect circulating glucocorticoid activity in ICU setting. The free cortisol index (FCI), defined as the ratio of total cortisol/CBG, correlates well with serum free cortisol.

Objective: We investigated whether the FCI is helpful in evaluating HPA status and predicting 30-day mortality of critical ill patients.

Methods: In a prospective fashion, 29 patients with septic shock (SS) and 19 patients with sepsis (S) in Seoul National University Hospital (SNUH) MICU were enrolled. As a control group, normal responders (n=14) in both a standard 250ug cosyntropin stimulation test (CST) and insulin tolerance test were enrolled. The levels in serum cortisol and CBG were measured during CST in all subjects. The relative adrenal insufficiency (RAI) was empirically defined as low cortisol increment ($< 9\mu\text{g/dL}$) during CST. Mann-Whitney rank sum test and Kruskal-Wallis one-way ANOVA on ranks were used. This study protocol was approved by the IRB of SUNH. Results: SS patients showed higher levels in basal cortisol, basal FCI and peak FCI than control group ($p<0.001$, $p<0.001$, $p=0.072$, respectively) but peak cortisol levels ($p=0.846$), while S patients did not show significant differences compared to control group. Despite similar levels in albumin and CBG, RAI patients showed lower FCI increments (0.19 ± 0.45 vs 0.64 ± 0.51 , respectively; $p<0.001$) than non-RAI patients. Seven patients with basal FCI above 0.82 (upper one third) of 23 patients with low albumin levels ($\leq 2.5\text{g/dL}$), showed a significant association with 30-day mortality ($p < 0.05$).

Conclusion: FCI is likely to be useful in evaluating HPA axis of SS patients in ICU. Moreover, high basal FCI may suggest a significant association with 30-day mortality in critical ill patients with low albumin levels.

Silent Corticotroph Adenomas: An Aggressive Clinico-pathologic Entity Distinct From Non-functioning Pituitary Tumors

Odelia Cooper, Vivien Bonert, William Yong, Robert Goodman, James Mirocha, Shlomo Melmed

Cedars-Sinai Medical Center, David Geffen Sch of Med at UCLA, Los Angeles, CA, United States

Introduction: Silent corticotroph adenomas (SCAs) have been considered to be variants of clinically non-functioning adenomas (NFAs). SCAs stain positively for ACTH without features of Cushing's disease but rather as NFAs as they do not actively hypersecrete ACTH. We compared clinical and pathological characteristics of SCAs with those of NFAs to determine if SCAs are indeed a distinct entity, with more aggressive biological behavior, as previously suggested.

Methods: We evaluated 106 consecutive patients with NFAs in a retrospective cohort study. Complete radiology information was available for 67 patients while comprehensive endocrine profiles were available for 52 patients. Wilcoxon test was used for analysis of continuous variables and Fisher exact test used for two group comparisons of categorical variables.

Results: Of 106 patients evaluated, 22 were identified as SCAs (21%). Both SCA and NFA patients presented with headache, visual field deficits, decreased libido, and fatigue. Pre-operatively, 48% of NFAs had hypopituitarism compared to 33% of SCAs, 30% of NFAs had growth hormone deficiency ($p<0.05$) compared to 0% in SCAs. Radiologically, the majority of SCAs presented initially as Hardy class 2 and 3 tumors while most NFAs presented as Class 2. Post-operatively, there were no differences in residual tumor or recurrences between SCAs and NFAs. However, 70% of SCAs developed new hypopituitarism compared to 31% of NFAs ($p<0.03$). Specifically, SCAs developed more adrenal insufficiency than NFAs (50% compared to 35%). Thus, SCAs develop new post-operative hypopituitarism.

Discussion: Over 20% of NFAs are in fact SCAs. SCAs exhibit distinct pathologic and clinical characteristics and exhibit increased incidence of post-operative hypopituitarism. SCAs require more vigorous post-operative surveillance and more aggressive pituitary trophic hormone testing.



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Complex Genetic Interactions of Background Strains C57BL/6J and 129S6/SvEvTac on the Pituitary Intermediate Lobe Phenotype of Dopamine D2 Receptor-Deficient Mice

Renee Bend¹, Damasia Becu-Villalobos², Malcolm Low¹

¹Oregon Health and Science University, Portland, OR, United States, ²Consejo Nacional de Investigaciones Cientificas y Tecnicas, Buenos Aires, Argentina

Dopamine D2 receptor-deficient mice (Drd2^{-/-}) develop prolactinomas, consistent with a role for dopamine in the regulation of lactotroph cell number. IL melanotrophs also express D2R, but the phenotype of the IL in different strains of Drd2^{-/-} mice has been controversial. To test our hypothesis that genetic background influences the effects of dopamine on melanotrophs we generated C57BL/6J (B6) and 129S6/SvEvTac (129) congenic strains of Drd2^{-/-} mice. At age 10 mo female B6.Drd2^{-/-} and 129.Drd2^{-/-} mice had equivalent increases in pituitary weight and serum prolactin compared to their respective congenic wildtype siblings, showing that there was no interaction of genetic background on the lactotroph response to loss of D2R inhibition. In contrast, there were significant interactions of genetic background and genotype on the volume of the IL, calculated from serial coronal sections of the glands. The IL of wildtype 129 mice were more than twice the size of wildtype B6 mice and were not further increased in 129.Drd2^{-/-} mice. However, the IL of B6.Drd2^{-/-} mice were significantly larger by two-fold compared to wildtype B6 in both sexes. A second cohort of animals was studied at age 5 mo, before the development of lactotroph hyperplasia. Plasma \pm -MSH was strikingly higher in female wildtype 129 compared to B6 mice (526 \pm 77 vs. 32 \pm 10 pmol/l) but was further increased in female 129.Drd2^{-/-} and particularly in female B6.Drd2^{-/-} mice (909 \pm 140 vs. 551 \pm 125 pmol/l). Pituitary content of \pm -MSH was four-fold greater in female wildtype 129 compared to B6 mice (1180 \pm 129 vs. 257 \pm 33 pmol), but was decreased in the Drd2^{-/-} mice (419 \pm 49 vs. 246 \pm 20 pmol). This combination of elevated plasma \pm -MSH levels but decreased IL content indicates that the absence of dopamine inhibition by D2R causes continued secretion that cannot be fully compensated by increased production. In conclusion, a chronic loss of dopamine inhibition results in IL melanotroph hyperplasia and hypersecretion of \pm -MSH in Drd2^{-/-} mice, but the phenotype is obscured in congenic 129 mice because this strain exhibits at baseline an enlarged IL and high circulating \pm -MSH levels. Together with recent data from other laboratories, our study reinforces the importance of considering genetic background in the analysis of neuroendocrine phenotypes of mutant mice.

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Cabergoline treatment in Cushing's Disease: Effect on Hypertension, Glucose Intolerance and Dyslipidemia

ROSARIO PIVONELLO¹, MARIA CRISTINA DE MARTINO¹, ANTONGIULIO FAGGIANO¹, MONICA DE LEO¹, GAETANO LOMBARDI¹, LEO J HOFLAND², STEVEN WJ LAMBERTS², ANNAMARIA COLAO¹

¹FEDERICO II UNIVERSITY, NAPLES, ITALY, ²ERASMUS MEDICAL CENTER, ROTTERDAM, NETHERLANDS

Cabergoline has been recently demonstrated to normalize cortisol secretion in more than one third of patients with Cushing's disease (CD). The aim of this study was to evaluate short-term (3-months) and long-term (12-24 months) effects of cabergoline treatment on the main systemic complications of CD, including hypertension, glucose intolerance and dyslipidemia. Twenty patients with CD unsuccessfully treated by neurosurgery entered the study. Cabergoline was administered at the initial dose of 1 mg/week and a maximal dose of 7 mg/week. At 3-months follow-up, 15 (75%) patients were responsive whereas 5 (25%) were resistant to cabergoline treatment. Systolic and diastolic blood pressure, serum glucose and insulin levels, HOMA index, and serum cholesterol levels significantly decreased in parallel with the normalization of cortisol secretion. A significant improvement of blood pressure and a slight improvement in glucose tolerance and cholesterol levels was found both in responsive and resistant patients. Cabergoline treatment was continued in the 15 responsive patients, although treatment escape was observed in 5 patients, so that the long-term study was performed in 10 patients, who was followed-up for 12-24 months. During long-term treatment, urinary cortisol levels remained within the normal range. Serum glucose and insulin levels, HOMA index and serum cholesterol levels further decreased. At the last follow-up, the

prevalence of hypertension decreased from 50% to 0%, glucose intolerance from 62.5% to 30%, and dyslipidemia from 33.3% to 0%. In conclusion, the results of the current study confirmed that cabergoline treatment is effective in controlling cortisol secretion for at least 1-2 years in more than one third of patients with CD, and demonstrated that it is able to improve hypertension, glucose intolerance and dyslipidemia in patients responsive and, partially, also in patients resistant to the treatment. Therefore, cabergoline is confirmed to be a useful treatment option in patients with CD unsuccessfully treated by neurosurgery.

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Immediate And Long-term Results Of Transsphenoidal Surgery In 132 Patients With Cushing's Disease: Career Experience Over 40 Years

IVAN CIRIC, SUNIT DAS, JIN ZHAO, WILLIAM KERR, ELENI DIMARAKI

NORTHWESTERN UNIVERSITY FEINBERG SCHOOL OF MEDICINE, EVANSTON, ILLINOIS, United States

132 patients with Cushing's disease underwent transsphenoidal microsurgery for Cushing's disease over a period of 40 years. The surgical technique has remained essentially the same, except for substituting the initial oro-rhino-septal approach with the endonasal submucous and in the last 10 years with a direct endonasal approach, and for the much improved optics and illumination source in the up-to-date operating microscopes. The surgical results were evaluated in relationship to the imaging diagnostic methods: polytomograms of the sella and the CT scans in the first half of the series and MRI imaging in the second. The results obtained were measured against the postoperative 24-hour urinary free cortisol excretion and plasma cortisols. A precipitous and symptomatic drop in the postoperative plasma cortisol to below 5 mcg/dl requiring replacement therapy and normalization of the 24-hour urinary free cortisol excretion has been equated with surgical remission of the disease. A sustained normalization of these levels in conjunction with complete symptomatic remission has been considered as surgical cure.

This review suggests that patients with endocrinologically documented Cushing's disease have a better than 85% chance of having their disease vanquished surgically and their anterior pituitary function preserved if there is a distinct microadenoma demonstrated on the preoperative MRI. In contrast, in the pre-MRI era and in patients in whom the MRI findings are inconclusive the surgical cure rate drops into the 50% range. The long-term results mirror the immediate post-operative results in this regard. There was no mortality. Surgical morbidity and recurrences will be discussed.

P57

Delayed Remission in Corticotrophin-secreting Cystic Pituitary Macroadenoma

Sylvia Velinova, Roberta Glick, Carmen Vanessa Villabona, Manuel Utset, Terry J. Keefer, Leon Fogelfeld, Rasa Kazlauskaite

Stroger Hospital of Cook County, Chicago, United States

OBJECTIVE: We report an unusual case of delayed remission (more than 2 weeks post-surgically) of corticotrophin-secreting pituitary macroadenoma

CASE REPORT: A 27-year-old woman presented to the emergency room with clinical stigmata of Cushing's, including severe hyperglycemia, resistant to high-dose insulin therapy. Her 24-hour urinary cortisol 68-times above the upper limit of the reference range with plasma corticotrophin level 132 pg/ml (5-27) led to finding pituitary macroadenoma (16 x 11 x 7 mm) with cystic change on magnetic resonance imaging. The diagnostic evaluation of the patient was confounded by incidentally discovered sarcomatoid renal cell carcinoma. After curative nephrectomy the patient underwent trans-sphenoidal resection of the macroadenoma. A single pituitary lesion was detected by neurosurgeon, which was completely removed without perioperative glucocorticoid coverage. Up to 7 days after surgery cortisol (serum and 24 hour urine) remained high. However, within one month post-operatively the patient has been normoglycemic off insulin (100 units/24h) and normotensive. Her 24 hour urinary cortisol remained low normal for the last 7 months after surgery (24 hour urinary cortisol 7.2, 10.3 and 13.3 mcg/24 h). After recovery the patient has no pituitary hormone deficiencies. No post-surgical adjuvant therapy or glucocorticoids were administered.

CONCLUSIONS: Delayed post-surgical remission of pituitary macroadenoma supports the assessment for remission 4-12 weeks after therapy, particularly in patients with macroadenoma who failed early assessment (2-14 days post-operatively). Recovery of the anterior pituitary function we attribute to decrease in circulating glucocorticoids and anterior pituitary decompression.



ABSTRACTS

Growth Hormone

P58

Effects of a long acting release formulation of pasireotide (SOM230 LAR) on hormone secretion in rats

Herbert Schmid, Josef Brueggen, Patrice Guitard

Novartis Instituts of Biomedical Research, Basel WKL-125.13.44, Switzerland

Ongoing hypersecretion of hormones, which underlies chronic diseases like acromegaly or GEP/NET tumors are currently treated successfully in most patients with somatostatin analogues like octreotide. The development of a long acting release formulation of octreotide (Sandostatin LAR) strongly increased convenience for these patients. Pasireotide (SOM230) is a new somatostatin analog which binds with nanomolar affinity to sst1, 2, 3 and 5 receptors, in contrast to octreotide, which binds preferably to sst2. In rats, pasireotide had a stronger inhibitory effect than octreotide on the secretion of GH, IGF-1, ACTH and corticosterone. These and other results suggested treatment options with pasireotide for those patients unresponsive or refractory to octreotide therapy and offers new treatment options in diseases, in which e.g. sst5 is heavily expressed as in patients with Cushing's disease. This study compared the effects of a single injection of octreotide LAR and the newly developed pasireotide LAR (4 and 8 mg/kg s.c.) on hormone secretion in rats and mice over 35 days. Pasireotide LAR 4 and 8mg/kg caused significant reductions in basal IGF-1 without tachyphylaxis (-50% after 35 days). In contrast the inhibitory effect of octreotide LAR was only significant on day 1 (-31%) and showed strong tachyphylaxis on subsequent days (-21% after 35 days). Body weight of pasireotide LAR treated animal remained stable over 35 days, whereas control rats and rats treated with vehicle and octreotide LAR gained 35% and 26%, respectively. Plasma glucose was not significantly affected (<10%) by pasireotide LAR or octreotide LAR at any time point or dose, although pasireotide LAR treated rats tended to have slightly higher glucose levels than octreotide LAR treated rats. Plasma insulin was inhibited similarly by both compounds (-30 to -74% at each time point and dose), however, none of these differences reached statistical significance. In contrast, glucagon levels were strongly and persistently inhibited (-75 to -96%) by octreotide LAR at each time point, whereas pasireotide LAR caused a moderate inhibitory effect on glucagon only on day 1 and had no effect at subsequent time points. These data suggest that in contrast to octreotide LAR, a single injection of pasireotide LAR can reduce IGF-1 secretion in rats up to 35 days without significant tachyphylaxis.

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Growth Hormone And Testosterone Exert Differential And Additive Effects On Lean Body Mass In Recreational Athletes

Vita Birzniece, Udo Meinhardt, Jennifer Hansen, Irene Walker, Amie Lau, Kin-Chuen Leung, Anne Nelson, Ken Ho

PRU, Garvan Institute of Medical Research and Department of Endocrinology, St. Vincent's Hospital, Darlinghurst, Sydney, NSW, Australia, -

GH and testosterone (T) are anabolic agents. As both hormones act through different mechanisms, it is likely that their effects on body composition, especially on components of lean body mass (LBM) such as extracellular water (ECW), may not be the same. Whether men and women respond equally to GH is unclear. The aims of this study were to compare (i) the effects of GH, T alone and in combination on body composition, and (ii) gender difference in response to GH.

97 recreational athletes participated in this placebo-controlled prospective double blind study of 8 weeks. 64 men were randomised into placebo, GH (2 mg/d), T (IM Sustanon 250 mg/wk), or GH+T; and 33 women to placebo or GH groups. Body composition was measured by DXA and ECW was estimated using the bromide dilution technique. Data are presented as change from baseline.

	Men				Women	
	Placebo	GH	T	GH+T	Placebo	GH
Δ FM kg	-1.1 ± 0.5	-1.8 ± 0.3	-1 ± 0.7	-2.1 ± 0.5	-0.1 ± 0.3	-2.4 ± 0.4 *
Δ LBM kg	0.8 ± 0.3 †	3.5 ± 0.5 *†	3.2 ± 0.4 *†	6.6 ± 0.6 *	0.5 ± 0.3	2.8 ± 0.5 *
Δ ECW l	-0.3 ± 0.6 †	2.1 ± 0.5 *	1.0 ± 0.7 †	3.3 ± 0.7 *	0.1 ± 0.4	1.2 ± 0.5

Mean ± SEM, p<0.01 * vs. placebo; † vs. GH+T.

In men, GH significantly increased both LBM and ECW, while T increased LBM only compared to placebo. The change in ECW with GH was twice that of T, however the difference did not reach statistical

significance. GH+T increased LBM more than with either treatment alone, while the increase in ECW was greater compared to T only. In women, GH significantly reduced FM, and increased LBM but not ECW. These changes were not significantly different from those in men. We conclude that GH and T exert differential and additive effects on LBM. As the non-ECW-component of LBM is body cell mass, T exerts a greater protein and cellular anabolic effect than GH. Supported by the World Anti-Doping Agency and Australian Government Anti-Doping Research Program. Novo Nordisk provided GH, and Organon provided testosterone.

P60

The Influence of Gender and Testosterone on the Response to GH of IGF Axis and Collagen Markers in Young Recreational Athletes: a Double-blind Placebo-controlled Study

Anne Nelson¹, Udo Meinhardt¹, Irene Walker¹, Jennifer Hansen¹, Glenn Stone², Markus Seibel³, Robert Baxter⁴, David Handelsman³, Minoru Irie⁵, Rymantas Kazlauskas⁶, Ken Ho¹

¹Garvan Institute of Medical Research, Sydney, Australia, ²CSIRO Mathematical and Information Sciences, Sydney, Australia,

³ANZAC Research Institute, Sydney, Australia, ⁴Kolling Institute of Medical Research, Sydney, Australia, ⁵Toho University, Tokyo, Japan, ⁶Australian Sports Drug Testing Laboratory, Sydney, Australia

Serum IGF axis and collagen proteins are useful markers of GH abuse. This study, approved by the local ethics committee, investigated whether their response to GH differed between men and women, or with combined administration of testosterone (T).

Young recreational athletes were administered GH (2 mg/day) and/or T (250 mg Sustanon IM/week) for 8 weeks followed by 6 weeks washout. 33 women were randomized to GH or placebo and 64 men to GH, T, GH+T or placebo. Statistical analysis of serum IGF-I, IGFBP-3, ALS and PIIINP was performed at week 8 compared to baseline, and all time points using a linear mixed effects model on log transformed data.

All markers increased significantly in response to GH. The GH response was significantly greater ($P < 0.001$) in men for all the markers considered across all time points. In men, treatment with T alone did not show a significant effect on IGF-I, IGFBP-3 and ALS and GH+T did not show a significant change compared to the response to GH alone. However PIIINP increased significantly in men in response to T, and GH+T significantly increased the response to GH alone.

Percentage change at week 8 compared to baseline, means (SE)

We conclude that these markers of GH abuse are more sensitive in men and that the sensitivity of the collagen marker PIIINP is increased by co-administration of T.

Supported by the World Anti-Doping Agency and Australian Government Anti-Doping Research Program. Novo Nordisk provided GH and Organon provided T.

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Immunocytochemical patterns of somatotrophs, mammotrophs, and mammosomatotrophs in the porcine anterior pituitary

Jin Lee¹, Marvin Stromer¹, Srdija Jeftinija¹, Ksenija Jeftinija¹, Colin Scanes², Lloyd Anderson¹

¹Iowa State University, Ames, Iowa, United States, ²Mississippi State University, Starkville, Mississippi, United States

A novel anterior pituitary cell type, the mammosomatotroph which contained both growth hormone (GH) and prolactin (PRL) was proposed to function as a transitional cell or progenitor cell between somatotrophs and mammotrophs under various physiological conditions. Fluorescence immunocytochemistry was used to identify spatial distribution patterns of somatotrophs, mammotrophs, and mammosomatotrophs by counting the number of immunopositive cells at three positions in each of 5 regions at 3 levels in the porcine anterior pituitary from newborn and prepubertal stages of pigs (day 1, day 45, and day 90). There were no significant differences among the total somatotrophs per counting area across the three age groups. However, significant increases were observed among the total numbers of mammotrophs and mammosomatotrophs across the three age groups (day 1: day 45 and day 1: day 90, $P < 0.01$). There were distinct spatial changes in these cell types across different regions, positions, and levels. Somatotrophs were densely distributed in lateral wings of the anterior lobe (regions 1 and 5) whereas mammotrophs were numerous in shoulder areas (regions 2 and 4) in all age groups. In the center (region 3), near the

intermediate lobe (positions a and b) at the proximal level, there was a significant decrease in the total number of somatotrophs from day 1 to day 90 ($P<0.01$). However, mammotrophs and mammosomatotrophs significantly increased from day 1 to day 90 ($P<0.01$). From proximal to distal level, in the center and the outer surface of the anterior lobe (position c), the number of somatotrophs, mammotrophs, and mammosomatotrophs significantly increased ($P<0.01$). The results of these studies strongly suggest regional specificity of cellular transformation or interconversion to facilitate GH and PRL secretion as the need for endocrine regulation during the rapid growth period in the young pig. Supported by research grant USDA/CSREES NRI 2003-35206-12817 (L.L.A., S.J., and C.G.S.).

P62

New Observations Of Growth Hormone's Involvement In Cerebrovascular Function Following Human Traumatic Brain Injury

Parham Moftakhar, Thomas C. Glenn, Christina Wang, Paul M. Vespa, Pejman Cohan, David McArthur, Neil A. Martin, Daniel F. Kelly

David Geffen School of Medicine, UCLA, LA, CA, United States

Introduction: Growth hormone (GH) has been shown in human and animal studies to augment blood flow and vascular reactivity by upregulating nitric oxide synthase (NOS). Using transcranial Doppler (TCD), we tested the hypothesis that low serum GH would be associated with impaired cerebrovascular function following human TBI.

Methods: Thirty-nine TBI patients (mean age 35 ± 16 ; mean GCS 7) were studied with morning bilateral TCD studies and serum GH levels performed on post-injury day 1-10. Middle cerebral artery (MCA) velocity, internal carotid artery end-diastolic (ICAED) velocity and pulsatility index (PI) were measured.

Results: Of the 128 serum samples collected for each subject, 91 (71%) were detectable (mean GH 2.29 ± 1.95 ng/ml) and 37 (29%) were non-detectable (defined as the lower limit of quantitation of the assay, <0.2 ng/ml). The mean ICAED velocity during periods of detectable GH (27.1 ± 8.2 cm/s) was higher than during periods of non-detectable GH (17.8 ± 3.1 cm/s, $p<0.0001$). Serum GH levels correlated with ICAED velocity ($R=0.76$, $p<0.001$), MCA velocity ($R=0.63$, $p<0.0001$) and with the Lindegaard ratio ($R=0.58$, $p<0.0001$). The ICAED velocity and LR were related to serum GH levels independent of the day after injury ($R^2=0.63$, $p<0.0001$). The pulsatility index during periods of detectable GH (0.7 ± 0.3) was lower than during periods of non-detectable GH (1.5 ± 0.4 , $p<0.0001$). During periods of detectable GH, 18 out of 91 TCD studies (19.7%) demonstrated vasospasm (MCA velocity range 121-195 cm/s, average Lindegaard ratio 4.19). There were no incidences of vasospasm in the patients with non-detectable GH samples.

Discussion: Acutely after moderate or severe TBI, low or non-detectable serum GH levels are associated with lower ICAED and MCA flow velocities and higher vascular resistance. Measurable levels of GH may be important in maintaining normal cerebral blood flow in TBI patients, although in some instances, higher GH levels may be associated with vasospasm.

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Low-Dose Growth Hormone Administration Mobilizes Endothelial Progenitor Cells In Healthy Adults

Jessica K. Devin¹, Douglas E. Vaughan¹, Lewis S. Blevins Jr.¹, Qingxia Chen², Joseph Covington¹, Denise K. Verity¹, Pampee P. Young³

¹Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, United States, ²Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN, United States, ³Department of Pathology, Vanderbilt University School of Medicine; Department of Veterans Affairs, Nashville, TN, United States

Objective: Endothelial progenitor cells (EPCs) are bone marrow-derived cells that mobilize secondary to a stimulus and home to sites of injury, where they differentiate into endothelial cells and contribute to the repair of damaged vasculature. Interventions that augment the number of circulating EPCs may represent a mechanism to enhance endothelial function, and thus vascular health. We hypothesized that growth hormone (GH) administration would increase circulating EPCs in healthy adults.

Methods: Six males and 4 females (mean 37 years, range 26-65), with an IGF-1 level in the lower half of the age-specific normal range, were enrolled following Institutional Review Board approval. Participants



received 0.03 mg/kg/week of GH subcutaneously for 4 weeks followed by 0.06 mg/kg/week for a maximum of 4 additional weeks. Primary outcomes included the number of circulating EPCs as assessed by colony-forming unit (CFU) assay and flow cytometry. Secondary outcomes included plasma measurements of known mediators of EPC mobilization (VEGF, SDF-1, erythropoietin, and estradiol) and indices of nitric oxide (NO). Outcomes were measured at baseline and study completion; data was analyzed by Wilcoxon signed-rank test.

Results: GH administration resulted in an increase in IGF-1 (143 ng/mL [IQR 121-164] to 222 [IQR 194-244]; $P=0.005$). The number of early-outgrowth EPCs increased (13 CFU per high-power field [IQR 6-24] to 19 [13-40]; $P=0.005$), whereas the number of late-outgrowth EPCs as well as CD34+, VEGFR2(KDR)+, and AC133+ cells did not significantly change. Other mediators of EPC mobilization remained stable while plasma nitrite trended upwards (1.3 $\mu\text{mol/L}$ [0-2.5] to 3.7 [2.2-8.9]; $P=0.052$).

Discussion: GH selectively augments the early-outgrowth EPC population, possibly through an NO-dependent mechanism. These findings support GH replacement in the setting of GH deficiency to maintain vascular integrity. Furthermore, the decrease in EPCs observed with aging may in part be explained by the declining somatotrophic axis, and thereby contribute to cardiovascular senescence.

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Standardized centile curves and reference intervals of serum insulin-like growth factor-1 (IGF-1) in normal adult Japanese population using LMS method

Akira Shimatsu¹, Kazuo Chihara², Naomi Hizuka², Akira Teramoto², Toshiaki Tanaka², Keita Tatsumi², Katsuhiko Tachibana³, Noriyuki Katsumata³, Susumu Yokoya³, Ken-ichi Fujieda³

¹Clinical Research Institute, Center for Endocrine and Metabolic Diseases, National Hospital Organization Kyoto Medical Center, Kyoto, Japan, ²The Study Committee for Hypothalamic-Pituitary Disorders, The Ministry of Health, Labour and Welfare, Tokyo, Japan, ³The GH and its Related Factors Study Committee, The Foundation for Growth Science, Tokyo, Japan

Introduction: Serum levels of insulin-like growth factor-1 (IGF-1) reflect endogenous growth hormone (GH) secretion. Measurements of IGF-1 are useful for diagnosis and management of patients with GH-related disorders. We have previously assessed the reference values of serum IGF-1 in Japanese in 1996. The aim of the study is to re-establish cross-sectional reference intervals of serum IGF-1 in normal adult Japanese population.

Methods: The study included 1110 healthy adult Japanese subjects (554 males, 556 females) aged 18 years to 83 years. None had diabetes or other endocrine disease or had received estrogen therapy. Height, weight and body mass index (BMI) were measured in all subjects. Serum samples were obtained during morning hours and IGF-1 was measured by two kinds of immunoassays (Daiichi RI and Bayer). The age-dependent reference intervals for serum IGF-1 concentrations were calculated using the LMS methods, which summarizes the centiles by three smooth curves representing skewness (L), the median (M) and coefficient of variation (S). The L, M, S values were smoothed for each age and gender using cubic spline curves.

Results: Median IGF-1 levels at 20 years of age were 243ng/ml and 259ng/ml in males and in females, respectively, which decline and reached 124ng/ml and 103ng/ml in males and females at the age of 70 years. There were a significant difference according to gender; males had higher levels than females over 35 years of age. The differences between two immunoassays were minimal and could be converted with each other using the formulated equation: $\text{Daiichi} = 18.03 \times 0.822 \times \text{Bayer}$.

Conclusion: We could establish the national standards of reference intervals of serum IGF-1 in normal adult Japanese populations and calculate the standard deviation score (Z-score) by one year intervals.



ABSTRACTS

Growth Hormone Deficiency



P65

Testosterone Stimulates Extra-hepatic But Not Hepatic Fat Oxidation At Systemic Replacement Doses In Hypopituitary Men

Vita Birzniece¹, Udo Meinhardt¹, Margot Hewett¹, Angela Peris¹, David Handelsman², Ken Ho¹

¹PRU, Garvan Institute of Medical Research and Department of Endocrinology, St. Vincent's Hospital, Darlinghurst, Sydney, NSW, Australia, - ²ANZAC Research Institute, Sydney, NSW, Australia, -

Fat is oxidised in the liver and in extra-hepatic tissues. As testosterone (T) enhances whole body fat oxidation (Fox) and resting energy expenditure (REE) in hypopituitary men, we aimed to determine whether this arises primarily from the liver by comparing the metabolic impact of T administered via oral route (using doses designed to achieve physiological concentrations in portal blood) compared with a standard transdermal T replacement dose. Twelve hypopituitary men (age 53.1 ± 4.1 y) participated in an open-label cross over study of 2 wk treatments of transdermal T (tdT 5 mg), followed by 2 wk washout period, and stepwise incremental doses of oral crystalline T (10, 20, 40, and 80 mg) in the absence of GH replacement. Serum T, IGF-I, metabolic effects (REE, Fox) and SHBG as a marker of excessive hepatic androgen exposure measured at the end of each treatment period were analysed by repeated measures ANOVA using Bonferroni's correction.

	tdT 5 mg	washout	oT 10 mg	oT 20 mg	oT 40 mg	oT 80 mg
T nmol/l	16.6 ± 3.1 †	4 ± 0.8	4 ± 0.9	4.6 ± 1.4	4.5 ± 0.9	6.2 ± 1.3
SHBG nmol/l	27.6 ± 3.6	32 ± 4.1	30.5 ± 4.5	31.3 ± 4.8	29.8 ± 3.9	23.9 ± 4.2 * ^
Fox mg/min	60.3 ± 5.7 *	46.4 ± 5.7	49.1 ± 5.8	40.6 ± 4.2	50.9 ± 5.8	46.8 ± 6.1

P<0.01 * vs. washout; ^ vs. oT 40 mg; † vs. all other treatment groups.

Mean blood T levels were not increased by 10, 20, 40 or 80 mg oral T, but was in the physiological range during transdermal delivery. Blood SHBG was unaffected by 10, 20, 40 mg oral and transdermal T but fell significantly with 80 mg oral T. None of T treatments changed plasma IGF-I or REE. Fox increased significantly with transdermal but not with any dose of oral T. In summary, oral T at doses sufficient to induce pharmacological hepatic androgenic effects without increasing systemic T concentration, had no effect on Fox, which was however stimulated by transdermal T at a standard replacement dose. In conclusion, T does not stimulate hepatic Fox but enhances whole body Fox by acting on extrahepatic tissues in the absence of GH replacement in hypopituitary men. (Supported by the NHMRC of Australia; Maynepharm provided Androderm)

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Major Impact of Sex and BMI on the Diagnosis of Growth Hormone Deficiency in Adults by the GHRH Arginine Test

Stephan Petersenn¹, Ricarda Althoff¹, Nicole Unger¹, Andrea Jäger¹, Christian Strasburger², Martin Bidlingmaier³, Klaus Mann¹

¹Division of Endocrinology, University of Duisburg-Essen, Essen, Germany, ²Charite Universitätsmedizin, Berlin, Germany,

³Medizinische Klinik - Innenstadt, Klinikum der Universität München, München, Germany

Background: The diagnosis of growth hormone (GH) deficiency in adults (GHD) is based on provocative testing. To improve the diagnostic accuracy of the GHRH plus Arginine test (GARG), we investigated the influence of age, BMI and sex on GH peak levels (pGH). Methods: 180 healthy subjects (C) were prospectively stratified into three age groups (18-30, 31-50, >51 years), three BMI groups (<25, 25-29.9, >=30kg/m²), and for both sexes. All subjects were tested by GARG. 102 patients with pituitary disease were studied by ITT and GARG. GH was measured with DPC Immulite 2000. ROC analysis was used to identify thresholds with at least 95% specificity. Results: Age, BMI, and sex accounted for 62% of the variability of pGH during GARG in normal subjects. All three parameter had significant influence on pGH, with BMI being the most relevant parameter (42.8%, p<0.001), followed by sex (36.6%, p<0.001) and age (15.8%, p<0.001). By ITT, 63 patients were diagnosed with severe GHD (pGH≤3ng/ml), in contrast to 39 GHS patients. Comparison of pGH during GARG between GHD and GHS+C revealed a threshold of 5.9 ng/ml (sensitivity 76%). Subgroup analysis determined thresholds (sensitivity) of 6.0 (73%), 14.2 (100%), 3.4 (77%), and 7.6 (91%) ng/ml for male and female subjects with BMI<30kg/m², and male and female subjects with BMI≥30 kg/m², respectively. Conclusions: The diagnostic accuracy of the GARG test is improved by adjusting thresholds according to BMI and sex. Especially the influence of the later parameter is currently neglected. Both variables have important impact on the correct diagnosis in patients with suspicion of GHD.



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Chronic Hypopituitarism after Complicated Mild, Moderate, or Severe Traumatic Brain Injury; A Prospective Study.

Sumati Bavisetty¹, Supriya Bavisetty¹, David L. McArthur², Joshua Dusick², Justin Wagner¹, Christina Wang⁴, Pejman Cohan³, John W. Boscardin⁵, Ronald Swerdloff⁴, Harvey Levin⁶, Daniel F. Kelly²

¹Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA, United States, ²Division of Neurosurgery, UCLA School of Medicine, Los Angeles, CA, United States, ³Division of Endocrinology and Gonda Diabetes Center, UCLA School of Medicine, Los Angeles, CA, United States, ⁴Division of Endocrinology, Harbor-UCLA Medical Center, Torrance, CA, United States, ⁵UCLA School of Public Health, Los Angeles, CA, United States, ⁶Baylor College of Medicine, Houston, TX, United States

Introduction: Chronic pituitary dysfunction is increasingly recognized as a sequelae of traumatic brain injury (TBI). We sought to define the incidence, risk factors and neurobehavioral consequences of chronic hormonal deficiencies after complicated mild, moderate or severe TBI.

Methods: TBI patients aged 14 to 65 years were prospectively enrolled acutely after injury and studied at 6-9 months post-injury with baseline and dynamic anterior pituitary stimulation testing to identify major and minor (sub-clinical) hormonal deficiencies. Neurobehavioral and quality of life measures were performed at 6-9 months post-injury and were compared in patients with versus without major hormonal deficiencies.

Results: Of 70 patients (mean age 32 years, median GCS of 7, 19% women) tested at 6-9 months post-injury, 21% had one or more major hormonal deficiencies including 16% with growth hormone deficiency, 10.5% with hypogonadism, and one with diabetes insipidus. Also, 28.6% of the patients had minor (untreated) deficiencies. Only 2 patients were above 65 years and they had no deficiencies. No patients required adrenal or thyroid replacement. Patients with major hormonal deficits at 6-9 months had more abnormal CT findings ($p=0.014$) and a worse Disability Rating Scale ($p=0.02$), but there were no other differences in terms of neurobehavioral or quality of life measures.

Conclusions: Chronic hypopituitarism requiring treatment occurs in approximately 21% of patients sustaining complicated mild, moderate and severe TBI and is associated with more severe brain injury as seen on acute CT scans. The somatotroph and gonadotroph axes appear most vulnerable to TBI and its associated insults while the thyrotroph, corticotroph and posterior pituitary axes appear more resilient. Long-term, these major hormonal deficits are associated with an increased rate of disability, but an overall similar outcome in terms of formal neurobehavioral and quality of life complaints. The clinical significance of minor hormonal deficits, which may occur in over 25% of patients, remains to be determined.

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Normal bone mineral apparent density (BMAD) and markers of bone turnover in GH deficient (GHD) young adults of childhood acute lymphoblastic leukaemia (ALL) treated with cranial irradiation.

Cecilia Follin, Katarina Link, Eva Marie Erfurth

Institution of internal medicine, Lund, Sweden

Background: Adults with childhood onset GHD have reduced BMD (Bone Mineral Density). Previously, it has been shown that BMD was reduced in young adult survivors treated with cranial radiation (CRT) for childhood ALL. As ALL patients are shorter their bones will have smaller width and also be thinner, i.e. volume corrections, using BMAD is therefore preferable.

Subjects and Methods: 44 former ALL patients (19-31 yr) treated with CRT (18-24 Gy) and chemotherapy with confirmed GHD (91%) or GH insufficiency, and matched controls were studied at baseline. A subgroup of 16 former ALL patients were treated with GH for 4 years and compared with the same controls as from baseline. BMAD and BMD were evaluated by DEXA and markers of bone turnover (crosslaps and osteocalcin) were analysed.

Results: Compared with controls, the former ALL patients were significantly shorter ($p < 0.001$) and had higher BMI ($p = 0.005$). Serum IGF-1 was significantly lower in the patients ($p = 0.004$). Compared to

controls, BMAD was not reduced in mid radius ($p = 0.07$), femoral neck ($p > 0.3$), lumbar spine ($p = 0.19$), or total body ($p = 0.3$). A small reduction in BMD of mid radius ($p=0.06$) and total body ($p=0.05$) was recorded among the patients, but with no differences in femoral neck, lumbar spine, or in markers of bone turnover ($p=0.3$). After 4 years of GH treatment the serum IGF-1 level increased significantly ($p=0.03$), but with no difference in BMAD, at any skeletal site. Serum levels of crosslaps increased significantly ($p=0.04$) after GH treatment, but with no difference in osteocalcin levels.

Conclusions: No difference in BMAD at any skeletal site, or in bone formation markers, was recorded in former ALL patients with GHD. After 4 years of GH treatment, BMAD was unchanged.

ABSTRACTS

Miscellaneous

P69

Sheehan's Syndrome: A review of 11 cases

Natalia Garcia Basavilbaso, Malena Musich, Clara Fritz, Marisa Morassutti, Mirtha Guitelman

Endocrinology Division , Durand Hospital, Buenos Aires, Argentina

Introduction : Originally described in 1957, Sheehan's Syndrome (SS) was characterized by pituitary necrosis, lactation failure and hypopituitarism in patients who have had postpartum hemorrhage (PPH). It is hypothesized that PPH leads to necrosis of the hyperplastic pituitary gland during pregnancy. In this study we report the long-term follow up of eleven patients with SS.

Patients and methods: The medical records of eleven patients with SS were reviewed retrospectively. Data on presentation, pituitary function and imaging were analyzed.

Results: Six patients had vaginal deliveries and five underwent caesarean section. All patients required blood transfusions due to PPH. Failure of postpartum lactation (agalactia) was seen in 90% of the women. All patients developed amenorrhea, 73% within the first five years of postpartum. Pituitary deficiency was found in all women. Time to diagnosis was between 15 and 35 years after delivery in 64% of patients. Reasons for presentation were anemia (18.2 %), hypothyroidism (18.2 %), clinical control (18.2%), hypothermia (9.1%), asthenia with impaired clinical status (9.1%), osteoporosis (9.1 %), and oligomenorrhea (18.7%). Two cases presented as endocrine emergencies: one as acute adrenal cortex insufficiency; the other, as consciousness alteration, probably due to secondary hypothyroidism. Magnetic resonance imaging of the pituitary gland showed empty sellae in the 8 evaluated patients. No women had diabetes insipidus. All patients responded successfully to replacement therapy.

Conclusion: In this series of patients with Sheehan's Syndrome, symptoms related to pituitary deficiency were present at the time of diagnosis. The lack of clinical suspicion leads to a delayed diagnosis, with a subsequent increase in the morbidity and mortality rate. Postpartum hemorrhage and agalactia could be the key in the detection of Sheehan's syndrome.

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Central Functional Hypernatremia/Hypodipsia In A Patient With Normal Imaging Of The Pituitary-Hypothalamic Region

VS Costa, CRL Scaglioni, L Higashi, MK Huayllas, KC Nogueira, B Liberman, A Cukiert

Hospital Brigadeiro, Sao Paulo SP, Brazil

Introduction: The hypernatremia/hypodipsia syndrome is usually associated to well documented lesions involving the pituitary/hypothalamic region. We report on a patient with hypernatremia/hypodipsia syndrome in whom imaging of this region was negative.

Case Report: A 17 years-old young girl was first examined by the age of 10 years. She presented with hypodipsia, repetitive episodes of severe dehydration and irritability. By that time, her biochemical investigation showed sodium plasma level of 170mEq/L, BUN of 130 mg/dL, creatinine of 1.9 mg/dL and plasma and urinary osmolarity of 330 mosm/L and 916mosm/L, respectively. She had palatal cleft and mental retardation. She needed many in-patient periods when she presented with severe hypernatremia (up to 192 mEq/L) and variable alterations in the level of consciousness over the last 7 years. MRI of the head showed septum pelucidum agenesis and normal pituitary/hypothalamic region. A normal-looking neurohypophysis was clearly seen. During this period, she has continuously shown inability to additionally concentrate urine in spite of very high serum osmolarity and no diabetes insipidus. She is currently being treated with DDAVP 30ug/day and a special water intake program.

Discussion: The hypernatremia/hypodipsia syndrome is usually associated to discrete lesions located in the pituitary/hypothalamic region, such as tumors, aneurysms, granulomatous disease etc. In these patients, there is deficiency of AVP release related to the topography of the lesion. AVP concentration is usually low, but not absent and overt diabetes insipidus is rare. Diabetes insipidus may occur when there is complete destruction of the neurohypophysis. Patients presenting with functional hypernatremia /hypodipsia syndrome and normal anatomy of the pituitary/hypothalamic region are extremely rare. MRI of this region was normal in our patient but she presented with two distinct midline defects (palatal cleft and septum pelucidum agenesis). We postulate that functional abnormalities related to midline anatomical alterations not adequately documented by MRI are the cause of this syndrome in our patient.



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Hypovitaminosis D Is Highly Prevalent in Hypopituitarism

Murray B. Gordon¹, Michael S. Gordon²

¹Division of Endocrinology, Allegheny General Hospital, Pittsburgh, Pennsylvania, United States, ²Endocrine-Hypertension Division, Brigham and Womens Hospital, Boston, Massachusetts, United States

Background: One of the characteristic features of hypopituitarism is low bone mass documented by use of DXA, a marker for future fracture risk. Several retrospective studies have documented an increased prevalence of fractures in hypopituitary adults. Vitamin D deficiency is a risk factor for osteopenia and bone fractures. Although hypovitaminosis D (25-hydroxy vitamin D level < 20 ng/ml) has been detected frequently in general medical inpatients in the United States (up to 57%) and in approximately 36% of otherwise healthy adults, the frequency of hypovitaminosis D in hypopituitary patients has not been well characterized. The primary aim of this study is to define the frequency of hypovitaminosis D in a group of hypopituitary patients.

Methods: We retrospectively analyzed the vitamin D status of 61 patients (24F, 37M, age 55.1±1.7 years) who had been diagnosed with hypopituitarism at Allegheny Neuroendocrinology Center in Pittsburgh, PA. 82% of the patients (50/61) had a pituitary macroadenoma. The mean number of pituitary hormone deficiencies was 3.2 ± 0.15. 58/61 had GHD. The data consisted of serum 25-hydroxy vitamin D (25-OHD), intact PTH (PTH), and bone mineral density DXA tests. Vitamin D deficiency was defined as a 25-OHD level <20 ng/ml and vitamin D insufficiency as a 25-OHD < 32 ng/ml.

Results: The mean 25-OH D concentration in our cohort was 24.55 ± 1.1 ng/dl. We found that 20 of the 61 patients (32.8%) were vitamin D deficient, and 47 of the patients (77%) were vitamin D insufficient. The mean PTH concentration was 40.4 ± 2.9 pg/ml. 29% of patients had secondary hyperparathyroidism (PTH > 46 pg/ml), all of whom had either vitamin D deficiency (25%) or vitamin D insufficiency (75%). Mean DXA T-scores were as follows: L1-L4 spine -0.71 ± 0.21, femoral neck -1.10 ± 0.15 and total hip -0.53 ± 0.16. 44% of patients had osteopenia (at least one T-score <-1 and >-2.5) and 17% had osteoporosis (at least one T-score <-2.5).

Conclusions: Vitamin D deficiency is highly prevalent in hypopituitarism. Because lack of vitamin D can negatively affect bone health and have extraskelatal adverse effects, screening for vitamin D deficiency should be performed in patients with hypopituitarism.

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Identification of a Novel Germline Mutation in the MEN1 Gene, Glu469STOP (E469X), and its Molecular Consequences.

Patricia Fainstein Day, Tomas Fernandez Gianotti, Maria Belen Benech, Luisa Plantalech, Marta Balzaretta, Andrea Kozak, Esteban Mocetti, Hernan Garcia Rivello

Hospital Italiano Buenos Aires, Buenos Aires, Argentina

Introduction: Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominantly inherited cancer syndrome characterized by tumors of the parathyroids, gastro-intestinal, anterior pituitary and other endocrine tissues. More than 300 mutations have been described along the MEN1 gene. Direct MEN1 gene sequencing allows mutations detection along the gene.

Objective: We report a novel germline MEN1 gene mutation and its possible mechanism of inhibition of menin physiological action.

Subject and Methods: A 42 years old woman with primary hyperparathyroidism diagnosis, without known familial endocrine tumors, was operated in April 1995. Anatomopathological studies informed parathyroid hyperplasia. In 1998 two non functional tumors in pancreas and adrenal gland were found and operated. Histological studies confirmed they were neuroendocrine tumors. Genomic DNA of the patient was obtained from peripheral blood leukocytes and was analysed by manual direct sequencing for the MEN1 gene using ddNTP33. A novel mutation was found in exon 10, and confirmed by PCR-RFLP using BseI. Immunocitochemistry of pancreatic tumor was performed using a commercial menin antibody generated against a peptide corresponding to the menin residues within exon 7.

Results: A non previously reported heterozygous germline MEN1 mutation, to our best knowledge, was found, E469X. This mutation would generate a stop codon and therefore a truncated protein of 468 amino-acid (aa), instead of 610 aa. The last 142 aa missed contain two nuclear localization signals that are needed

for menin to be carried into the nucleus. Immunocytochemistry studies showed that truncated menin was expressed in cytoplasm of pancreatic tumoral cells, but not in the nucleus.

Conclusions: We described a novel mutation in MEN1 gene and showed the mechanism by which mutated menin would not play its tumor suppressor action.

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Developmental control of pituitary proliferation, cell cycle exit and differentiation

Steve Bilodeau, Audrey Roussel, Jacques Drouin

Laboratoire de génétique moléculaire. Institut de recherches cliniques de Montréal (IRCM), Montréal, Québec, Canada

Organogenesis is the result of a tight balance between control of cell proliferation, cell cycle exit and differentiation. Indeed, the formation of many tissues includes a phase of active proliferation that is followed by differentiation of postmitotic cells. This scheme implies a tight cross-talk between the two processes and it is often believed that patterning and differentiation signals drive the developmental program including cell cycle exit of proliferating progenitors.

Using BrdU immunofluorescence together with analysis of expression profiles of many cell cycle regulators during pituitary organogenesis, we have defined a population of proliferating progenitors that do not express any markers of differentiation during development. Then, taking advantage of the spatial and temporal separation of proliferating and differentiated cells within the developing anterior pituitary gland, we identified a population of non-cycling precursor cells that have not yet entered the differentiation program. We identified a cell cycle regulator that controls progenitor cell cycle exit independently of the differentiation program. Conversely, blockade of cell differentiation as achieved in *Tpit^{-/-}* pituitaries does not prevent cell cycle exit but rather leads to accumulation of non-cycling undifferentiated precursors in the adult pituitary intermediate lobe. These data indicate that during normal development, cell cycle exit is controlled independently of differentiation. Furthermore, this new paradigm could be relevant in many other biological systems including control of stem cell potential in regenerative medicine. With regards to pituitary, this work suggests models for tumor formation from either differentiated cells or from a presumptive pituitary stem cell.

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Body Composition in Morbid Obese Patients undergoing Laparoscopic- Adjustable Silicone Gastric Banding

Carolina Di Somma¹, Luigi Angrisani², Francesca Rota¹, Maria Cristina Savanelli¹, Teresa Cascella¹, Annamaria Belfiore³, Francesco Orio¹, Annamaria Colao¹, Silvia Savastano¹, Gaetano Lombardi¹

¹Department of Molecular and Clinical Endocrinology and Oncology Federico II University, Napoli, Italy, ²Department of Surgery, San Giovanni Bosco Hospital, Napoli, Italy, ³Department of Neuroscience, Unit of Physiology Federico II University, Napoli, Italy

Morbidly obese subjects are characterized by abnormalities in Growth Hormone (GH)/Insulin-like growth Factor (IGF)-I axis. Although GH/IGF-I axis has been previously evaluated after malabsorptive bariatric procedures, no data have been provided after laparoscopic-adjustable silicone gastric banding (LASGB), a purely restrictive bariatric procedure. The aim of this study was to explore the relationship between the GH/IGF-I axis, body composition and nutritional state in morbidly obese subjects before and after LASGB. This study included 72 morbidly obese females (BMI: 44.8 4.7; mean age: 33.1 11.3 yrs) evaluated before and 6 months after LASGB. IGF-I, IGF binding protein (IGFBP)-3, and acid-labile subunit (ALS) levels were investigated, and the GH secretion was assessed with the GHRH plus arginine test. Body composition was evaluated by conventional Bioelectrical Impedance Analysis (BIA). After surgery, BMI, waist circumference (W), Fat Mass (FM), Free Fat Mass (FFM), and HOMA index were significantly reduced ($p=0.000$). The percent decrement of FM was greater than that of FFM ($p=0.000$). Before surgery, considering a cut off of 4.2 g/l at GHRH+ ARG in obese subjects, 22 (31%) subjects were GH deficient, while 16 (22%) had IGF-I levels below the normal values for age and sex. After surgery, 15 (20.8%) subjects were GH deficient, while 25 (34.7%) had IGF-I levels below the normal values for age and sex. Thus, even in case of non malabsorptive bariatric surgery low IGF-I levels represent a possible marker of an underlying persistent state of hypercatabolism and are also associated to unfavourable body composition changes.



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Sex-Specific Effects of Long Term Oral Opioids on Hypothalamus-Pituitary-Gonadal Axis in Patients with Chronic Non-Cancer Pain

Stan Van Uum¹, Deric Morrison¹, Lisa-Ann McKeough¹, Terri Paul¹, Sonya Tokmakejian², Yves Bureau⁴, Pat Morley-Forster³

¹Dept. of Medicine, University of Western Ontario, London, Ontario, Canada, ²Dept. of Biochemistry, University of Western Ontario, London, Ontario, Canada, ³Interdisciplinary Pain Program, University of Western Ontario, London, Ontario, Canada, ⁴Dept. of Psychology, University of Western Ontario, London, Ontario, Canada

INTRODUCTION Chronic non-malignant pain (CNP), persisting at least 6 months is common and affects up to 15-30% of the population. This population is increasingly treated with chronic opioid therapy. It is well-known that heroin and oral opioids suppress testosterone levels in male patients. Less information is available on the effect of oral opioids on the hypothalamus-pituitary-gonadal (HPA) axis in women with CNP.

METHODS We included men aged 18-60 years and premenopausal women aged 18-50 yrs, all gave written informed consent. Exclusion criteria were: pregnancy, sex steroids, selective estrogen receptor modulators. A study physician obtained a history and performed a physical exam. A fasting blood sample was obtained for LH, FSH, Estradiol (women), Total Testosterone, Free Androgen Index (men). Hypogonadism was defined for women as an Estradiol level below the normal range throughout the menstrual cycle, for men as a Testosterone below the normal range. Results were analyzed using the non-paired Student-test and the Chi-Square test. The study was approved by the local Research Ethics Board, and conducted in accordance with the Declaration of Helsinki.

RESULTS (mean±SD) in men (n=11) and women (n=15) were as follows: age 45±6 and 38±7 years (p<0.05); Body Mass Index 29.2±4 and 28.2±7 kg/m² (p=ns); opioid dose 689±550 and 585±661mg/day (p=ns); duration opioid use 7.4±3.9 and 4.0±1.8 years (p<0.05); LH 2.5±1.3 and 8.7±7.2 IU/L (p<0.05); FSH 3.8±1.3 and 6.4±5.1 IU/L (p=ns); LH/FSH ratio 0.7±0.37 and 1.48±0.94 (p<0.05). Total testosterone (men) was 7.1 ± 4.3 nmol/L, estradiol (women) was 345 ± 363 nmol/L. Hypogonadism was present in 9 (82%) men and in 5 (33%) women (p<0.05).

CONCLUSION: This study in patients taking long-term oral opioids for CNP suggests that hypogonadism is common and more prevalent in men than in women. This may increase the risk of sexual dysfunction and decreased bone mineral density in this patient population.

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Down-Regulation of Long Form Prolactin Receptor by Short Form 1b

Ameae Walker, Dunyong Tan

Div. of Biomedical Sciences, University of California, Riverside, California, United States

Alternative splicing leads to the production of a number of different prolactin receptors. In the human, the best studied include a long form (LF), a so-called intermediate form and two short forms (SF1a and SF1b) consisting of 598, 325, 352 and 264 amino acids, respectively. Each has the same extracellular and transmembrane sequence, but differs in the signaling region. Studies in different species have suggested that SFs of the receptor act as dominant negatives for prolactin effector function through the LF. This is proposed to be due to LF-SF heterodimerization and resultant interference with LF-LF dimer signaling. We and others have provided evidence for LF-SF heterodimerization of the human receptors in support of this general concept. Signaling from the SF does occur, but is different from LF signaling. To further investigate the influence of SF on LF function, we prepared humanized expression constructs coding for untagged SF1b cDNA or LF cDNA tagged either with green fluorescent protein (GFP) or renilla luciferase. Each of these tagged constructs has normal prolactin binding and signaling through Jak2-Stat5. The SF1b and LF constructs were transiently co-transfected into HEK 293 cells (equivalent total plasmid was achieved with an irrelevant protein-expressing construct, pHcRed-Tandem). Forty eight hours post-transfection, expression of LF protein was assessed by GFP intensity or luciferase activity, and mRNA by RTPCR using form-specific primers. Results showed that SF1b decreased GFP intensity, luciferase activity and LF mRNA >10 fold. Similar co-transfections adding untagged LF did not reduce GFP intensity or luciferase activity, thereby demonstrating that the result was not due to over taxation of the rough endoplasmic reticulum translational and processing machinery. These results demonstrate a novel additional mechanism whereby a short form of the receptor may affect signaling through the long form of the receptor. This work was supported by BCRP grant 10PB-0127

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Please see Brief Summary of Prescribing Information on adjacent page.

† Includes both ongoing and completed trials.

‡ Combined use of immediate release Sandostatin[®] Injection and Sandostatin LAR[®] Depot.

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