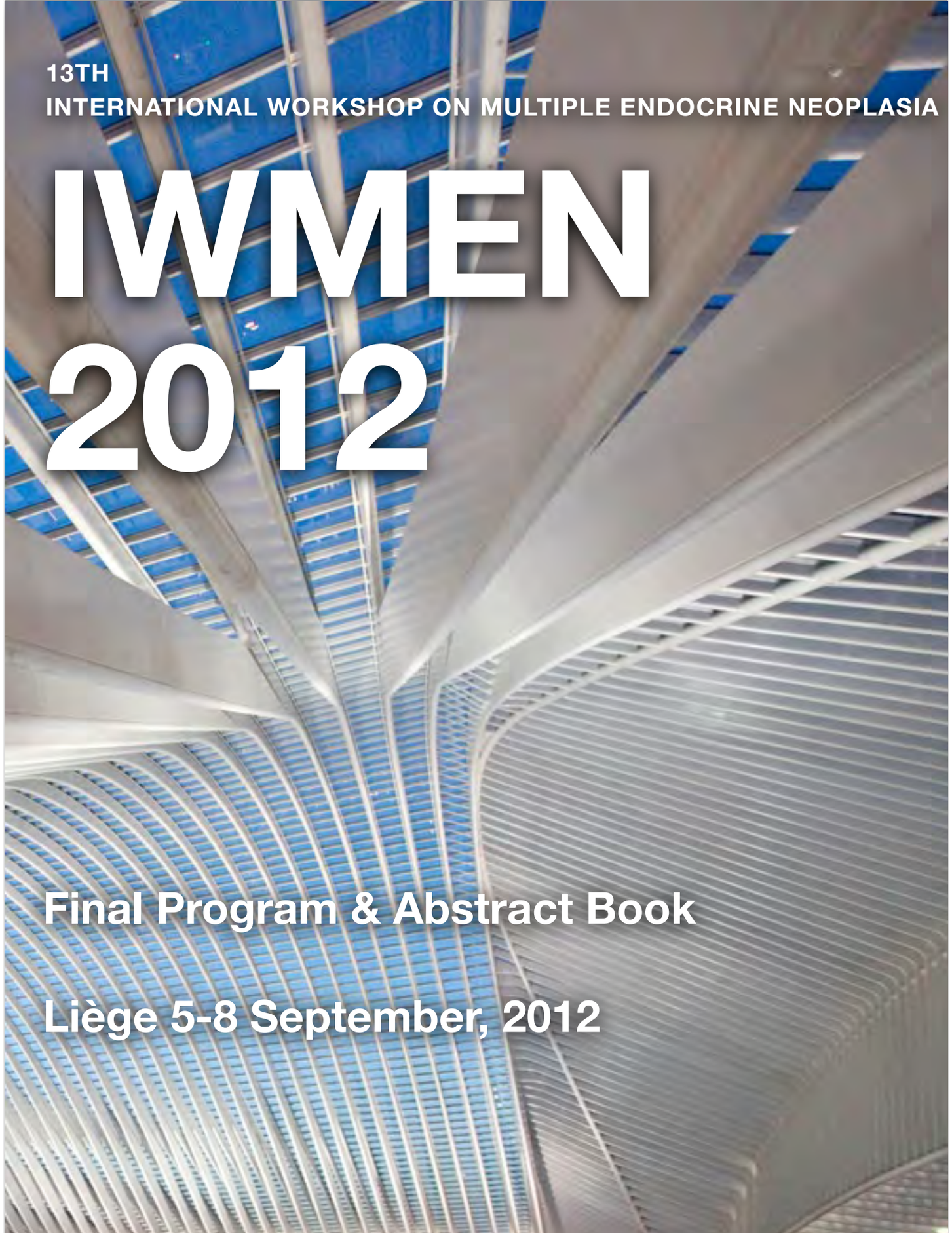
13TH
INTERNATIONAL WORKSHOP ON MULTIPLE ENDOCRINE NEOPLASIA

IWMEN 2012



Final Program & Abstract Book

Liège 5-8 September, 2012

IWMEN 2012

Liège, Belgium

13th International Workshop on
Multiple Endocrine Neoplasia

5-8, September, 2012

Palais de Congrès

Liège, Belgium

Welcome Message from the Chairman

Dear Friends and Colleagues,

It gives me the greatest pleasure to welcome you all as participants, speakers and guests to the 13th International Workshop on Multiple Endocrine Neoplasia (IWMEN). In particular I am delighted to welcome you to Liège, where I trust you will enjoy your stay in our inviting city.

The IWMEN is held every two years and is an ideal opportunity to update ourselves about the newest findings in multiple endocrine neoplasia research. This year I am very pleased that we are offering an unbeatable program of Symposia and Plenary Lectures, which together cover the full spectrum of pathology, disease and treatment. This is truly an international meeting with 29 countries represented. As such, the Workshop is an unparalleled opportunity to meet and discuss topics of common interest with world leading figures. I would also like to point out the range of new results being presented in Oral and Poster form during the Workshop and we have selected 4 topics of specific interest for more complete presentation during the Hot Topics session on Friday 7th September.

The Local Organizing Committee gratefully acknowledges the generous contribution made by the Sponsors to support us in hosting such a complete scientific and clinical Program.

On behalf of the Local and Regional Organizing Committees and the Board of the IWMEN, I wish you a productive, insightful and rewarding stay in Liège!

Prof. Albert Beckers

Chairman, Local Organizing Committee IWMEN 2012, Liège, Belgium

13th International Workshop on Multiple Endocrine Neoplasia

Liège, Belgium

5-8 September, 2012

IWMEN Committees

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References:
1) Ruszniewski et al., Neuroendrocrinology (2004) 80 244-251
2) Andersen, Exp. Rev. Endo. Met (2007) 2(4) 433-441
3) Caron P., Therapy (2007) 4(1) 9-29
4) Alexopoulou et al. Eur. J. of Endocrinology (2004) 151 317-324

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IWMEN 2012: Practical Information

Venue & Getting There

Palais des Congrès

The Liege convention centre has three levels, over twenty variable halls, with the largest auditorium able to receive up to one thousand people comfortably. Its two enormous floors offer a panoramic view of the river.

Recently renovated, the convention centre combines modern comfort and specific hi-tech facilities.

Address

Esplanade de l'Europe, 2
B-4020 Liège (Belgium)
Tel.: +32.4.340.38.88
Fax: +32.4.343.20.85

http://www.palaisdescongresliege.be

For a Google Map of Useful Places and Sites for IWMEN 2012
Click Here

Getting there

By train / TGV (High Speed Train)

Station "Liege Guillemins" (central station). More info on trains: www.b-rail.be (Belgium) or www.tgv-europe.be (Europe)

At Guillemins station, take the bus line TEC 17 or 38b directly to Palais des Congrès (every 15 minutes). It will take about 7 minutes, with direct access. More info on: www.infotec.be.

By plane

Brussels International Airport (Zaventem): At the airport, you can take the train to Brussels North station or to Louvain/Leuven (2 trains per hour). From there, change to the train to Liège Guillemins. Total duration of the journey: +/- 75 min. Price: 15€/ one way. More info and tickets on www.b-rail.be
At Guillemins station, take the bus line TEC 17 or 38b directly to Palais des Congrès (every 15 minutes) or take a short taxi ride. www.infotec.be.

Brussels South Charleroi Airport (Charleroi): At the airport, take the city bus Line A (stop is outside of the departure hall), which costs 3€ one way to Charleroi-Sud (south) train station, then the train to Liège-Guillemins. Train departs once every hour from 5am. Last train leaves at 23:00. The trip takes approximately 1 hour and 10 minutes.
At Guillemins station, take the bus line TEC 17 or 38b directly to Palais des Congrès (every 15 minutes) or a short taxi ride. www.infotec.be

Liège Airport (Bierset): By public transportation: Take the bus line TEC 57 to Guillemins station. Duration: 20 minutes. Price: 1,60€. IMPORTANT NOTE: there are about 6 connections per day. Check the schedules on www.infotec.be. At Guillemins station, take the bus line TEC 17 or 38b directly to Palais des Congrès (every 15 minutes) or a short taxi ride.

By car: see TRAFIROUTES for real time driving conditions

Parking: There is free parking in front of the venue. However, this might be full within a short time. In that case, you can park in the public parking "Mypark Médiacité" (13€ for 24 hours).

Car Service: Transport by car from Brussels and Local Airports to and from the Congress Centre/Hotels can be arranged. Please contact the Secretariat at registration@iwmen.org

About Liège

As the most important tourist city in Wallonia, Liège has many riches in store waiting to be discovered.

The characteristic districts, the river Meuse, which transects from South to North, the abrupt and wooded hills surrounding provide a multitude of original perspectives.

A very lively culture with a positive-minded population, always ready to celebrate, and a large number of restaurants all combine to make this an essential part of any trip to Belgium.

The surrounding area also offers a vast array of options for walking and tourist visits.

For full information on the city please click here

For a Google Map of Useful Places and Sites for IWMEN 2012
Click Here

Registration and Hotels

Registration fees [EUR]

Category	Excl. VAT/IVA/TVA	Incl. VAT/IVA/TVA
Participant	500.00	605.00
Fellow/Trainee	300.00	363.00

Registration can be completed online at www.iwmen.org up until August 31st, 2012. Thereafter, registration can be completed on-site at the Congress Venue.

Hotels

For Hotel Bookings, these can be made up until August 31st, 2012 using the online Registration system at www.iwmen.org. Thereafter, attendees will need to make their own hotel reservations directly with hotels in the city:

Liège Hotels
HUSA de la Couronne***
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4000 Liège
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http://www.hoteldelacouronne.be

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Tel. : +32.(0)4.252.44.14
Fax. : +32.(0)4.252.44.15
info@hotellesnations.be
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Speaker Information and Posters

Oral presentations, Symposium and Plenary Lectures:

All presentations should be prepared as a series of Powerpoint slides. The format for the projection will be Windows-based PC. To avoid problems, please check your slides and content (e.g. images, video) for compatibility if converted from other programs like Keynote or if using specialized formats.

In order to be fair to all speakers we will have to be strict about timing and duration and the Chairs of each session have been instructed to be firm about over-running of allotted time. Please prepare your presentation with this in mind.

For Oral presentations of accepted abstracts, the total time per presentation is 10 minutes, so introductions should ideally be limited to a single or a couple of slides to allow you more time to dedicate to the results and their implications. If you have any relevant Disclosures of funding for your research, please make them known at the beginning of your presentation. There will be time for a short question or two after each presentation.

For all Speakers, please bring your slides to the speakers booth at least the day before your presentation in order for them to be loaded into the Audiovisual system in advance.

Posters

Posters should be made available for viewing from 16.00 on Wednesday 5th of September, and may be removed at the close of the evening session on Friday September 7th (19.40). Poster boards are numbered and materials will be provided to mount the poster.

Please note that poster boards are in PORTRAIT style, with dimensions for accommodating posters up to A0 (1189 mm high by 841 mm wide).

We would encourage your presence with the posters for at least 30 minutes during the lunch breaks on the 6th and 7th of September, in order to allow for questions and answers from fellow attendees. You might also include a contact email or

message on your poster should colleagues wish to follow up with questions after the meeting is over.

Following the end of the meeting, posters that remain on the poster boards will be taken down and disposed of and we regret that there is no possibility to keep or return posters that are inadvertently left behind.

CME and Attendance

EACCME
European Accreditation Council for Continuing Medical Education

The International Workshop on Multiple Endocrine Neoplasia is accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide the following CME activity for medical specialists. The EACCME is an institution of the European Union of Medical Specialists (UEMS).

EACCME credits are recognized by the American Medical Association towards the Physician's Recognition Award (PRA). To convert EACCME credit to AMA PRA category 1 credit, contact the AMA.

The International Workshop on Multiple Endocrine Neoplasia is also accredited by the Accreditation Council of Oncology in Europe (ACOE). The primary function of the ACOE is the accreditation of European CME activities in oncology.

In relation to this function, ACOE reviews and evaluates CME activities in the field of oncology, more specifically ACOE assess the quality and the educational value of the scientific program of the activity. The ACOE accreditation label provides delegates with a guarantee of a high quality and unbiaised educational activity.

Please check at the Registration Desk for CME Assessment Forms and Information

Attendance Certificates can be obtained also at the Registration Desk

Contact

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http://www.mindstream.be

IWMEN 2012: Program Day by Day

Wednesday September 5th

Wednesday September 5th, 2012

SPECIAL FOCUS SESSION: AGGRESSIVE PITUITARY ADENOMAS & CARCINOMAS
Chairs: Philippe Chanson (Paris, France) & Albert Beckers (Liège, Belgium)

Time	Title	Presenter
11.00-11.20	Pathological markers of aggression in pituitary adenomas: focus on MEN1	Jacqueline Trouillas (Lyon, France)
11.20-11.40	Are there imaging markers for pituitary tumor aggressiveness?	Jean-François Bonneville (Besançon, France)
11.40-12.00	AIP status as clinical indicator of aggressiveness in pituitary adenoma patients	Marie-Lise Jaffrain-Rea (L'Aquila, Italy)
12.00-12.20	Break	
12.20-12.40	Aggression & Resistance in Somatotropinoma: Molecular Mechanisms	Marily Theodoropoulou (Münich, Germany)
12.40-13.00	Pituitary carcinoma: Current status of temozolomide	Gérald Raverot (Lyon, France)
13.00-13.35	The Liège Acromegaly Survey: First data in 3000 patients	Patrick Petrossians (Liège, Belgium)
13.35-14.15	Rational use of genetic testing in patients with pituitary adenomas and their families	Albert Beckers (Liège, Belgium)

Wednesday September 5th, 2012

OPENING CEREMONY		
Time	**Title**	**Presenter**
15.35-15.45	Welcome to the 13th IWMEN, Liège 2012	Albert Beckers (Chairman, Local Organizing Committee, Liège, Belgium)
F.I.R.M.O. FOUNDATION MEDAL PRESENTATION		
Time	**Title**	**Presenter**
15.45-16.00	Presentation of the Parathyroid Medal to Raj Thakker, University of Oxford	Maria-Luisa Brandi (Florence)
SYMPOSIUM 1: PANCREATIC & GASTROINTESTINAL NEUROENDOCRINE TUMORS		
Time	**Title**	**Presenter**
16.00-16.25	S1.1: Optimum treatment regimens for advanced pancreatic neuroendocrine tumors.	P. Ruszniewski (Hôpital Beaujon, Clichy, France)
16.25-16.50	S1.2: Pancreas-preserving pancreatectomy for neuroendocrine tumors.	H. Dralle (Univ. Halle-Wittenberg, Germany)
16.50-17.15	S1.3: Combination therapy for the management of advanced gastrointestinal neuroendocrine tumors.	B. Wiedenmann (Charité Berlin, Germany)

Wednesday September 5th, 2012

PLENARY LECTURE 1		
Time	**Title**	**Presenter**
17.15-17.45	The molecular and clinical genetics of familial and syndromic parathyroid disorders.	R. Thakker (Univ. Oxford, United Kingdom)

IPSEN SPONSORED SYMPOSIUM : DEFINING THE BEST CANDIDATES FOR SOMATOSTATIN ANALOGUE TREATMENT IN NET IN 2012 AND BEYOND **Chair:** Bertram Wiedenmann (Berlin, Germany)		
Time	**Title**	**Presenter**
	Chairman's Welcome & Introduction	Bertram Wiedenmann (Berlin, Germany)
18.00-18.25	MEN1: a model for predicting the course of pNET	Gerlof Valk (Utrecht, The Netherlands)
18.25-18.50	Predictors of tumor response in NET patients treated with SSAs	Eric Baudin (Villejuif, France)
18.50-19.15	How will the management of NET with SSA evolve in the future?	Ivan Borbath (Leuven, Belgium)

WELCOME COCKTAIL & BUFFET 19.30
Musée d'Art moderne et d'Art contemporain (MAMAC), Liège

Section 2

Thursday September 6th

Thursday September 6th, 2012

SYMPOSIUM 2: MEN-1 & MENIN		
Time	**Title**	**Presenter**
9.00-9.25	S2.1: Update on the structure and interactions of menin	X. Hua (Univ. of Pennsylvania, USA)
9.25-9.50	S2.2: Mortality in MEN-1: findings of the French registry	P. Goudet (CHU Dijon, France)
9.50-10.15	S2.3: Optimal management of parathyroid disease in MEN-1	B. Niederle (Univ. of Vienna, Austria)
Break		
10.15-10.30		

PLENARY LECTURE 2		
Time	**Title**	**Presenter**
10.30-11.00	Newer pheochromocytoma and paraganglioma susceptibility genes.	M. Robledo (National Cancer Center and CIBERER, Madrid, Spain)

Thursday September 6th, 2012

SYMPOSIUM 3: MULTIPLE KINASE INHIBITORS IN ENDOCRINE NEOPLASIAS		
Time	**Title**	**Presenter**
11.15-11.40	S3.1: Clinical experience with vandetanib for the treatment of advanced or metastatic hereditary medullary thyroid cancer.	E. Baudin (Institut Gustave Roussy, Villejuif, France)
11.40-12.05	S3.2: Profile of cabozantinib (XL 184), an oral tyrosine kinase inhibitor in medullary thyroid cancer and other endocrine tumors.	R.F. Gagel (Univ. of Texas, MD Anderson Cancer Center, Houston, U.S.A.)
12.05-12.30	S3.3: Integrating multiple kinase inhibitors into the clinical management of hereditary and multiple endocrine neoplasias.	B.G. Robinson (Univ. of Syndey, Australia)

Lunch (Posters are available for viewing at this time)
12.30-14.00

PLENARY LECTURE 3		
Time	**Title**	**Presenter**
14.00-14.30	Improving the management of gastroenteropancreatic neuroendocrine tumors: optimizing surgical and medical cooperation.	W.W. de Herder (Erasmus Medical Center, Rotterdam, Netherlands)

Thursday September 6th, 2012

SYMPOSIUM 4: ADRENAL NEOPLASIA		
Time	**Title**	**Presenter**
14.30-14.55	S4.1: Pathophysiology of macronodular adrenal hyperplasia	A. Lacroix (Univ. of Montreal, Canada)
14.55-15.20	S4.2: Genetics of bilateral tumors of the adrenal cortex.	J. Bertherat (Institut Cochin/Univ. of Paris, France)
15.20-15.45	S4.3: KCNJ5: from sporadic to familial hyperaldosteronism	M.C. Zennaro (Univ. of Paris/AP-HP/INSERM, Paris, France)

Break
15.45-16.00

PLENARY LECTURE 4		
Time	**Title**	**Presenter**
16.00-16.30	MEN4, MEN5?, MEN6?: Cyclin dependent kinase inhibitors in multiple endocrine neoplasias.	S. Marx (NIH, Bethesda, U.S.A.)

Thursday September 6th, 2012

SYMPOSIUM 5: PARAGANGLIOMA & PHEOCHROMOCYTOMA		
Time	**Title**	**Presenter**
16.30-16.55	S5.1: Molecular classification of paraganglioma and pheochromocytoma by integrative genomic approaches.	A.P. Gimenez-Roqueplo (Hôpital Europeen Georges Pompidou/INSERM/ Univ. of Paris, France)
16.55-17.20	S5.2: The Paraganglioma Valley: Epidemiology of paragangliomas.	G. Opocher (Univ. of Padova, Italy)
17.20-17.45	S5.3: SDH immunohistochemistry in the diagnosis of hereditary or multiple endocrine neoplasias.	Francine van Nederveen (Erasmus Medical Center, Rotterdam, Netherlands)

ORAL SESSION 1 (18.00-19.20)		
Time (10 min. per presentation)	**Title**	**Presenter**
	OP1.1: Creation of a locus-specific database for mutations in the AIP gene	Giampaolo Trevellin (Bart's, London, U.K.)
	OP1.2: The clinical and genetic characteristics of patients with gigantism	Liliya Rostomyan (Univ. Liège, Belgium)
	OP1.3: Should we screen MEN1 gene in addition to AIP, in young patient with isolated sporadic pituitary macroadenoma?	Anne Barlier (Hôpital de la Conception, Marseilles, France)
	OP1.4: Performance of immunohistochemistry vs. conventional genetic screening to detect SDHx mutations in patients with pheochromocytoma and paraganglioma from Belgium	Selda Aydin (Cliniques Universitaires Saint-Luc, Brussels, Belgium)
	OP1.5: Co-existence of pituitary adenoma and phaeochromocytoma/ paraganglioma (PHAEO/PGL) does it represent a new syndrome with a heterogeneous genetic pathogenesis?	Judit Denes (Military Hospital, Hungarian Defence Forces, Budapest, Hungary)

Thursday September 6th, 2012

ORAL SESSION 1 (Continued)		
Time (10 min. per presentation)	**Title**	**Presenter**
	OP1.6: Identification of chromosome 11 parental origin in a population with endemic PGL1 syndrome	Sara Bobisse (Istituto Oncologico Veneto, Padova, Itoly)
	OP1.7: A rare gain-of-function mutation in an inhibitory uORF in the CDKN1B gene causes MEN4 phenotype	Gianluca Occhi (Univ. Padova, Padova, Italy)

Friday September 7th

Friday September 7th, 2012

SYMPOSIUM 6: MEN-2 & RET		
Time	**Title**	**Presenter**
9.00-9.25	S6.1: Molecular genetics of RET and prognosis in MEN-2.	F. Raue (Heidelberg, Germany)
9.25-9.50	S6.2: Natural history of pheochromocytomas in MEN-2A diagnosed by familial genetic screening.	F. Castinetti (Univ. of Marseilles, France)
9.50-10.15	S6.3: Adrenal function-preserving adrenalectomy in MEN-2.	M. Brauckhoff (Univ of Bergen, Norway)
Break		
10.15-10.30		

PLENARY LECTURE 5		
Time	**Title**	**Presenter**
10.30-11.00	What can mice tell us about men(in).	C.X. Zhang (Lyon Cancer Research Center, Lyon, France)

Friday September 7th, 2012

SYMPOSIUM 7: PARATHYROID DISEASE		
Time	**Title**	**Presenter**
11.15-11.40	S7.1: Diagnostic and therapeutic challenges in parathyroid carcinoma management.	N. Hamdy (Univ. of Leiden, Netherlands)
11.40-12.05	S7.2: Management of chronic post-surgical hypoparathyroidism.	M.L. Brandi (Univ. of Florence, Italy)
12.05-12.30	S7.3: HRPT2 genotype-phenotype correlations in parathyroid disease.	L. Groussin (Institut Cochin/Univ. of Paris 5, France)

Lunch (Posters are available for viewing at this time)
12.30-13.30

PLENARY LECTURE 6		
Time	**Title**	**Presenter**
13.30-14.00	Genetically-determined disorders of cAMP signaling in endocrine neoplasia syndromes.	C. A. Stratakis (NICHD, Bethesda, U.S.A.)

Friday September 7th, 2012

SYMPOSIUM 8: IMAGING **Chairs:**		
Time	**Title**	**Presenter**
14.00-14.25	S8.1: Nuclear imaging to aid peptide receptor radiotherapy of pancreatic neuroendocrine tumors in MEN-1.	J. Teunissen (Erasmus Medical Center Rotterdam, Netherlands)
14.25-14.50	S8.2: Endoscopic ultrasound of the Pancreas in MEN-1 patients.	D. O'Toole (Trinity College, Dublin, Ireland)

Break
14.50-15.10

ORAL SESSION 2 (15.10-17.40)		
Time (10 min. per presentation)	**Title**	**Presenter**
	OP2.1: Measurements of CALCA transcripts are useful for the evaluation of asymptomatic RET-mutation carriers and in the follow-up of patients with medullary thyroid cancer and may replace the pentagastrin test	Cleber Camacho (UNIFESP, Sao Paolo, Brazil)

Friday September 7th, 2012

ORAL SESSION 2 Continued (15.10-17.40)		
Time (10 min per presentation)	**Title**	**Presenter**
	OP2.2: The role of tyrosine kinase inhibitors in a MEN2B patient with metastatic medullary thyroid carcinoma	Monica Tomé (Univ. Liège, Belgium)
	OP2.3: Profile of patients treated for Medullary Thyroid Carcinoma at São Paulo Medical School	Marcos Tavares (Univ. São Paulo School of Medicine, São Paulo, Brazil)
	OP2.4: Pheochromocytoma in Multiple Endocrine Neoplasia Type 2 in Japan: Analysis of a Multicenter Database	Tsuneo Imai (Nagoya University, Nagoya, Japan)
	OP2.5: A study of the Spanish national register of Multiple Endocrine Neoplasia type 2A	Nuria Valdes (Hospital Central de Asturias, Oviedo, Spain)
	Break	
	OP2.6: Could the long-acting somatostatin analogues modify the therapeutic strategy in patients with early stage MEN1-related duodenopancreatic neuroendocrine tumors (NET)s?	Antongiulio Faggiano ("Federico II" University of Naples, Naples, Italy)
	OP2.7: Diagnostic accuracy of chromogranin A, pancreas polypeptide and glucagon in the screening for pancreatic neuroendocrine tumors in Multiple Endocrine Neoplasia type 1 patients	Joanne Marieke de Laat (University Medical Center Utrecht, Utrecht, Netherlands)
	OP2.8: Analysis of penetrance and clinical impact resultant of the diagnosis of multiple endocrine neoplasia type 1-related tumors during childhood and adolescence.	Lourenco Junior Delmar Muniz (School of Medicine, Univ. São Paulo, São Paulo, Brazil)
	OP2.9: Changing manifestations of MEN1 (Burin): increase in occurrence of PNETs	Jane Green (Memorial University, St. John's, Canada)
	OP2.10: Functional characterization of mutations in the Multiple Endocrine Neoplasia type 1 (MEN1) gene suggest therapeutic strategies	Geoffrey Hendy (McGill University, Montreal, Canada)

Friday September 7th, 2012

ORAL SESSION 2 Continued (15.10-17.40)		
Time (10 min per presentation)	**Title**	**Presenter**
	OP2.11: Association of Type-O Blood with Neuroendocrine Tumors in Multiple Endocrine Neoplasia Type 1	Allison Weisbrod (NCI, NIH, Bethesda, U.S.A.)
	OP2.12: Comparison between managements of MEN1 and MEN2	Stephen Marx (NIH, Bethesda, U.S.A.)
	OP2.13: Oncoprotein MafB is switched on in early mouse Men1 b-cell neoplastic lesions	Mr Rémy Bonnavion (Cancer Research Center-Lyon Inserm U1052/CNRS 5286, Lyon, France)
	OP2.14: Quinazoline sensitive binding sites are promising new targets for chemotherapy of neuroendocrine tumors	Robert Fuchs (Medical University of Graz, Graz, Austria)
Break		
17.40-18.00		

HOT TOPICS (18.00-19.40)		
Time (25 min per presentation)	**Title**	**Presenter**
	HT1: Genome-wide characterization of menin-dependent H3K4me3 in mouse embryonic stem cells undifferentiated or differentiated in vitro into islet-like endocrine cells	Sunita Agarwal (National Institutes of Health, Bethesda, U.S.A.)
	HT2: The Cdk5-p25 holoenzyme causes neuroendocrine cancer in the thyroid	James Bibb (Univ. Texas Southwestern Medical Center, Dallas, Texas, U.S.A.)

Friday September 7th, 2012

HOT TOPICS (Continued)		
Time (20 min per presentation)	**Title**	**Presenter**
	HT3: Survival and natural course of patients with thymic and bronchopulmonary neuroendocrine tumor in Multiple Endocrine Neoplasia type 1	Joanne Marieke de Laat (University Medical Center Utrecht, Utrecht, Netherlands)
	HT4: Menin is required to maintain bone mass in older mice	Geoffrey Hendy (McGill University, Montreal, Canada)

CONGRESS DINNER 20.00
Palais de Princes Evêques, Liège

Section 4

Saturday September 8th

Saturday September 8th, 2012

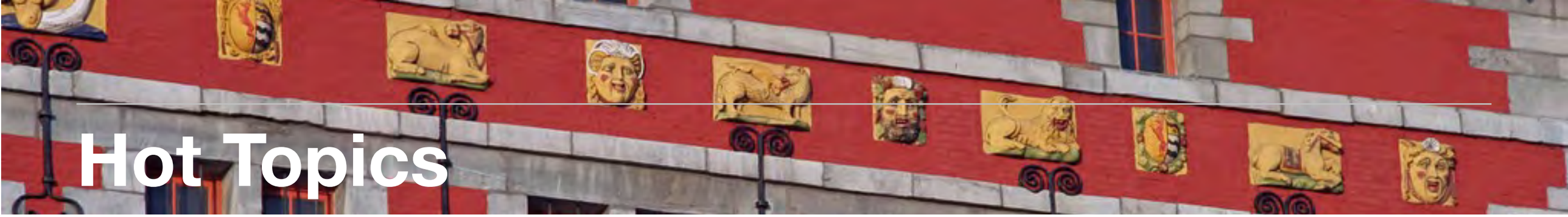
ORAL SESSION 3 (9.10-9.30)		
Time (10 min per presentation)	**Title**	**Presenter**
	OP3.1: Radiofrequency ablation of pulmonary metastases in parathyroid carcinoma: an alternative therapy for severe refractory hypercalcemia.	Lourenco Junior Delmar Muniz (School of Medicine, Univ. São Paulo, São Paulo, Brazil)
	OP3.2: Clinical features of primary hyperparathyroidism (PHPT) in patients with parathyroid carcinoma.	Natalia Mokrysheva (Endocrinology Research Centre, Moscow, Russia)

Saturday September 8th, 2012

SPECIAL FOCUS SESSION: PARATHYROID CARCINOMA RESEARCH **Chairs:** Raj Thakker (Oxford, U.K.) and Albert Beckers (Liège, Belgium)		
Time	**Title**	**Presenter**
9.30-9.50	Diagnosis of parathyroid carcinoma: potential role of PTH laboratory assays	Etienne Cavalier (Univ. of Liège, Liège, Belgium)
9.50-10.10	Molecular genetics of parathyroid carcinoma	Raj Thakker (Univ. Oxford, U.K.)
10.10-10.25	Break	
10.25-10.45	Diagnosis of parathyroid carcinoma: pathological and molecular genetic challenges	Hans Morreau/Naveen Hamdy (Univ Leiden, Netherlands)
10.45-11.05	Therapy of parathyroid carcinoma: update on anti-PTH immunotherapy	Daniela Betea (Univ. of Liège, Liège, Belgium)
11.05-11.30	Discussion on tools for improved research and collaboration: patient databases, tumor biobanks, common goals and purposes	Roundtable discussion

CLOSING CEREMONY		
Time	**Title**	**Presenter**
11.40-11.45	Closure of the 13th IWMEN, Liège 2012	Albert Beckers (Chairman, Local Organizing Committee, Liège, Belgium)

IWMEN 2012: Abstracts



Hot Topics

HT1

Genome-wide characterization of menin-dependent H3K4me3 in mouse embryonic stem cells undifferentiated or differentiated in vitro into islet-like endocrine cells

Sunita K. Agarwal1, and Raja Jothi2 1Metabolic Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland 20892, USA 2Biostatistics Branch, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina 27709, USA

Inactivation of the MEN1 gene encoding menin is causative for the MEN1 syndrome and for a fraction of similar sporadic endocrine tumors. Among other interactions, menin partners with histone-modifying protein-complexes. Menin is found in the MLL1/MLL2-complexes that trimethylate (me3) histone-H3 at lysine-4 (K4), a histone-modification (H3K4me3) for active gene-expression. Identifying genes that undergo tissue-specific menin-dependent H3K4me3 could provide insights into tissue-specific tumor-suppressive actions of menin as well as targets causative for endocrine tumorigenesis. Men1-knockout mouse embryonic stem cells (menin-null ESCs) display defective in vitro hematopoietic differentiation. However, it is not known whether menin-null ESCs could differentiate in vitro into pancreatic islet cells or other endocrine cell-types affected in MEN1. We found that similar to wild-type (WT) ESCs, menin-null ESCs could differentiate in vitro into pancreatic islet-like endocrine cells (PILECs). We compared WT and menin-null ESCs before and after in vitro differentiation into PILECs to identify menin-dependent H3K4me3 and gene-expression changes by H3K4me3-ChIP-Seq and gene-expression microarray. In ESCs the gene most affected by menin loss was Meg3 (maternally expressed gene 3), which showed 16-fold reduction in H3K4me3 and 14-fold reduction in gene-expression. In menin-null ESCs 3-9-fold reduced expression of several genes at the Dlk1-Meg3 locus was also observed. MEG3 is an imprinted long-non-coding-RNA that functions as a tumor-suppressor by p53-dependent and p53-independent pathways. Interestingly, sporadic pituitary tumors show somatic silencing of MEG3 and many genes at the DLK1-MEG3 locus. In PILECs the genes most affected by menin loss were Hox genes that showed 4-16-fold reduction in H3K4me3 and 3-13-fold reduction in gene-expression. The 39 HOX genes are homeodomain-containing transcription factors that regulate cellular identity during development, and some act as potential tumor-suppressors or oncogenes. Interestingly, parathyroid tumors show abnormal expression of several HOX genes. Therefore, although HOX genes were dispensable for the development of menin-null PILECs, their abnormal expression may impact islet-tumor development. Our investigation demonstrates that WT and menin-null ESCs differentiated in vitro into PILECs could be used effectively for genome-wide analysis of menin-dependent gene-regulation. Methods for in vitro differentiation of ESCs into MEN1 target tissues, such as the parathyroids and anterior pituitary, could be applied to generate homogeneous populations of WT and menin-null cells for similar studies. Such investigations will advance our understanding of molecular and cellular events associated with normal and pathological endocrine cell proliferation and function.

HT2

The Cdk5-p25 holoenzyme causes neuroendocrine cancer in the thyroid

Karine Pozo*1,, Chunfeng Tan1, Florian Plattner1, Herbert Chen2, Fiemu E. Nwariaku3, Roswitha Pfragner4, James A Bibb1,5,6 1Department of Psychiatry, The University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9070, 2 Endocrine Surgery Research Laboratory, Department of Surgery and the University of Wisconsin Carbone Cancer Center, Madison, WI 53792 3 Department of Surgery, The University of Texas Southwestern Medical Center 4 Institute of Pathophysiology and Immunology, Medical University of Graz, Graz, Austria 5 Department of Neurology, The University of Texas Southwestern Medical Center 6 The Harold B. Simmons Comprehensive Cancer Center, The University of Texas Southwestern Medical Center

Synaptic remodeling during cognition may considered a special adaptation of the cell cycle. The protein kinase Cdk5 is a member of the cyclin-dependent kinase family, which is highly expressed in neurons. Its activity is dependent upon association with a cofactor p35. Cdk5/p35 contributes to synaptic remodeling and numerous other neuronal functions. However, when p35 is truncated by calpain, the resulting Cdk5/p25 complex engenders aberrant activity and is neurotoxic. To model neurodegeneration, we derived an inducible transgenic mouse that overexpresses GFP-tagged p25 under the control of the neuronal specific enolase promoter. Surprisingly, these mice rapidly developed medullary thyroid carcinoma (MTC) with 100% lethality. Medullary thyroid cancer (MTC), the most common neuroendocrine tumor, arises from calcitonin-producing parafollicular or C-cells and metastasizes frequently to regional lymph nodes, and to the lungs, liver, and brain. Neurons and neuroendocrine cells share a common ontogenic linage and functions such as calcium-dependent secretion. The inducible/arrestable tumors of our mouse model exhibit the characteristics of human MTC. They express calcitonin, are invasive and metastatic. We found that Cdk5 and p35/p25 were normally expressed in mouse and human C-cells and that their levels and Cdk5 activity were prevalent in both human sporadic and familial medullary tumors. Moreover, both human and mouse MTC cell proliferation was dependent upon aberrant Cdk5 activity. Phosphoproteomic analysis implicated retinoblastoma protein as a critical downstream effector of aberrant Cdk5 activity in proliferating tumors. This target was validated using a novel small interfering peptide that disrupted this mechanism. These findings suggest that targeting p25/Cdk5 or its downstream effectors may be a valuable therapeutic strategy for the treatment of MTC and provide a new perspective on the relationship between the mechanisms of synaptic remodeling and oncogenesis.

HT3

Survival and natural course of patients with thymic and bronchopulmonary neuroendocrine tumor in Multiple Endocrine Neoplasia type 1

Joanne M. de Laat, MD[1], Carolina R.C. Pieterman, MD[1], Jos W.R. Twisk PhD[2], Medard F.M. van den Broek[1], Ad R. Hermus, MD PhD[3], Olaf M. Dekkers, MD PhD[4], Wouter W. de Herder, MD PhD[5], Anouk N. A. van der Horst-Schrivers, MD PhD [6], Madeleine L. Drent, MD PhD[7], Peter H. Bisschop, MD PhD[8], Bas Havekes, MD PhD[9], Menno R. Vriens, MD PhD[10], Gerlof D. Valk, MD PhD[1]

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Thymic- and bronchopulmonary neuroendocrine tumors (Th- and Bp-NET) are non often occurring MEN1 manifestations, and available data concerning the natural course, screening and care of patients with these tumors is limited.

To describe the clinical characteristics and survival of patients with Th- and Bp-NET and to assess the growth rate of Bp-NET on imaging.Longitudinal observational study based on the Dutch national MEN1 database including more than 90% of the total MEN1 population in the Netherlands. MEN1 patients >16 years, with a confirmed MEN 1 mutation, who were under the care of the Dutch University Medical Centers (<1990-2010) (n=296). Linear mixed models were applied to assess changes over time in size of Bp-NET. Time was treated as continuous variable and defined in quarters. Logaritmic transformation of the size of the bronchopulmonary nodules was performed, because of non-normal data distribution. MEN1 patients with histologically proven or radiologically suspected metastasis from other NETs were excluded from the analysis. Gender, the presence of a missense or frameshift mutation in exon 2,9 or 10 and size of Bp-NET ≥ or <10 mm at the first radiograph were investigated for effect modification. Prevalence and survival of Bp- and Th-NET, change in Bp-NET size over time.The prevalence of Th-NET in MEN1 patients was 3.7%, with a 10-year survival of 25% (SE 14.8%). Ninety-one percent of patients with Th-NET were male (p=0.001). Diagnostic screening of the thorax for Bp-NET was performed in 184 MEN1 patients (62%). Bp-NET was identified in 42 of those patients (22.8%) with a 10 year survival of 69% (SE 13.0%). Fifty-two percent of Bp-NET patients were female (p=0.55). Fifteen patients were operated on (36%). Tumor volume of Bp-NET increased 4% per quarter (p=0.0001) leading to a tumor doubling time of six years. Bp-NET growth was significantly higher in male patients (p= 0.054). Bp-NET growth was not significantly associated with type of mutation (p= 0.471) and baseline tumor size (<1 or ≥ 1 cm, p= 0.816).

Th-NET was an aggressive manifestation with a poor prognosis. Bp-NET was more common manifestation with a relatively indolent course and a good prognosis.

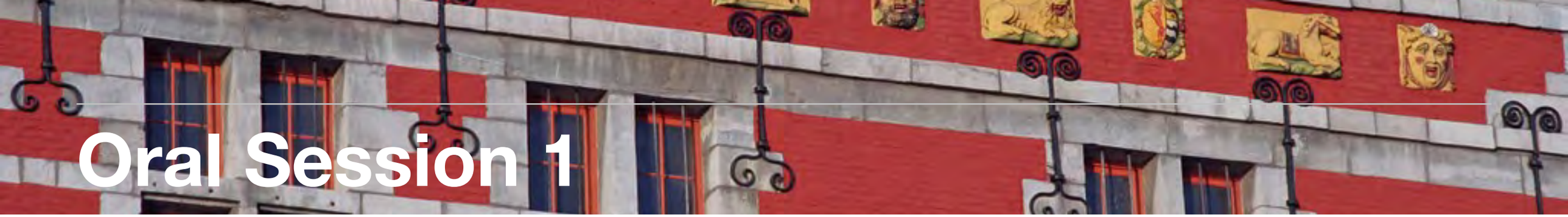
HT4

Menin is required to maintain bone mass in older mice

Ippei Kanazawa, Lucie Canaff, Monzur Murshed and Geoffrey N. Hendy. McGill University, Montreal, Quebec, Canada

Background: The tumor suppressor menin is expressed in all mouse tissues examined, including bone, Homozygous Men1 inactivation in mice is embryonic lethal at 12 days and the small fetuses exhibit defects in cranial and facial development, suggestive of a role for menin in bone formation. The heterozygous Men1 phenotype is similar to that of the multiple endocrine neoplasia type 1 (MEN1) syndrome in humans with endocrine tumors developing later in life. Although previous in vitro studies have shown that menin does have an important role in osteoblastogenesis and osteoblast differentiation, little is known about the in vivo role of menin in bone accumulation. Methods and Results: We conditionally inactivated Men1 in postnatal mature osteoblasts by crossing osteocalcin-Cre mice with floxed Men1 mice to generate mice lacking Men1 exon 3 to 8 in osteoblasts (Men1osb−/− mice). Nine-month-old Men1osb−/− mice displayed significant reduction in bone mineral density by dual-energy X-ray absorptiometry and in trabecular bone volume and cortical bone thickness by micro-computed tomography (CT) analysis. Micro-CT images indicated abnormality of trabecular bone formation in Men1osb−/− mice. By histomorphometric analysis bone volume/total volume, osteoblast and osteoclast number, as well as mineral apposition rate (MAR) were significantly reduced in Men1osb−/− mice. The mRNA expression of osteoblast genes, OPG, RANKL, BMP-2, Runx2, Osx, Dlx2, Dlx5, and cyclin-dependent kinase inhibitors, p15, p18, p21 and p27, were all reduced, whereas that of cyclin dependent kinases, CDK2 and CDK4, were increased in isolated osteoblasts from Men1osb−/− mice compared to controls. These data are consistent with the menin-deficient osteoblasts having reduced responsiveness to TGF-β and/or BMP-2 and loss of Smad signaling. In contrast to the knockout mice, 12-month-old transgenic mice overexpressing the human menin cDNA in

osteoblasts (Men1osbTG/+ mice) driven by the 2.3 kb Col1a1 promoter, showed a gain of bone mass by micro-CT and histomorphometric analysis. Osteoblast number and MAR were significantly increased in Men1osbTG/+ mice. Conclusion: Taken together, depletion of menin in the osteoblast leads to decreased osteoblast and osteoclast numbers as well as impaired bone remodeling, resulting in a reduction in trabecular and cortical bone whereas overexpression increases bone volume by enhancing bone formation. Therefore, maintenance of menin expression and function in the osteoblast is important to avoid decreased bone mass.



Oral Session 1

OR1.1

Creation of a locus-specific database for mutations in the AIP gene

Giampaolo Trivellin1, Katarzyna Wertheim-Tysarowska2, Plamena Gabrovska1, Karen Stals3, Serban Radian1,4, Márta Korbonits1 1Department of Endocrinology, Queen Mary University of London, London, UK 2Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland 3Department of Molecular Genetics, Royal Devon and Exeter Foundation Trust, Exeter, UK 4C.I. Parhon Institute of Endocrinology, Bucharest, Romania

The increasing number of sequence variations reported in the human genome and the consequent necessity to establish their clinical significance has recently led to the creation of locus-specific databases (LSDBs). LSDBs are useful and widely used publicly available on-line resources. Several genes causing endocrine syndromes have LSDBs available, such as MEN1, VHL, RET, GNAS and the SDH subunits. We are developing a curated, freely accessible AIP-LSDB which will be soon available at http://www.fipapatients.org/aip/. The aim of this project is to collect reported and unpublished variants related to FIPA and their clinical information in order to deepen our knowledge of the disease. The database is generated using PHP and Ajax scripting languages, and MySQL relational database management system. The core database is hosted on the Queen Mary University of London server. Molecular defects will be named according to the GenBank's NG_008969.1 RefSeq strictly following HGVS (Human Genome Variation Society) recommendations. In order to submit variants a clinical description module which allows for a comprehensive description of the patient's genotype and phenotype will be provided upon free registration. The core of the database is a graphic view of the AIP gene structure, divided in exons and introns. The number and type of variants in each region of the gene are reported in a pop-up window while hovering with the cursor over the corresponding fragment. After clicking on the region of interest, a detailed pop-up window with the nucleotide and amino acid sequence opens and all the reported variants are showed as red dots below the sequence. Clicking on each dot enables to see all the genetic and clinical details available for that specific variant. Moreover, the database allows for the addition of data from patients harbouring the same variant. A flexible data selection tool for statistical analysis will be implemented, but the user can also download all data in a CSV format and perform further statistical analyses. This database will assist clinicians as well as researchers in the interpretation of AIP variants and in genetic counselling and will help to avoid unnecessary testing. Moreover, we believe that the AIP-LSDB will help to examine the structure–function and the genotype-phenotype correlations, if any, in AIP mutation positive patients and will enable scientists worldwide to collaborate on future research projects.

OR1.2

THE CLINICAL AND GENETIC CHARACTERISTICS OF PATIENTS WITH GIGANTISM

Liliya Rostomyan1, Adrian F. Daly1, Maria Tichomirova1, Luciana A. Naves2, Anne-Lise Lecoq3, Emil Nachev4, Andreas Moraitis5, Dianne Kranenburg6, Ian Holdaway7, Caroline Sievers8, Ekaterina Sorkina9, Daria Gusakova10, Elena Malchiodi11, Elena Nazzi12, Margaret Zacharin13, Roberto Salvatori14, Renata Auriemma15, Jakob Dal16, Silvia Filipponi1, Irena Ilovaiskaya17, Dominique Maiter18, Ann McCormack19, Klaus von Werder20, Françoise Borson-Chazot21, Sandrine Laboureau Soares22, Jens Otto L. Jorgensen16, Annamaria Colao15, Diego Ferone12, Paolo Beck-Peccoz11, Vyacheslav Pronin9, Gunter K. Stalla8, Sebastian Neggers6, Constantine A. Stratakis5, Sabina Zacharieva4, Patrick Petrossians1, Philippe Chanson3, Albert Beckers1 1Department of Endocrinology, CHU de Liège, University of Liège, 4000 Liège, Belgium 2Endocrinology, University of Brasilia, Brasila, Brazil 3Endocrinology, CHU Le Kremlin-Bicetre, Le Kremlin Bicetre, France 4Clinical Center of Endocrinology and Gerontology (M.Y., S.Z.), Medical University, 1431, Sofia, Bulgaria 5Section on

Endocrinology Genetics, Program on Developmental Endocrinology Genetics (PDEGEN), Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD), National Institute of Health (NIH), Bethesda, MD 20892, USA 6Section of Endocrinology (S.N., W.D.H.), Department of Internal Medicine, Erasmus Medical Centre, 3015 GD Rotterdam, The Netherlands 7Department of Endocrinology (I.H.), Greenlane Clinical Centre, 1051 Auckland, New Zealand 8Department of Endocrinology (G.K.S.), Max Planck Institute of Psychiatry, 80804 Munich, Germany 9I.M. Sechenov First Moscow State Medical University, The Endocrinology Clinic, Moscow, Russian Federation. 10Scientific and Research Institute of Urology, Moscow, Russian Federation 11Endocrinology, Fondazione IRCCS Osp. Maggiore Policlinico Mangiagalli Regina Elena, Milan, Italy 12Department of Endocrinology and Medical Sciences, Center of Excellence for Biomedical Research, University of Genoa, Genoa, Italy 13Murdoch Childrens Research Institute, Royal Children's Hospital and University of Melbourne, Parkville, Victoria, Australia. 14Division of Endocrinology (R.S.), Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, USA 15Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University of Naples Naples, Italy 16Department of Endocrinology and Internal Medicine, NBG/THG, Aarhus University Hospital, Aarhus, Denmark 17Moscow Regional Research & Clinical Institute named by MF Vladimirsky, Moscow, Russian Federation 18Department of Endocrinology, St. Luc University Hospital, Université Catholique de Louvain, B-1200 Brussels, Belgium 19Cancer Genetics Unit, Hormones and Cancer Group, Kolling Institute of Medical Research, Royal North Shore Hospital, Sydney, NSW, Australia 20Innere Medizin/Endokrinologie, Max-Planck-Institut für Psychiatrie, München 21Endocrinology, CHU de Lyon, Lyon, France 22Département d’endocrinologie diabétologie nutrition, CHU d’Angers, 4, rue Larrey, 49933 Angers cedex 9, France

Objective: To analyse a large series of patients with gigantism. Design: A multicentre retrospective study. Patients: The gigantism was considered in patients with current/previous abnormal, progressive, excessively rapid growth velocity for age and/or a height greater than 2 SD above normal for their population and/or absolute height more than 200 cm or greatly (>5 cm) in excess of the calculated midparental height in the absence of constitutional tall stature. 118 patients were included. Data of patients systematically collected in selected centres was recorded in case report forms. Results: In most of cases gigantism was due to GH-overproduction by pituitary adenomas (PA) (88%), in 4% by pituitary hyperplasia, in 1% because of ectopic GHRH production, 4% without visualisation of PA and 3% in Klinefelter syndrome. Males/Females - 93/21. 36% of patients were taller than 200cm (max 231mm). Median age of rapid growth velocity was 12yr. 86 patients stopped growing at age of 20yr. [18;22] with the height 196 cm [188;202]. 18 still growing patients had height 193cm [178;205]. 32.8% (22/67) had tall relatives, 50% of them had features of genetic syndromes. Age at first symptoms - 14 yr [10;17]. 92% had facial changes and/or acral overgrowth at time of diagnosis. Age at diagnosis PA in females were younger than in males (14.9yr vs 22yr) with delay in diagnosis of PA ? 21 yr. Most of PA were macroadenomas (76%, even giant PA in 12%) and in 19% microadenomas with Max tumor size- 20 mm [12;34]. In more than half of cases there was extrasellar extension (70%) and invasion (52%). Pituitary hyperplasia in 6%. There was no difference between men and women in GH (26.75ng/ml vs 45ng/ml, p=0.36) and IGF-1 (258%ULN vs 314%ULN, p=0.98) levels at diagnosis. Prolactin co-secretion observed in 42%. Median follow up on treatment was 50.5months. 87 patients were operated with the remission in 11%, Multimodal treatment approach was in 46% with control in 20%. Overall control of the disease was achieved in 33%. Genetic/syndromic features presented in 36% (7 FIPA, 6 AIPmut cases, 5 McCune-Albright, 4 Klinefelter syndrome, 3 Carney complex, 1 MYH-polyposis with GHRH secretion by pheocromocytoma). Mutations in AIP gene were found in 37.5% (9/24). Conclusion: In most of cases gigantism was due to PA, which are mostly large (with extrasellar extension and invasion in >1/2 of cases) and difficult to control. Syndromic features are presented in 1/3 of cases and AIP mutations are common in gigantism.

OR1.3

Should we screen MEN1 gene in addition to AIP, in young patient with isolated sporadic pituitary macroadenoma ?

Thomas Cuny, Morgane Pertuit, Mouna Sahnoun-Fatahla, Adrian Daly, Marie Françoise Odou, Antoine Tabarin, Marie Laure Nunes, Brigitte Delemer, Rachel Desailloud, Véronique Kerlan, Olivier Chabre, Jean-Louis Sadoul, Muriel Cognes, Philippe Caron, Christine Cortet-Rudelli, Anne Lienhardt, Isabelle Raingeard, Anne-Marie Guedj, Thierry Brue, Albert Beckers, Georges Weryha, Alain Enjalbert, Anne Barlier

Context: whereas germline mutations in the AIP gene had been extensively studied in patient with sporadic pituitary macroadenoma, there are few datas about the prevalence of MEN1 mutations in such population. Design: we assessed the prevalence of both AIP and MEN1 genetic abnormalities in 174 young patients (age < 30 years) from Endocrinology Department of 16 French University Hospital Centers. Each patient was diagnosed with isolated and sporadic pituitary

macroadenomas, namely without MEN1-associated lesion and/or familial history of pituitary disease. The respective entire coding sequence of AIP and MEN1 was screened for mutation, in DNA of peripheral blood leukocytes. In case of negative screening, multiplex ligation-dependent probe amplification was performed for detection of large gene deletion (LGD). Results: 21/174 (12%) patients bore AIP (AIPmut, n=14) or MEN1 mutations (MEN1mut, n=7). Among pediatric patients (less than 18 yrs), it reaches 24% (11/46). According to previous report, acromegalic patients count for most of the AIPmut population (7/14), with high prevalence of acromegalogigantism (3/6 patients). MEN1mut were identified in 4, 2 and 1 patient with prolactinomas, somatotropinomas and non –secreting adenoma respectively. One patient was identified with de novo mutation of MEN1 in our cohort. Interestingly, 4/12 (33%) patients with non-secreting adenomas bore AIPmut or MEN1mut, whereas none of the 8 corticotroph-adenomas and the one single thyrotropinoma were mutated. In negative-screening patients, no LGD were identified. Conclusion: Beside AIP, mutations in MEN1 can be found in up to 4% of young patient with isolated sporadic pituitary macroadenoma, therefore suggesting to systematically perform this genetic analysis in such population.

OR1.4

PERFORMANCE OF IMMUNOHISTOCHEMISTRY VS. CONVENTIONAL GENETIC SCREENING TO DETECT SDHx MUTATIONS IN PATIENTS WITH PHEOCHROMOCYTOMA AND PARAGANGLIOMA FROM BELGIUM.

Selda Aydin, Francien H van Nederveen, Nathalie Lannoy, Francesca Severino, Thomas Papathomas, Dominique Maiter, Marc Hamoir, Miikka Vikkula, Ronald de Krijger, Alexandre Persu.

Background. Mutations in predisposing genes are detected in 30-40% of patients with paraganglioma/pheochromocytoma (PGL/PHEO), including 10-20% of mutations in SDHx genes, encoding the subunits of succinate dehydrogenase. In Belgium, SDHx mutations are frequent, especially in head and neck PGLs (40%) (Persu et al., Horm Metab Res 2012). Immunohistochemistry (IHC) of SDHB and SDHA subunits was shown to be a straightforward and effective method to detect such mutations (van Nederveen at al., Lancet Oncol 2009). However this approach still needs further validation in new cohorts. Aim. To look for the proportion of PGL/PHEO tumors without SDHB and/or SDHA expression and to assess the sensitivity and specificity of the IHC method for detection of SDHx mutations versus conventional genetic screening in a multicentric series of PGL/PHEOs from Belgium. Methods. Immunohistochemistry for SDHB and SDHA was done on 75 tumors from 69 patients. In 55 patients, genetic screening for SDHx mutations had been already performed. In 14 additional samples, genetic status was unknown at the time of IHC. The pathologists in charge of interpreting the IHC were unaware of the results of genetic screening in either subgroup. Results. Expression of SDHB was abolished in tumors from 42% (n=23) of patients previously genotyped for SDHx genes. Of these, 15 were previously known to harbor mutations in SDHB (n=3) or SDHD (n=12), while genetic screening was negative in the remaining eight. Noteworthy, SDHB expression was abolished in all patients known to harbor SDHx mutations. Furthermore SDHB expression was abolished in 29% (n=4) of the 14 patients of unknown genetic status. In one of them, expression of SDHA was also absent, suggesting the existence of a mutation in SDHA. Genetic analysis of this additional subset is under way. Conclusion. We report the first Belgian series of patients with PGL/PHEOs in whom the presence of SDHx mutations was assessed both by conventional genetic analysis and IHC. As expression of SDHB was abolished in all patients with known SDHx mutations, the sensitivity of IHC was 100%. The absence of SDHB staining in 8 patients screened negative for SDHx genes might either reflect a lack of specificity of the IHC method, or more likely the presence of mutations in the promoter or other unexplored regions of the corresponding genes. In the near future, IHC might be offered to all patients operated from a PHEO and/or a PGL, genetic analysis of SDHx genes being reserved to subjects without expression of SDHB and/or SDHA by IHC.

OR1.5

Co-existence of pituitary adenoma and phaeochromocytoma/paraganglioma (PHAEO/PGL) – does it represent a new syndrome with a heterogeneous genetic pathogenesis?

Judit Dénes, Francesca Swords, Paraskevi Xekouki, Ajith Kumar, Eamonn Maher, Christopher Wassif, Naomi Fersht, Stephanie Baldeweg, Damian Morris, Stafford Lightman, Amar Agha, Aled Rees, Joan Grieve, Michael Powell, Cesar Luiz Boguszewski, Cristina Preda, Jacqueline Trouillas, Nadezhda Dalantaeva, Antônio Ribeiro- Oliveira Jr, Sian Ellard, Eleanor Rattenberry, Karen Stals, Constantine A. Stratakis, Ashley B. Grossman, Márta Korbonits

Pituitary adenoma and PHAEO/PGL can very rarely occur in the same patient or in the same family. One possible cause has been previously described, when these 2

tumours occur as a PHAEO/PGL which secretes GHRH and thus cause somatotroph hyperplasia and acromegaly. However, we have hypothesised several other mechanism which could be involved in the development of these two tumour types together: a PHAEO/PGL gene which also causes pituitary adenoma, a pituitary tumour gene which also causes PHAEO/PGL, a digenic disease, a new gene(s) causing both diseases, or the development of the 2 tumours together can be a pure coincidence. We found 44 cases in the literature with this combination of diseases, but only 7 of them had a confirmed genetic diagnosis. We studied 21 new patients with the combination of pituitary adenoma and PHAEO/PGL. PHAEO/PGL causing genes (SDH A-D, SDHAF2, RET, VHL, TMEM127, MAX) and pituitary adenoma genes (MEN1, AIP, p27) were sequenced using next generation or Sanger sequencing, and loss of heterozygosity was studied in the tumours where available. We identified mutations in SDHB, SDHC, SDHD, MEN1, RET and VHL in some patients and families with PHAEO/PGL and pituitary adenomas (3 SDHB, 2 VHL, 1 SDHC, 1 SDHD and 1 MEN1). Loss of heterozygosity for the relevant gene was shown in all the cases where tissue was available. These data suggest that mutations in some PHAEO/PGL and pituitary genes can affect both these tissue types.

OR1.6

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Paraganglioma syndrome type 1 (PGL1) is a rare autosomal dominant disease with maternal imprinting characterised by the development of head-and-neck paragangliomas and pheochromocytoma, associated with germ-line mutations of SDHD gene. We identified a PGL1 founder effect caused by the SDHD c.341A>G p.Tyr114Cys mutation. We recruited 540 individuals from 95 affected kindreds: 287 carried the mutation. Of this group, 184 individuals inherited the mutation from the father, 77 from mother, while 26 were with an unknown inheritance. The genetic evaluation of 4025 resident volunteers allowed to identify 59 carriers of the founder mutation, resulting in a prevalence of 1.5% among the general population. The identification of a large numbers of carriers with an unknown inheritance highlights the lack of a tool to discriminate the parental origin of the mutated chromosome. To this aim we isolated the chromosome 11 using the conversion technology and analysed the methylation pattern of the 11p15.5 region. Hybrids were generated by PEG-mediated fusion of lymphoblastoid cell lines with mouse RAG cell line and were cultured in a selective medium. After hybrid clones were grown, we had determined by genotyping which were haploid for chromosome 11. Each of these clones were characterised for the presence of the founder mutation with an allelic discrimination taqman assay and for the methylation pattern of the 11p15.5 region with the MLPA kit ME030-B2. Using lymphoblastoid cell lines from two carriers who inherited the mutation from the father and from the mother, respectively, we obtained 136 hybrid clones, among which 9 were haploid for chromosome 11. The analysis of the methylation pattern with the MLPA kit ME030-B2 confirmed the parental origin of the chromosome 11. Using this validated method, we therefore investigated a patient with an unknown inheritance. In particular, we screened 77 hybrid clones, among which 11 maintained a single copy of chromosome 11. MLPA analysis revealed the maternal origin of the mutation. Preliminary results indicate that this approach may be useful to demonstrate parental origin of the SDHD mutation, allowing paraganglioma risk estimation in individuals with unknown inheritance.

OR1.7

A rare gain-of-function mutation in an inhibitory uORF in the CDKN1B gene causes MEN4 phenotype

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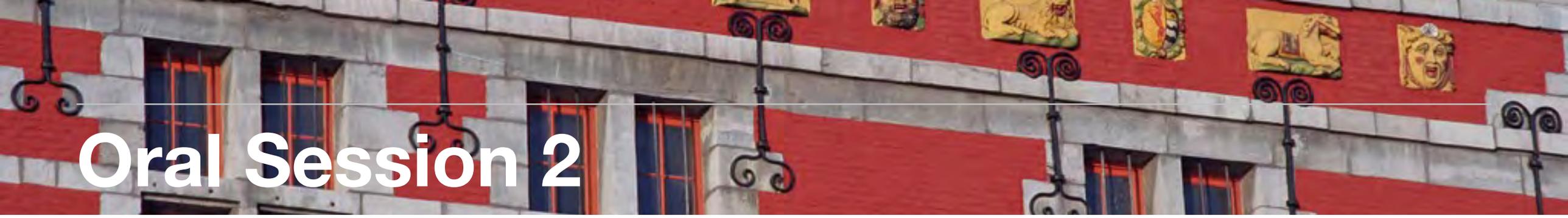
The CDKN1B gene encodes for the cyclin-dependent kinase inhibitor p27KIP1 that is a key player in many cell processes including proliferation and differentiation. Impaired p27KIP1 expression and/or localization is often observed in tumor cells, further confirming its central role in regulating the cell cycle. Recently, germline mutations in CDKN1B have been associated with the inherited multiple endocrine neoplasia syndrome type 4 (MEN4, MIM610755). MEN4 is a rare autosomal dominant syndrome characterized by varying combinations of tumors that affect at least two endocrine organs. Aim of present study was to determine the possible causes of multiple endocrine tumors in 25 MEN1-like patients by analyzing the entire CDKN1B gene for point mutations and large rearrangements in germline DNA. A c.-456_-453delCCTT was identified in a regulatory upstream ORF (uORF) in the 5'UTR, in a 62 year old acromegalic

patient with a pancreatic endocrine neoplasm. The 4-bp deletion causes the shift of the uORF termination codon with the consequent lengthening of the uORF encoded peptide and the drastic shortening of the intercistronic space. Our functional studies clearly prove a negative influence of such deletion on the translation reinitiation at the CDKN1B starting site. LOH analysis from the pancreatic lesion revealed no loss of the second allele, confirming that p27KIP1 may act as haploinsufficient tumor suppressor. Importantly, our in-silico analyses showed a very high level of inter-species conservation of the uORF within the CDKN1B gene (both at nucleotide and protein level), strongly supporting a physiological role of the uORF-encoded peptide in modulating p27KIP1 translation. Although regulatory uORFs are very common in human and mouse genes, being observed in about half of cases, CDKN1B gene represents the fourth case for which an uORF affecting mutation have been clearly associated to human diseases. In addition, our findings demonstrate for the first time that, besides the already described degradation by the ubiquitin/proteasome pathway and non-covalent sequestration, p27KIP1 activity can also be modulated by an uORF, and that mutations in this region may have deleterious consequences.



Oral Session 2

OR2.1

MEASUREMENT OF CALCA TRANSCRIPTS ARE USEFUL FOR THE EVALUATION OF ASYMPTOMATIC *RET*-MUTATION CARRIERS AND IN THE FOLLOW-UP OF PATIENTS WITH MEDULLARY THYROID CANCER AND MAY REPLACE THE PENTAGASTRIN TEST. Cléber P. Camacho, Susan C. Lindsey, Maria Clara C. Melo, Rosa Paula M. Biscolla, José Gilberto H. Vieira, Janete M. Cerutti, Magnus R. Dias-da-Silva and Rui M. B. Maciel

Introduction: Calcitonin is the main tumor marker for medullary thyroid cancer (MTC), but it has certain limitations. In some clinical situations, the pentagastrin stimulation test is desirable, such as in the screening for MTC in *RET* mutation-carrying relatives or in the postoperative follow-up of MTC patients. Notwithstanding, the pentagastrin stimulation test is expensive, time-consuming and uncomfortable for patients; besides, pentagastrin is not world wide available. **Objective**: We aimed to 1) investigate the applicability of measuring *CALCA* gene transcripts (CT-CALCA and CGRP-CALCA) in patients with MTC and in relatives diagnosed with a *RET* mutation and 2) suggest an alternative molecular diagnostic tool for the pentagastrin stimulation test. **Setting and Patients**: Twenty-six individuals consecutively evaluated for MTC at the Thyroid Clinic of the Federal University of São Paulo were selected, including patients with sporadic or hereditary MTC (with or without evidence of the disease) and *RET* mutation-carrying relatives. **Results**: We detected CT-CALCA mRNAs and CGRP-CALCA mRNAs in the blood samples and observed a positive correlation between them (r: 0.970 and p <0.0001). Both mRNAs also correlated with the serum CT (sCT) (CT-CALCA - r: 0.708 and p: <0.0001 / CGRP-CALCA - r: 0.702 and p: <0.0001). The relative expression (RE) of CT-CALCA and CGRP-CALCA presented a higher sensitivity (85.71 and 100, respectively), specificity (83.33 and 91.67, respectively), positive predictive value (85.71 and 93.33, respectively) and a higher negative predictive value (83.33 and 100, respectively), than did the isolated sCT (78.57, 75, 78.57 and 75, respectively). In addition, CGRP-CALCA presented a higher sensitivity, specificity, PPV and NPV than did CT-CALCA. The CALCA-transcript measurement mirrors the response to the pentagastrin stimulation test. **Conclusion**: We demonstrated the measurement of CALCA gene transcripts in the bloodstream—refining the test in the follow-up of patients with MTC and *RET* mutation-carrying relatives—and herein suggest the application of this molecular diagnostic tool as an alternative to the pentagastrin stimulation test.

OR2.2

THE ROLE OF TYROSINE KINASE INHIBITORS IN A MEN2B PATIENT WITH METASTATIC MEDULLARY THYROID CARCINOMAMartin-Hernandez Tomas[a], Tome-Garcia Monica[b], Gonzalez-Rivera Natividad[a], Virizuela JA[c], Beato Carmen[c], Gentil-Baldrich Alfonso[a], Sendon-Perez Angel[a]aDepartment of Endocrinology. University Hospital Virgen Macarena. Seville. Spain.bService d´Endocrinologie. Centre Hospitalier Universitaire de Liège. Belgium.cDepartment of Oncology. University Hospital Virgen Macarena. Seville. Spain.

Medullary thyroid carcinoma (MTC) is present in up to 100% of patients with multiple endocrine neoplasia syndrome type 2 (MEN2). Traditional chemotherapy or external beam radiation have shown limited effects. Development of tyrosine kinase inhibitors open a new era in the management of the disease. A 23-year-old man with marfanoid phenotype was admitted to emergency room with pneumothorax. Surgery was performed and anatomopathologic diagnosis of lung samples was metastatic MTC. The patient underwent total thyroidectomy and cervical lymphadenectomy. Genetic test was positive for protoncogene RET Met918Tr mutation. The extension study included a whole body scan with ^{111}In-octreotide, PET-CT scan with ^{18}F-FDG, whole body scincigraphy with ^{99m}Tc-DMSA and cervical ultrasound. The results confirmed extension of local and distant metastases in the right lung, the liver, mesenteric and retroperitoneal lymph nodes and vertebral spine. Biochemical study presented an elevation in CEA 59ng/ml (NV <5ng/ml), calcitonin (CT) 2000pg/ml (N<18pg/ml), and an increase in

urinary free catecholamines. Pheochromocytoma was excluded by imaging methods and repeated catecholamines. Clinically the patient had malnutrition, diarrhea, dyspnea and cervical nodules and calcitonin was increasing rapidly (CT 10,128pg/ml). Dual treatment with octreotide LAR 20mg/28d and sorafenib 400mg/12h was started with clinical relief and a decrease in CT levels (2,000pg/ml). Seven months later tumor progression was observed with an increase in calcitonin (26,753pg/ml) and everolimus was used instead of sorafenib. No decrease in CEA or CT was observed with everolimus and moreover, the patient experienced limited clinical tolerance. Some months later compassionate treatment with sunitinib 37.5 mg/d was started in addition to the octreotide LAR 20mg/28d. Four months later after the 3rd sunitinib cycle, a reduction in CEA and CT levels was seen (CEA 72ng/ml, CT 19.249pg/ml). Again, four months later, clinical and biochemical tumor progression prompted a change to vandetanib 200mg/24h with a significant reduction in CT (2000pg/ml). Seven months later, vandetanib is still ongoing with maintained reduction in CEA and CT and an improvement in clinical condition.:Tyrosine kinase inhibitors are promising drugs for the treatment of metastatic MTC. A possible mechanism of tolerance causing loss of efficacy has led to sequential treatment being proposed. The effective chronic use of these drugs enhances the need for personalized treatment to weigh the risk/benefit ratio.

OR2.3

Profile of patients treated for Medullary Thyroid Carcinoma at São Paulo Medical School

Marcos R Tavares, Sergio PA Toledo, Rodrigo A Toledo, Antonio Carlos Ferrete Jr, Fernanda L D Ribeiro, Gabriel L Valente, Lenine G Brandão

Medullary thyroid carcinoma (MTC) behavior in MEN2 present variations, ranging from indolent MTC, as frequently seem in cases harboring intra-cellular “weak” mutations, to highly aggressive disease, with RET mutations in codons 634 and 918. This retrospective chart review examines the profile and outcome of the patients treated at our institution. Patients and methods: The charts of 67 MTC patients, 26 sporadic (38.8%) and 41 carriers of RET mutations (61.2%) operated at our institution from January 2002 to December 2011 were retrospectively reviewed. Surgical and pathologic reports were accessed. Follow up ranged from 1 to 65 months (average 10.9 months). Patients were tested for RET hot spot mutations, pre- and postoperative calcitonin (Ct), carcinoembryonic antigen (CEA), parathyroid hormone (PTH) and calcium. Patients were stratified in 3 groups according to Ct serum concentration in the last evaluation: a) cured: undetectable Ct; b) Ct≤40pg/mL; c) Ct>40pg/mL. Results: Forty two patients were included in the study group: 28 individuals (68.3%) from a large familial MTC harboring a germline RET Cys620Arg mutation, 10 patients (24.4%) with a C634Y/Y791F double mutation; two patients (4.9%) with the V804M and one (2.4%) with the C634Y mutation. Nineteen patients (46.3%) achieved cure (Group a). According to RET mutation, there were 14 (50%) with C620R; 4 (40%) with C634Y/Y791F and 1 (50%) with V804M. The only patient C634Y did not reach cure. Age at treatment was an important factor related to cure. Younger patients had better prognosis (p=0.02). There was no statistical difference for preoperative calcitonin and cure, although there was a trend to higher levels in patients not cured. Calcitonin ranged from 4.5 to 1367pg/mL (average 124.5) in group “a”; from 9 to 180149 (average 124.5) in group b and from 98 to 8358 (average 2587) in group c. CEA levels ranged from 0.3 a18.4ng/mL (average 5.2) for group a; from 1.1 to 82.9 (average 12.4) for group b and from 2.6 a 641.7 (average 169.3) for group c. The extension of surgery was not related to cure. TT TT+CND TT+UND TT+BND Group a 5 10 2 2 Group b 0 11 4 3 Group c 0 3 1 0 TT: Total thyroidectomy CND: Central neck dissection (ND) UND: Unilateral ND BND: Bilateral ND Fifteen patients (36.6%) presented postoperative hypoparathyroidism, not related to cure. Conclusion: Genetic test is the most important exam to perform in MTC patients, since age at treatment prevails as important prognostic factor.

OR2.4

Pheochromocytoma in Multiple Endocrine Neoplasia Type 2 in Japan: Analysis of a Multicenter Database

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[Introduction] Approximately 50% of patients with multiple endocrine neoplasia type 2 (MEN2) develop pheochromocytomas. While the clinical features and optimal management of MEN2 are well characterized in Western countries, they are less well recognized in Asian countries, including Japan. The aim of this study was to clarify the clinical features and outcomes of the treatments of pheochromocytoma in Japanese MEN2 patients. [Patients and Methods] We established a MEN study group designated the “MEN Consortium of Japan” in 2008, and asked physicians and surgeons to provide clinical and genetic information on patients they had treated. Of 493 registered MEN2 patients, 212

pheochromocytomas were analyzed. The questionnaire included the following questions: 1) Was there a positive diagnosis of pheochromocytoma? If yes; age at diagnosis. 2) Was surgical intervention performed? If yes; age at surgery. 3) Was there unilateral or bilateral involvement? 4) Which particular surgical procedures were performed? 5) Was there a malignant feature? 6) Were multiple surgeries performed? 7) Was genetic testing performed? 8) Is the patient alive? [Results] The median age at primary adrenalectomy for pheochromocytoma was 37. There were 85 males and 126 females. Adrenal surgery was performed on 187 (88%) of the 212 patients. Unilateral adrenalectomy was performed in 72 patients. Bilateral adrenalectomy was performed in 104 patients, 71 patients had one stage bilateral adrenalectomy and 33 patients had two stage bilateral adrenalectomy. Laparoscopic surgery was performed in 48 patients. Clinical diagnosis including follow-up analysis confirmed pheochromocytoma in all but one case of malignant pheochromocytoma. Multiple operations were performed on 36 patients. The RET mutation positive rate was 95.4% among 153 patients with pheochromocytoma. There were 25 deaths among the 212 patients, of which six were due to pheochromocytoma. The median age of death was 39 in pheochromocytoma patients, which was younger than that in other causes of death. [Discussion] We have established the first extensive database of pheochromocytoma in Asian MEN2 patients. Although malignant pheochromocytoma was very rare in MEN2, complications arising from untreated pheochromocytoma or Addisonian crisis after bilateral total adrenalectomy were critical in MEN2 patients.

OR2.5

A STUDY OF THE SPANISH NATIONAL REGISTER OF MULTIPLE ENDOCRINE NEOPLASIA TYPE 2A

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Introduction: MUltiple endocrine neoplasia type 2 is a genetic rare disease due to germline RET mutations. MEN 2A is a syndrome of thyroid medullary carcinoma (MTC) in 90% of adult gene carriers, unilateral or bilateral pheochromocytoma (PHEO) in 50%, and multigland parathyroid tumors (HPT) in 20-30%. Objective: The aim of this study was to analyse the clinical and genetic characteristics of Spanish MEN 2A patients. Methods: The Spanish Group for the Study of Multiple Endocrine Neoplasia designed an online national database, which was launched in January 2009.Data was collected from January 2009 to December 2011. Results: There were data on 233 genetic carriers from 84 unrelated families. The mean age at diagnosis of any MEN 2 component was 27.74±18.19 years (6 months-75 years). 198 (84.97%) patients had MTC: 28 index cases were diagnosed at 42.32±14.53 (15-71) years, 36 patients were diagnosed by biochemical screening at 33.30±16.33 (8-68) years, and 134 patients were diagnosed by genetic screening at 27.55±16.11 (1-76) years. At the time of diagnosis 65 patients (32.82%) had metastases. 32 (13.73%) patients were diagnosed of thyroid C-cell hyperplasia by genetic screening at 9.93±7.4 (8 months- 43 years) years. PHEO was detected in 104 (45.21%) patients: 19 index cases were diagnosed at 43.21±16.63 (20-73) years, 20 patients were diagnosed by biochemical screening at 42.10±15.81 (19-68) years, and 65 patients were detected by genetic screening at 34.56±13.14 (13-72) years. HPT was present in 12 (5.15%) patients at 32.75±17.50 (14-71) years. Only 2 patients had cutaneous lichen amyloidosis. Three patients, at the ages of 2, 3 and 4 years, were asymptomatic genetic carriers. Two (2.38%) families were negative for germline RET mutations. 89% of RET gene mutations were located in codon 634 and the most common RET amino acid substitution was Cys634Tyr (75%). Conclusions: The clinical characteristics of Spanish MEN 2A patients are similar to those of European patients but the prevalence of mutations in 634 codon is higher than in European families, and specially the Cys634Tyr mutation, which suggests a "founder effect".

OR2.6

COULD THE LONG-ACTING SOMATOSTATIN ANALOGUES MODIFY THE THERAPEUTIC STRATEGY IN PATIENTS WITH EARLY STAGE MEN1-RELATED DUODENO-PANCREATIC NEUROENDOCRINE TUMORS (NET)S?

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Background: Somatostatin analogues (SSA) represent one of the main therapeutic option in patients affected with functioning well-differentiated neuroendocrine tumors (NET). There are no studies specifically focusing on NET associated to Multiple Endocrine Neoplasia type 1 (MEN1). Aim: To evaluate the efficacy of long-acting somatostatin analogues in MEN1 patients affected with duodeno-pancreatic NET. Patients & Methods: All first-degree relatives of MEN1 subjects, genetically diagnosed for MEN1 before the clinical diagnosis of NET and with evidence of one or more duodeno-pancreatic NET less than 15 mm in size were enrolled. Twenty-two patients with MEN1-related duodeno-pancreatic NET (age range 21-42 yrs) were treated with octreotide LAR (30 mg / 28 days). Treatment duration ranged 1-7 yrs. At the radiological evaluation (performed by multidetector-row computed tomography and endoscopic ultrasound), multiple duodeno-pancreatic NET (range 1-8), sized 3–14 mm, were detected. Results: An objective tumor response was observed in 18%, stable disease in 78% and progression of disease in 4% of cases. In five patients with abnormally increased chromogranin-A and/or gastrin serum concentrations, a significant hormonal response occurred in 100% cases and was stable along the time. Conclusions: Therapy with SSA is highly effective in patients with early stage MEN1 duodeno-pancreatic NET, resulting in long-time suppression of tumor and hormonal activity and 18% objective response. This suggest a change in therapeutic strategy in patients with early stage MEN1.

OR2.7

Diagnostic accuracy of chromogranin A, pancreas polypeptide and glucagon in the screening for pancreatic neuroendocrine tumors in Multiple Endocrine Neoplasia type 1 patients

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Context Periodic assessment of tumor manifestations and subsequent treatment of MEN1 mutation carriers prevents complications and seems to lead to a more favourable course of the disease. At present tumor markers are used in the periodic screening for pancreatic neuroendocrine tumors (pNET) in Multiple Endocrine Neoplasia type 1 (MEN1) patients, but have never been validated for this purpose. Objective To assess the diagnostic accuracy of chromogranin A (CgA), pancreatic polypeptide (PP) and glucagon in the screening for pNET in MEN1 patients. Design A diagnostic study as part of the Dutch national MEN1 database, which includes more than 90% of the total MEN1 population in the Netherlands. Setting Patients >16 years with a confirmed MEN1 mutation under the care of the Dutch University Medical Centers (2008-2010) (n=253) Patients and methods Reference standard for pNET diagnosis was pathology and, if not available, detection on MRI, CT or EUS with confirmation at least once on further imaging. For CgA patients with pulmonary, thymic, or stomach NET were excluded and analysis were stratified for proton pump inhibitor (PPI) use. The diagnostic accuracy for identifying metastatic disease in MEN1 patients was also assessed. Main outcome measures Area under the curve (AUC), positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity were calculated for each marker. Results 155 patients were eligible for assessing the reference standard, and measurement of the tumor markers were available in combination with the reference standard for PP in n=70 and for glucagon in n=91. CgA measurement in combination with the reference standard was available in n=78. The AUC of CgA without PPI was 0.58 (95% Confidence Interval (CI) 0.44 - 0.73), for glucagon 0.57 (95% CI 0.45 – 0.70) and for PP 0.61 (95% CI 0.47 – 0.76). For diagnosing metastatic disease the AUC of CgA and PP was 0.69 (95% CI 0.52 – 0.86) and 0.69 (95% CI 0.50-0.88) respectively, with significant NPVs of 90% or higher for all three tumor markers. Combined measurement of the three individual tumor markers led to an AUC of 0.67 (95% CI 0.52 – 0.82) for identifying pNET. Conclusion Tumor markers are not useful in the screening for pNET in MEN1. Because of the high NPV, CgA and other markers seem to be more useful for excluding metastatic disease in MEN1 patients.

OR2.8

Analysis of penetrance and clinical impact resultant of the diagnosis of multiple endocrine neoplasia type 1-related tumors during childhood and adolescence.

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Background: In order to reach an early diagnosis, the Consensus on MEN1 (2001/2012) established periodic hormonal/radiological exams in carriers of MEN1germline mutation with beginning to each tumor type based on younger case age reported. This recommendation is resultant of absence of consistent data reported on penetrance, prevalence, phenotype and clinical impact of the MEN1-related tumors in adolescents. Aims: To recognize the penetrance/prevalence of MEN1 tumors during 2nd decade reaching a more appropriate screening. Patients/design: One hundred and fifteen MEN1 cases harboring germline MEN1 mutations were studied. Two subgroups were selected: group 1, 27 cases approached during 2nd decade; group 2: 24 cases diagnosed with MEN1 after 21 years but presenting suggestive clinical history of MEN1 during 2nd decade. Results: The penetrance and prevalence of HPT, PET and PIT in 27 cases of the group 1 were respectively 55.6 %, 44.4 %, 44% and 71.4 %, 57.1%, 55%. Indeed, the penetrance of prolactinoma, NF-PIT, NF-PET and insulinoma were 34.6%, 8%, 44.4% and 7.4%, The overall penetrance during 2nd decade of HPT, insulinoma, gastrinoma, non-functioning PET, PIT, prolactinoma and non-functioning PIT in 114 MEN1 cases were respectively 26.9, 2.6, 0, 44.4, 16.5, 15 and 8%. Half of young cases diagnosed were asymptomatic and predominant symptoms were related to prolactinoma (82%), insulinoma (18.2%) and HPT (9%). During follow-up, 26.4% of young cases with asymptomatic HPT presented urolithiasis before 21 years. Prolactinoma was the more prevalent pituitary tumor (78%) and 44.5% were macroadenoma. Non-functioning PITs are less frequent (22%) presenting as incipient microadenomas. Non-functioning PETs are frequent into 2nd decade (57%) and relevant clinically (55%, surgical indication). Clinical/surgical treatment was conducted to one or more MEN1-related tumors in 44.4% (12/27) of the adolescents. Conclusions: Our data concerning penetrance/prevalence/phenotype of MEN1 tumors reveal prolactinomas, insulinomas, HPT and NF-PETs as significant clinically tumors in adolescents. We proposed an intensive periodic clinical/hormonal screening to early diagnosis of these tumors. In opposite, considering a very low penetrance of NF-PIT and an irrelevant clinical impact in adolescents, we recommend basal pituitary MRI 10 years later (15-20y-old) than suggested for actual Consensus or before only if pituitary hormones will be altered. Our screening approaching 27 patients reveal high penetrance of NF-PET and surgical indication in 15% (4/27) of these young cases. In addition, a periodic radiological approach including MRI (10-15 y-old) and endoscopic ultrasonography and/or MRI (15-20 y-old) is recommended to active investigation of NF-PETs. We indicate basal radiological screening between 10-15 y-old and valid the suggestion of the Consensus on MEN1 (2012) to begin this screening at 10 y-old based in case report of two adolescents presenting NF-PET.

OR2.9

Changing manifestations of MEN1 (Burin): increase in occurrence of PNETs

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Introduction: Four families with Multiple Endocrine Neoplasia type 1 (MEN1) from the Burin Peninsula of Newfoundland (NL) were identified in the 1980s (N= 25 affected). They were called the Prolactinoma variant of MEN1, or MEN1 (Burin), because the majority of patients presented with prolactinomas; pancreatic tumours were very rare; and carcinoid tumours of the lung or thymus were more common than in typical MEN1. Objectives: i) to offer clinical screening and genetic testing to all affected and at risk; ii) to prospectively document the natural history of MEN1 (Burin) including age at diagnosis, order and frequency of manifestations, and treatment required; iii) to modify the screening protocol as necessary. Methods: Clinical screening was introduced in 1987, and genetic testing has been offered since 1997 when a common mutation (R460X) was identified in menin. All results of screening and testing were recorded in a database. Results: A common ancestor of the four families has been identified through genealogical studies. There are 139 individuals now known to be affected with MEN1 (Burin). The frequencies of hyperparathyroidism (95%), prolactinomas (38%), and carcinoid tumours (13%) have remained constant but pancreatic tumours have increased (7% >35%). These are mainly pancreatic neuroendocrine tumours (PNETs); gastrinomas and glucagonomas remain rare. Discussion: The PNETs are non-functioning so only detected with CT or MR imaging. They have been identified since 2000 and have caused three deaths from metastatic disease. The increase in frequency of PNETs is not just increased recognition of previously present tumours because abdominal CT and MRI have been used regularly for screening since 1990. The screening protocol now includes blood work for biochemical screening from age 10, MRI of the head from age 10, imaging of the abdomen from age 15, and MRI of the chest from age 20. The radiologists recommend alternating triple phase CT and MRI of the abdomen to facilitate diagnosis of PNETs while reducing radiation exposure. Interestingly the large VHL family in NL

has had a similar increase in PNETs (from 0-29%) in the same time period, suggesting the possibility of an environmental component to the predisposition.

OR2.10

Functional characterization of mutations in the Multiple Endocrine Neoplasia type 1 (MEN1) gene suggest therapeutic strategies

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Menin is the product of the multiple endocrine neoplasia type 1 (MEN1) gene which when inactivated causes an autosomal dominant disorder characterized by tumors of the parathyroids, endocrine pancreas and anterior pituitary. We identified an MEN1 splice-site mutation leading to a menin Δ(184-218) mutant having an in-frame deletion of 35 amino acids, but otherwise of wild-type sequence. The transfected mutant was well expressed, and like wild-type menin, interacted directly with the transcriptional regulators JunD and NF-κB and inhibited their activities. However, the mutant had lost the normal interaction with Smad3 and was defective in mediating TGF-β-stimulated Smad3 transcriptional activity, stimulation of the cyclin dependent kinase inhibitors (CDKIs), p15 and p21, and cytostatic activity. Thus the mutant was stable, had selective loss of TGF-β signaling and growth inhibition and, importantly, identified the menin/Smad3 interacting region on a homology model of the human menin structure. These studies suggest the menin/Smad3 interface as a potential therapeutic target. We functionally characterized a panel of 16 menin missense mutants, including W423R and S443Y identified in new MEN1 families and that are poorly expressed. Proteasome inhibitors, MG132 and PS-341, and inhibition of the chaperone, heat shock protein 70 (Hsp70), or the ubiquitin ligase, COOH-terminus of Hsp70-interacting protein (CHIP), by specific siRNAs, restored the levels of the mutants whereas that of wild-type menin was unaffected. Inhibition of CHIP restored the ability of mutants to mediate normal functions of menin – TGF-β upregulation of p15 and p21, as well as TGF-β inhibition of cell proliferation. Potentially, targeting specific components of the proteasome chaperone pathway could be beneficial in treating a subset of MEN1 cases.

OR2.11

Association of Type-O Blood with Neuroendocrine Tumors in Multiple Endocrine Neoplasia Type 1

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Context: The ABO blood type system describes the expression of human blood group antigens found on both erythrocytes and normal tissue throughout the body. We recently reported an association between O blood type and the manifestation of pancreatic neuroendocrine tumors in a cohort of patients with Von Hippel-Lindau (VHL) syndrome. Objective: To determine if there is an association of ABO blood type with the development of neuroendocrine tumors in patients with multiple endocrine neoplasia, type 1 (MEN-1). Design: A retrospective analysis of 105 patients with MEN-1 was performed. Demographic, clinical and biochemical data were analyzed by ABO blood type. A Fisher Exact test was used to determine association between ABO blood type and manifestation of neuroendocrine tumor. Results: We found an association between O blood type and the manifestation of a primary neuroendocrine tumor of the gastrointestinal tract, lung, pancreas and thymus in patients with MEN-1 (p=0.01). Sixteen of 17 (94%) patients with metastatic neuroendocrine tumors had type-O blood as compared to 11 of 32 (74%) who had a benign tumor with non-O blood type. Demographic and clinical characteristics were similar amongst blood type cohorts. Conclusions: Our findings suggest an association between O blood type and the manifestation of a primary neuroendocrine tumor in patients with MEN-1. Prospective clinical trials are warranted to see if patient blood type status may be a useful addition to current screening and surveillance practices.

OR2.12

COMPARISON BETWEEN MANAGEMENTS OF MEN1 AND MEN2

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BACKGROUND: There have been no direct comparisons of all of the major management issues in MEN1 versus MEN2. METHODS: We assembled the

concepts about major managements of MEN1 and MEN2. Then, we subdivided these into clinical-based and mutation-based managements. Next, we showed our judgments about increasing and parallel ranking of importance of the managements within the four groups. Clinical-based managements strong in MEN1: *(a) surgery is improved by novel strategy (subtotal parathyroidectomy with thymectomy); *(b) excellent immunoassays (for carrier diagnosis or tumor monitoring or tumor localization); *(c) surgery guided by (expression and stage of tumors). *(d) excellent medical treatments (for gastric hyperacidity). Clinical-based managements strong in MEN2: *(a) surgery is improved by novel strategy (easy access and timing for thyroidectomy); *(b) excellent immunoassays (for carrier diagnosis or tumor monitoring); *(c) surgery guided by (determination of the carrier state). *(d) excellent medical treatments (for removed thyroid). Mutation-based managements strong in MEN1: *(a) unusual expressions (pheochromocytma or leydig cell tumor): *(b) non-hormonal expressions (angiofibomas or collagenomas); *(c) absence of identifiable MEN1 gene mutation in germ-line mimics of MEN1 (isolated parathyroid tumors, isolated pituitary tumor, isolated neuro-endocrine tumor, or isolated and simultaneous sporadic tumor in both the parathyroids and pituitary); *(d) variant of MEN1 with identified MEN1 gene germline mutation (MEN1-Burin); *(e) variants of MEN1 without identified MEN1 gene germline mutation: (~2% from identified genes [some CDKIs]), ~40% from germline mutations of MEN1 and/or unidentified genes), Mutation-based managements strong in MEN2: *(a) unusual expressions (Hirschsprung); *(b) non-hormonal expressions (neurofibromas); *(c) absence of identifiable RET gene germiline mutation in germ-line mimics of MEN2 (isolated C-cell cancer or isolated pheochromocytoma); *(d) variants of MEN2 with identified RET gene germlinemutation (MEN2a, MEN2b, FMTC); *(e) variants of MEN2 without identified RET gene germlinemutation: (these are rare). CONCLUSIONS: Clinical-based managements in MEN1 and MEN2 differ but have changed little. These benefit from immunoassays; they have excellent surgical and medical managements. Mutation-based tests assist managements (early and accurate diagnosis of carriers; help to identify variants of syndromes). Overall, the clinical-based or mutation-based managements differ strikingly.

OR2.13

Oncoprotein MafB is switched on in early mouse Men1 &#946-cell neoplastic lesions. R Bonnavion1 Z Hamze1, J Lu1,3,4, , N Herath2, C Pouponnot2, F Assade1, S Fontanière1, P Bertolino1, M Cordier-Bussat1, and CX Zhang1,3, 1 Team4, Cancer Research Center of Lyon, INSERM U1052, CNRS UMR5286, Lyon FRANCE 2 Institut Curie, Centre de Recherche, Orsay F-91405 France and CNRS, UMR 146 ; 3The E-Institute of Shanghai, Sino-French Life Science and Genomic Center, Ruijin Hospital, Shanghai, China. 4 Shanghai Clinical Center for Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao-Tong University, Shanghai, 200025 China;

Multiple Endocrine Neoplasia Type1 is a hereditary tumor disorder affecting mainly endocrine organs. MEN1 patients are, in general, carriers of a germinal mutation of the predisposing gene, MEN1, a tumor-suppressor that is completely inactivated in tumors by loss-of heterozygosity. In MEN1 patients, pancreatic endocrine tumors are the major cause of death due to malignancy. To better study the mechanisms underlying the tumorigenesis of endocrine cells related to MEN1 inactivation, our group had previously generated β-cell specific Men1-KO mice that develop insulinomas at 6 months of age, several months after Men1 ablation. This suggests that Men1-disruption itself is not sufficient to trigger tumor development. It is, therefore, crucial to uncover the subsequent molecular alterations following Men1 inactivation that may contribute specifically to endocrine tumorigenesis. The transcription factor MafB is essential for embryonic and terminal differentiation of pancreatic α and β-cells. Considering its oncogenic potential in several other tissues, we investigated the possible alteration of its expression in our β-cell specific Men1-KO mouse model. We found that MafB, normally silenced in mouse β-cells, was reactivated in the early mouse Men1 β-cell lesions and insulinomas that developed in these mutant mice. Importantly, our data further demonstrated that MafB expression could be induced following Men1 knockdown by siRNA in cultured INS-1E cells. Moreover, MafB overexpression in cultured βTC3 cells enhanced cell foci formation both in culture medium and on soft-agar, accompanied with the increased expression of Cyclin B1 and D2. Conversely, MafB down-regulation by siRNA transfection reduced BrdU incorporation in INS-1E cells. Taken together, our data reveal that Men1 inactivation leads to MafB re-expression in mouse β-cells in vivo, and provides evidence that deregulated ectopic MafB expression may play a hitherto unknown role in adult β-cell proliferation and Men1-related tumorigenesis in the mouse.

OR2.14

Quinazoline sensitive binding sites are promising new targets for chemotherapy of neuroendocrine tumors

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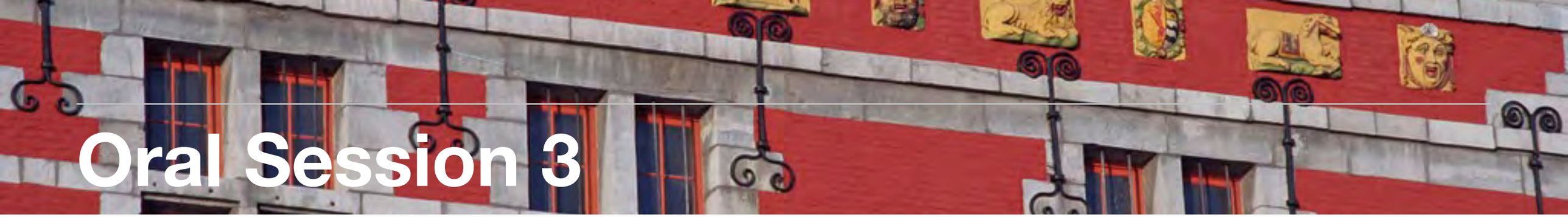
Background: Recently we demonstrated that the quinazoline-based alpha1-adrenergic blocker prazosin inhibits the growth and induces apoptosis in the medullary thyroid cancer (MTC) cell lines MTC-SK and TT. Similar to other cell types, the pro-apoptotic effect of prazosin on MTC-cells is independent of alpha1-adrenoceptors. In addition to apoptosis, prazosin treated MTC-cells show prominent alterations of cell morphology, including needle like protrusions and a dramatic increase of cell size. Aims: To further characterize the effects of prazosin on MTC-cells, we analyzed the DNA-content of cells using flow cytometry and performed live cell imaging to observe the formation of protrusions. For identification of the target-organelle of prazosin, we used the fluorescent prazosin derivate QAPB and organelle specific dyes. Furthermore, we tested, whether prazosin also induces apoptosis in the SI-NET cell line KRJ-I in order to extend the field of application of quinazoline based drugs to other neuroendocrine tumors. Results: We discovered that prazosin treated MTC-SK cells exhibit a significantly higher content of tetraploid cells. Furthermore, an accumulation of cells arrested in the G2/M phase of the cell cycle was evident. Analysis of TT cells by Live Cell Imaging showed that the needle like protrusions formed in response to prazosin treatment are involved in cell fusion which seems to be a frequently happening process in the TT cell line. Microscopic analysis of TT cells expressing GFP-actin revealed that the formed protrusions are positive for GFP-actin. Using QAPB and confocal microscopy, we observed colocalization of QAPB and the lysosomal specific dye LysoTracker®Red. Pretreatment of cells with chloroquine, which affects the mass and pH of lysosomes, protected MTC-SK cells against the toxity of prazosin. Assays testing proliferation and apoptosis showed that prazosin also induces cell death in the SI-NET cell line KRJ-I. Conclusion: We demonstrate that treatment of MTC-cells with prazosin induces apoptosis as well as polyploidy. The induction of polyploidy seems to be closely associated with alterations of the actin-cytoskeleton of the cells which may result in a defect of cytokinesis and/or enhanced cell fusion. The lysosomes may act as a buffer which protects cells against the toxic effects of prazosin. The identification of the still unknown quinazoline binding sites which seem to be associated with the cytoskeleton, might open the possibility to design new cancer drugs for the treatment of neuroendocrine tumors.



Oral Session 3

OR3.1

Radiofrequency ablation of pulmonary metastases in parathyroid carcinoma: an alternative therapy for severe refractory hypercalcemia.

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Background: Parathyroid carcinoma (PCA) is a rare disease accounting for 0.005% of all malignances and less than 2% of primary hyperparathyroidism (HPT) cases. HRPT2 gene mutations play a central role in the pathogenesis of parathyroid carcinoma and are observed not only in sporadic but also in familial cases. Parathyroid carcinoma is an aggressive disease associated with significant morbidity and mortality. Patients present with severe hypercalcemia, renal insufficiency and debilitating bone manifestations including fractures and brown tumors. Surgery is the main treatment but unfortunately recurrent disease is extremely common. With the goal of disease control and improvement of hypercalcemia, the treatment of metastatic PCA includes surgery of metastatic lesions, bisphosphonates, radiotherapy, chemotherapy and calcimimetics. Despite a multidisciplinary approach, death from uncontrolled hypercalcemia is common. Recently, radiofrequency ablation of metastatic PCA has been proposed as an alternative approach to control disease. In this report, we describe 3 patients with metastatic parathyroid carcinoma that failed conventional therapy and underwent radiofrequency ablation of pulmonary lesions. All patients had severe hypercalcemia resistant to hydration, calcitonin and zoledronic acid. One patient was treated with cinacalcet but failed to tolerate it. All patients had multiple bilateral pulmonary metastases not amenable for surgery. Severe bone disease (fractures, brown tumors) was observed in two cases and the last presented recurrent pancreatitis. Pre-procedure serum calcium ranged from 14.8 mg/dL to 19.3 mg/dL and serum PTH ranged from 1519 pg/mL to 6118 pg/mL. Radiofrequency ablation was performed in at least two steps to treat lesions from both lungs. Normalization of serum calcium and significant reduction of PTH was observed in all 3 patients. Information on long-term control is available in 1 patient who remained stable for 7 months when a repeat ablation was performed and resulted in calcium normalization and disease control for another 8 months. The other 2 patients were recently treated. Conclusion: Radiofrequency ablation of metastatic parathyroid carcinoma to lungs is an alternative approach to control refractory hypercalcemia.

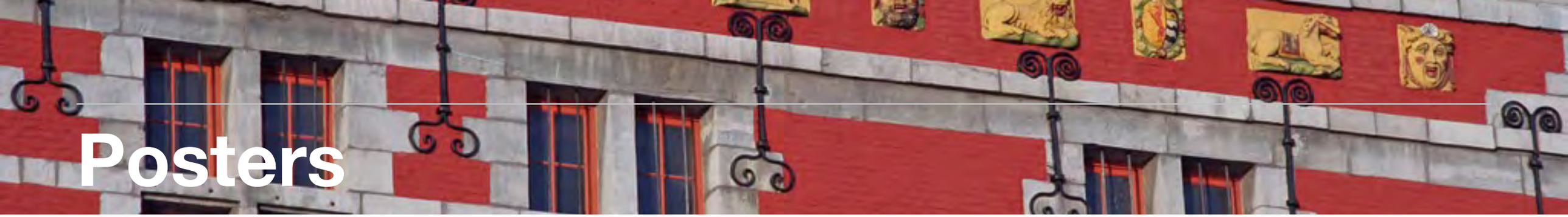
OR3.2

Clinical features of primary hyperparathyroidism (PHPT) in patients with parathyroid carcinoma.

Mokrysheva N.G., RozhinskayaL.Ya.

Objective: To determine pre-operative predictors of parathyroid carcinoma (PC). Materials and methods: The pre-operative diagnosis of malignancy is very difficult to obtain, and, thus, intra-operative recognition of PC is mandatory. A retrospective review was performed based on the medical records of 358 patients with primary hyperparathyroidism who underwent surgical treatment between 2000 and 2009. Data of serum levels of calcium and phosphorus, urinary excretion of calcium, serum levels of CTx, osteocalcin and alkaline phosphatase, creatinine, urea, serum and urine osmolality and GFR (MDRD) were available for all patients. Body composition was determined by X-ray and DEXA. The ultrasonograms of kidneys, ECG and EGD of all patients were studied for the evaluation of possible complications. Ultrasonography and isotope scanning were used for regional exclusion. Recurrence rates were evaluated for 214 patients 5 years after surgery. Results: Parathyroid adenoma occurred in 76% (275/358) patients, parathyroid hyperplasia – in 19% (66/258) patients and PC – in 4.7% (17/358) patients. Male/female ratio was significantly smaller in patients with PC (1:2.5) in comparison with patients with parathyroid adenoma (1:4) and hyperplasia (1:10). No significant differences were found between the two groups with regards to age. The median serum levels of PTH (590[252;1650] vs 243[150;462], р=0,03) and ionized calcium (1,6±0,2 vs 1,4 ±0,24 mmol/l)

were significantly greater in patients with PC. Prevalence of severe complications was also significantly greater in patients with PC (88%) in comparison with patients with parathyroid adenoma (52%) and hyperplasia (37%), р1=0,04, р2=0,03. Overall recurrence rate 5 years after surgery was 5% (20% for PC, 7.5% for hyperplasia and 3% for adenoma). PC are larger than other parathyroid lesions (hyperplasia, adenoma) (p=0.005). The risk of malignancy is 3 times more in patients with parathyroid lesion bigger than 6 sm3. Conclusions: The pre-operative predictors of PC are extremely high serum levels of calcium, severe complications of PHPT and size of parathyroid lesion more than 6 sm3.



Posters

P1

ASYNCHRONUS DEBUT OF THE ADRENAL CUSHING´S SYNDROME BY BILATERAL MACRONODULAR ADRENAL HYPERPLASIA

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INTRODUCTION: ACTH-independent macronodular adrenal hyperplasia (AIMAH) is a rare cause of Cushing's syndrome. Bilateral adrenalectomy is considered the treatment of choice, with subsequent lifetime steroid replacement, but unilateral adrenalectomy has been recently proposed to reduce the secreting tissue. We presented two patients witch AIMAH to evaluate the 5-10 years results of unilateral adrenalectomy concerning the main laboratory, MRI and clinical abnormalities. METHODS: The patients with confirmed AIMAH underwent unilateral adrenalectomy of the largest gland. ACTH and cortisol levels, arterial blood pressure (BP), glycometabolic parameters and MRI, were measured preoperatively and annually during your evolution. RESULTS: Six months after unilateral adrenalectomy, clinical improvement was evident. Free urinary cortisol was normalized as well the morning ACTH and cortisol and low/high dexamethasone suppression, in the beginning and after a follow-up (5 and10 years). The size of the contralateral adrenal appeared quite stable. CONCLUSIONS: Unilateral adrenalectomy of the largest gland can be an effective and safe treatment for AIMAH in case of asymmetric involvement. It may achieve long-term remission of Cushing's syndrome.

P2

Surprising clinical presentation of a family with germline SDHD mutation.

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Introduction: Germline SDHD mutations have been associated with the presence of multifocal head&neck paragangliomas. Most cases are hormonally inactive and benign. Papillary thyroid cancer, renal cell cancer and GIST are reported as other associated tumors. SDH mutations are transmitted in an autosomal dominant manner (with paternal transmission of tumor susceptibility). Therefore, genetic testing should be offered to all first degree relatives. Case description: A 32-year old Caucasian female was diagnosed with a thyroid nodule. By co-incidence, at neck ultrasound bilateral neck paragangliomas were detected. Thyroid nodule cytology was inconclusive (follicular neoplasia). After negative functional screening for catecholamine excess the patient underwent total thyroidectomy to obtain definitive histological diagnosis and resection of one paraganglioma because of local disturbance. Histological analysis revealed an invasive follicular thyroid cancer (insular subtype) and a paraganglioma. Postoperatively, radioactive iodine ablation was given. No metastases were observed on post-therapeutic whole-body 131I-scintigraphy. Molecular genetic testing revealed a frameshift mutation in the SDHD gene (c.4.5delC,p.Phe136LeufsX32). CT imaging of thorax and abdomen did not show other paraganglial or extraparaganglial tumors. At genetic testing, both the 63-year old father and unique 40-year-old brother were detected as carriers and further clinically screened. In contrast to the father who was free of clinical disease (suggesting maternal transmission of the mutation), the asymptomatic brother was diagnosed with multifocal small head&neck non-secreting paragangliomas and diffuse lung metastases (FDG-PET and Ga-68-Dotatoc-PET positive) without evidence for another primary tumor, apart from a very small (5 mm) nodule in the thyroid with inconclusive fine needle cytology. Diagnostic resection of a lung metastatic lesion showed typical features of metastatic paraganglioma. Because of the diffuse lung lesions, the absence of evidence for hormonal hypersecretrion, the absence of symptoms and the expected slow tumor growth, a watchful waiting approach was proposed with control CT imaging after three months. SDHx immunohistochemistry on the

different tumor tissues available is scheduled. Especially in case of the thyroid cancer this would allow to support the association between SDHD inactivation and follicular thyroid carcinogenesis. Conclusion: We describe a remarkable kindred with germline SDHD mutation: besides the presence of multifocal non-functional bilateral head&neck paragangliomas, one case presented with a concurrent thyroid cancer of the follicular subtype and one case presented with lung metastases without evidence for another primary tumor. In the near future the clinical spectrum of individuals harboring a pathogenic germline SDHD mutation will be probably further broadened.

P3

Clinical profile of 25 patients with bilateral pheochromocytoma (PCC) presented to a single tertiary care center in India

Rajeev kasaliwal,Sweta budyal, Harshal kakade Reshma Pandit,Anurag lila, Tushar Bangar,Nalini Shah

Title: Clinical profile of 25 patients with bilateral pheochromocytoma (PCC) presented to a single tertiary care center in India Objective: To study clinical profile of patient presented with bilateral PCC presented to a single tertiary care center in India Patients and methods: Medical record of 25 patients diagnosed with bilateral PCC from 1995 to 2012 were reviewed and analysed. Results: Of 25 patients, 12 were male and 13 were female. Mean age of onset of symptom was 23.0±11.7 years (6-50 yrs), and the mean age of diagnosis is 24.0 ± 11.2 (7-53 yrs). The mean interval between age of onset and diagnosis was 16 ± 5.5 months (1-72 months). Typical history of paroxysmal symptoms was present in 17 patients while it was incidentally diagnosed in 6 patients, In 2 patients; PCC was diagnosed during screening due to positive family history. Family history of PCC was present in 9 patients while 16 patients were apparently sporadic. Plasma free metanephrines were available in 18 patients. 14 patients had normetanephrine secreting biochemical phenotype while 4 patients had metanephrine secreting phenotype. Conclusion: Average age of presentation for bilateral PCC was younger. 24% patients with bilateral PCC were diagnosed incidentally. Though family history was present in only 36% of our patient, all patients with bilateral PCC should be subjected to genetic analysis as they have high likelihood of having genetic abnormality.

P4

Malignant abdominal paraganglioma treated by 131I-MIBG (a case report)

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Introduction: paragangliomas are rare neuroendocrine tumors that arised from extra-adrenal sympathetic and parasympathetic paraganglia that derived from the embryonic neural crest. 30% of paragangliomas are genetically determined. They are often multiple, malignant, recidivant in familial cases. The first line treatment is surgical sometimes combined with therapeutic 131I-MIBG. Case presentation: A 77-year-old women with a familial history of carotid body tumor and Recklinghausen disease in whom a left para-aortic paraganglioma was diagnosed in 2000 based on clinical, biological and radiological data. Clinical signs were: paroxystic hypertension, palpitations, headaches, excess sweating and abdominal pain. Urinary methanephrines were high up to 6 fold the normal range. The abdominal scan revealed a solid left para-aortic mass measuring 60mm in diameter, the MIBG scintigraphy showed a unique left abdominal uptake. In the same year of diagnosis, the patient underwent a laparotomy with resection of a mass measuring 60 mm in diameter. Histologic examination confirmed the diagnosis of paraganglioma. Post-operatively, hypertension improved and urinary metanephrines fall into normal range. 8 years later, the patient presented with spinal pain. A spinal MRI discovered a vertebral metastasis in D11. The patient underwent in 2009 a laminectomy with vertebral arthrodesis. Histologic examination diagnosed a vertebral metastasis of the paraganglioma. An angioscann revealed a paravertebral solid mass of 35 mm in diameter with vascular invasion and high suspicion of malignancy. This mass demonstrated an intense MIBG uptake. Plasmatic chromogranin was increased. The mass was unresectable because the tumor showed an important vascular invasion and tissue adhesions. In an attempt to palliate the metastatic disease, a therapeutic complement with MIBG was given. The patient received 59 mci of Iode131-MIBG by slow IV infusion. Toxicity was limited to an abdominal pain in day 2 after MIBG therapy. The control octreoscan revealed many uptakes in the abdomino-pelvic region however the MIBG scintigraphy didn’t show any uptake. Conclusion: metastasis of malignant paraganglioma can arise many years after the diagnosis was made. Familial cases are often malignant, bilateral and recurrent. The follow-up should last all the life. Genetic testing could be of great interest for this patient and their relatives in order to make an early diagnosis and an early management.

P5

Paraganglioma syndrome: a SDHD mutation family, with a wide spectrum of penetrance

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Familial paraganglioma (PGL) syndrome is an autosomal dominant disorder due to mutations in genes encoding for subunits of respiratory chain complexes or their assembly factors. We recently characterized a family affected with PGL syndrome type 1 due to a new succinate dehydrogenase complex subunit D (SDHD) gene mutation c.437A>T p.Asp146Val. The in silico analysis through Pathogenic Or Not – Pipeline attributes a probability of pathogenicity of 98% (standard error: 2.0e-02). The pathogenicity of this mutation is sustained by the negative SDHB immunohistochemistry of the proband tumor. The proband, male 43 yo, had a surgical intervention for a basicranial right jugulotimpanic PGL at age 24, and recent surgical operations for bilateral carotid bodies. A post-surgery F-PET-DOPA scan depicted a 20 mm hepatic mass, suspected to be a malignant PGL liver metastasis, which is waiting to be removed by DaVinci robotic surgery. His father, 82 yo has been operated for right carotid body 40 years ago, while the left has been recently localized, simultaneously he was found to be carrier of the same SDH mutation. Genetic analysis has been extended to all relatives with no prior evidence of PGL, showing the mutation in one out of three siblings (male, 50 yo) and in two out of three children (male 4 yo; female 11 yo). The proband's brother is now undergoing radiological imaging and radioreceptorial scan, whereas the son, due to young age, is not yet under investigation. To date, the daughter underwent to an initial PGL screening by neck and abdomen ultrasound scan that was negative. Clinically, she was affected with congenital psychomotor retardation, and during childhood she manifested severe visus impairment, ataxia, dystonia, hearing loss, language and mental retardation, although a definitive diagnosis has not yet been made. Interestingly, most of these findings were described also in Leigh syndrome, a clinically heterogeneous mitochondrial disorder due to dysfunctions of the respiratory chain complexes, including succinate dehydrogenase, coenzyme Q, and pyruvate dehydrogenase complex. The molecular analysis has been extended to SDHA, B, C, D, and AF2 genes. No mutations has been detected in the coding regions. The mRNA of SDH genes is under evaluation.Further studies on mitochondrial DNA mutations are ongoing to clarify a genotype-phenotype correlation.

P6

IDENTIFICATION OF SDHA MUTATION IN A PATIENT WITH HEAD AND NECK PARAGANGLIOMA

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SDHA gene is located on chromosome 5p15 and encodes the flavoprotein subunit of succinate dehydrogenase. In 2010, the first heterozygous germ-line mutation associated with a catecholamine-secreting extra-adrenal PGL was identified, and showed that tumor associated with SDHA mutation displays negative staining for SDHA as well as SDHB. More recently, using an immunohistochemical procedure, other two different SDHA mutations in five non-related patients were identified: one with pheochromocytoma, two with sympathetic PGL and two with head and neck PGL. It has been demonstrated that SDHA is a tumor suppressor gene associated to Paraganglioma syndrome type 5. Case report In 2009, a 28-years-old man presented with an evident mass on the neck; there was no known history of familial syndromes associated to head and neck paraganglioma. The patient underwent computed tomography examination which demonstrated a 2,5 cm right carotid body solid mass. In 2009 a non secreting paraganglioma (2,5x1,2x1,1) was removed. There was evidence of malignancy in the lymphonodes. In 2010 and 2011 the patient underwent additional surgeries for local recurrences. In order to detect alterations related to a heritable disorder, complete genetic testing for the known genetic loci associated with paraganglioma, SDHB, SDHC, SDHD and SDHAF2 genes, has been performed. Large genomic deletion or duplication

analysis was analyzed by multiplex ligation-dependent probe amplification (MLPA). No pathogenic sequence variant has been identified. Recently, immunohistochemistry was carried out for SDHB and SDHA and both resulted negative for protein expression. Sequence analysis of SDHA was performed on germ-line DNA, identifying a point mutation affecting the splice acceptor site of exon 14 (c.1795-1G>T). Sequence splicing variation was analyzed using Human Splicing Finder and Splice Site Prediction by Neural Network, software that compares normal with variant sequences for differences in potential regulatory signals, including donor and acceptor sites. In silico analysis indicated that the SDHA splicing mutation was predicted to disrupt the acceptor splice site. The effects of the mutation on RNA splicing and the study of the loss of heterozygosity in the tumor are ongoing. In case of a patient without familial or clinical indications for a particular type of PGL syndrome, it's possible to combine molecular analysis strategy with SDHA-SDHB staining on paraffin-embedded tumors to identify patients carrying germ-line SDHA mutations. This case report describes the seventh patient affected by PGL5, the fourth SDHA mutation and the first case with evidence of malignancy associated to PGL5.

P7

Identification and functional analysis of novel variants of CDKN1B (encoding p27Kip1) in sporadic parathyroid tumors from an Italian cohort

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Mutations in the MEN1 gene not only cause the MEN1 syndrome but are also found in sporadic hyperparathyroid tumors. Recently, a novel MEN-like syndrome, MEN4, was described, caused by heterozygous germline inactivating mutations of the CDKN1B gene, encoding the cyclin-dependent kinase inhibitor, p27kip1. In addition, somatic CDKN1B mutations have been reported in sporadic parathyroid adenomas. To further investigate the contribution of such abnormalities, we performed direct sequencing on CDKN1B exons and exon/intron boundaries in 69 samples comprising parathyroid carcinoma (CA, n=6), atypical adenoma (AA, n=9) and typical adenoma (TA, n=54) in an Italian cohort of primary hyperparathyroid patients having no mutations in the MEN1 gene. LOH analysis for the CDKN1B locus (chr 12p13.1) was performed in a subset of samples, and promoter hypermethylation was also assessed. In a CDC73 mutation-negative CA and in two TAs, we identified 3 heterozygous coding variants – S100I, c.50delAinsGG, F62V, respectively. Clearly the c.50delAinsGG mutation involving a frameshift (p.D17del/ins/fsX124) would be deleterious. Moreover, an intronic variant (IVS1+30G>A) was observed in a CA case. The germline CDKN1B sequence of all tested samples was normal. None of the variant CDKN1B sequences was found in 2000 previously reported alleles from normal individuals (1000 Genomes Database). LOH was detected in 6 of 33 cases (18,2%) but promoter hypermethylation was not observed. Flag-tagged p27Kip1 mutant F62V and S100I constructs transfected into HEK293 cells were as well expressed as wild-type and were unaffected by the proteasome inhibitor MG132. Our data suggest that the CDKN1B gene can be involved, albeit rarely, in the pathogenesis of sporadic parathyroid tumours. Additional functional analyses are ongoing.

P8

CDKN1B V109G POLYMORPHISM AS A NEW PUTATIVE PROGNOSTIC FACTOR IN MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 (MEN 1) PATIENTS

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Background: CDKN1B encodes the cyclin-dependent kinase (Cdk) inhibitor p27 (Kip1), an important cell-cycle regulatory protein that inhibits the progression from G1 to the S phase. Loss of p27 function may contribute to tumorigenesis. A total of 21 single nucleotide polymorphisms in CDKN1B have been described. Of these, only one single nucleotide polymorphism (T/G) causes a glycine for a valine substitution at codon 109. This V109G polymorphism affects p27 degradation in

vivo and appears to be associated with several cancers, suggesting that it may play a role in tumor progression. Furthermore, expanded pedigree analysis showed that p27 mutations are associated with the development of a MEN-like phenotype in multiple generations. In a previous study, we demonstrated that the V109G polymorphism is associated with a better prognosis in patients with sporadic medullary thyroid cancer. Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal dominant syndrome, with inter- and intra-familial variability, without a known genotype-phenotype correlation. This emphasizes the need for new prognostic markers to better understand the outcome. Aim: To evaluate the frequency of the CDKN1B V109G polymorphism in patients with MEN1 and correlate its presence with disease progression and outcome. Patients & Methods: 31 consecutive MEN 1 patients followed at the Neuroendocrine Tumors Unit of the Federico II University of Naples, were enrolled. The V109G polymorphism was examined in all cases on the germline DNA, using PCR amplification of exon 1, followed by direct sequencing of the amplicon. Tumor type, severity and follow-up data were collected and related to the genetic data. Results: Out of 31 MEN1 patients, 10 were positive for the V109G polymorphism. Among them, 2 required surgery because of a G2-type proliferating tumor (1 pancreatic neuroendocrine tumor, 1 atypical thymic carcinoid) and 1 had a pancreatic neuroendocrine tumor which progressed under therapy. Out of the remaining 7 patients, 6 had stable pancreatic neuroendocrine tumors and 1 had no pancreatic neuroendocrine tumor. Twenty-one patients tested negative for the polymorphism, 13 of which showed stable pancreatic neuroendocrine tumors, without progressive disease or metastasis, and 8 had no pancreatic or thymic neuroendocrine tumors. Conclusions: The present data suggest that CDKN1B V109G polymorphism may play a critical role in the outcome of MEN1 patients not addressed so far. Further studies are needed to better elucidate the mechanisms underlying the interplay of this polymorphism with the MEN1-gene pathway.

P9

Potential Benefits of PET-CT Imaging in the Management of MEN1 Syndrome

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Introduction The management of MEN 1 syndrome requires screening for the presence of multiple tumours both through biochemicals evaluations and the use of different imaging techniques. The role of the PET-CT imaging in evaluating this syndrome is still to be determined. Clinical case study A 49-year old man, suspect of MEN1, was referred to our department, for persistent primary hyperparathyroidism, already operated twice, unsuccessfully. A 99mTc MIBI scintigraphy suggested the presence of pathologic parathyroid tissue in the area of the thyroid isthmus and the anterior mediastinum. A 11C-methionine PET-CT was performed and revealed two hypermetabolic lesions, one in the right retrotracheal area, the other in the thymic region. A 18F- FDG PET-CT revealed that the thymic lesion was highly hypermetabolic while the retrotracheal mass captured only slightly FDG. Surgery was performed: a benign parathyroid adenoma was removed. The final pathology examination of the thymic mass revealed a B3-type thymoma. The MEN1 syndrome was confirmed by genetic testing (c.1328delC mutation) Conclusion Thymic carcinoids occur in 2 to 5 % of MEN 1 patients, but the occurrence of a thymoma as in our patient is much rarer. In our case, thymoma and parathyroid adenoma had the same avidity for methionine, but the avidity of the thymoma for FDG was higher. This permitted us to speculate not only on the nature but also on the degree of malignity of these two different tumours.

P10

Primary hyperparathyroidism confirmed by histology: sensitivity and predictors of 99mTc-Sestamibi / CT scan.

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OBJECTIVE: The purpose of this study is to evaluate the sensitivity, the positive predictive value (PPV) and predictors of 99mTc-Sestamibi / CT scan dual phase in patients with primary hyperparathyroidism (PPH) with histologic confirmation. PATIENTS AND METHODS: Since 2011 to 2012, we have investigated 35 consecutive patients with (PPH). They underwent biological studies, cervical ultrasonography and a neck-chest CTscan/99mTc-MIBI scintigraphy. A total of 19/35 patients had an histological confirmation after cervical exploration. Sensitivity and positive predictive value (probability that the disease is present when the test is positive) of this method were calculated. RESULTS: At diagnosis, the average age of the patients (15F / 4 H) was 54±14 years. 99MTc-MIBI CT scan was positive in 14/19 cases: sensitivity = 74% and (VPP) = 100%. The left/right lateralization predicted by the scan (and found by the surgeon) was concordant in 14/19 cases and discordant in 3/13 cases. In 5 cases with negative scan, cervical

exploration found in 4 cases an adenoma and in another case, three hyperplastic glands. The probability of a positive scan is not associated with age, gender or weight of parathyroid ($p > 0.1$). CONCLUSIONS: 99mTc-Sestamibi / CT scan has a high VPP for identification of pathological parathyroid glands. However, a negative scan does not exclude a parathyroid adenoma. A frequent growth pattern of superior polar adenomas through the inferior thyroid pole is a potential misleading diagnosis.

P11

Neuroendocrine tumors: additional value of new molecular imaging tracers in the detection of recurrent/residual disease

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Context: Recent studies have proposed the use of new molecular imaging tracers, such as 18F-DOPA and 68Ga-DOTANOC, in studying neuroendocrine tumors (NET) and in particular for the detection of recurrent or residual disease in case of negative conventional imaging modalities. Objective: to evaluate sensitivity of 18F-DOPA PET/CT and 68Ga- DOTANOC PET/CT in the detection of residual/recurrent NET. Patients: 15 patients (M:F=7:8; median age 51, range 27-79), with histologically proven NET were prospectively enrolled. In all cases, there was a biochemical evidence of recurrent/residual disease, not evident with conventional imaging techniques (^{111}In-DTPA-octreotide scan, MRI and/or TC). Six patients had medullary thyroid carcinoma (MTC) with post-surgical persistence of increased calcitonin (3 MEN2A, 1 FMTC, 2 sporadic), 2 patients had a suspect of pheocromocytoma with increased urinary metanephrines/normetanephrines (MEN2A), 5 were affected with head-and-neck non-secreting paraganglioma (PGL), SDHB and SDHD mutation gene carriers underlying multiple/malignant PGL syndrome, and two patients had MEN1 (Hyperparathyroidism, one pancreatic GHRH-secreting, and the other with bronchopulmonary NET). Intervention: All patients underwent investigation with 18F-DOPA PET/CT (13 cases) or 68Ga-DOTANOC PET/CT (2 cases). Results: Among patients undergone to 18F-DOPA PET/CT, the presence of disease was identified in 9/13 cases (69%), whereas the two MEN 1 patients investigated with 68Ga-DOTANOC PET/CT resulted negative for residual disease, in contrast with the presurgical imaging that localized multiple pancreatic GHRH secreting lesions. In particular, in 4/6 cases with MTC (66.7%), 18F-DOPA PET/CT detected loco-regional recurrence in the thyroid bed, upper mediastinum, laterocervical region. Two patients with suspect of pheocromocytoma (MEN2A) showed a positive uptake in the adrenal gland. Whereas 4 patients out of the 5 cases with PGL (80%) had a documented recurrent/residual disease at 18F-DOPA PET/CT in the head-and-neck district (cervical, pharyngeal) and one showed a liver metastasis. Discussion: The identification of limited residual/recurrent disease in NET is crucial for patient management and therapeutic optimization. In the present study, residual/recurrent disease was documented in 69% of the overall NET population and in 80% of patients affected with PGL, strongly suggesting that the introduction of new molecular imaging tracers, such as 18F-DOPA and 68Ga-DOTANOC permits an early diagnosis of minimal disease even in case of negative conventional imaging modalities.

P12

Multiple endocrine neoplasia type 1: initial experience and management

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Multiple endocrine neoplasia syndrome, type 1 (MEN1) is an underdiagnosed autosomal dominant inherited cancer syndrome. Disease is caused by mutations in the MEN1 gene on chromosome 11. Patients with MEN1 have a shorter life expectancy than the general population. Recognition of the syndrome is important both for treatment and for evaluation of family members. Aim We present our experience of management of genetically confirmed MEN1 family members from Lithuania. This is the first set of well characterized MEN1 mutation carriers presented from Lithuania. Materials and Methods Retrospective case study of MEN1 patient and his family was performed. Data collected from medical records included anamnesis of illness, clinical characteristics and findings, treatment, surgical procedures and genealogy. Results The 22-year-old man was operated for adhesive intestinal obstruction and metastases of neuroendocrine carcinoma

in the lymph nodes at the stomach were found. During esophagogastroduodenoscopy the primary tumor in the gastric body was found. Gastric resection by Billroth I was performed and the diagnosis of well-differentiated neuroendocrine tumor secreting gastrin was established. Following a computer tomography scan of the abdomen multiple foci in the pancreas were found. Hyperparathyroidism and hypercalcaemia remained. We clinically suspected and oncogeneticist confirmed diagnosis of MEN1. Well-differentiated pancreatic neuroendocrine tumors (somatostatinoma, gastrinoma) were enucleated from caput, corpus and tale of pancreas. Recently subtotal parathyroidectomy for hyperparathyroidism was performed. In total 8 additional clinically asymptomatic family members were found to be mutation carriers. Four of them were found to have characteristic biochemical abnormalities after laboratory investigations. They are followed-up and treated. Discussion At present the diagnosis must be established by direct mutation testing. MEN1 patients, their relatives and patients suspected of MEN1 are eligible for mutation testing. MEN1 patients and mutation carriers should be subjected to periodic screening in order to detect manifestations in an early stage. Early genetic diagnosis and subsequent periodic screening is associated with less morbidity and mortality at follow-up.

P13

Laboratory management of Multiple Endocrine Neoplasia Type 1 genetic test: results and considerations of two decades of activity

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Since the identification of MEN1 gene, our Laboratory has set up and performed mutation analysis, by PCR-based sequencing of coding region (exon 2-10) and intron-exon junctions. We identified 73 different mutations: 31 frameshift (42.5%), 20 missense (27.4%), 12 nonsense (16.4%) and 10 splicing site (13.7%), confirming percentage data from literature [1]. 57 mutations was found only in one family or one single case. We identified a family with two different mutations (in exon 4 and in exon 8), both inherited by the same parental lineage. No correlation between genotype and phenotype has been found, in our series, for any mutation. In about 30% of clinically affected individuals we failed to find any mutation in coding region and intron-exon junctions of MEN1 gene. When in a MEN1 pedigree no MEN1 germline mutation is identified, genetic ascertainment can be performed by 11q13 haplotype analysis in at least two different generations of affected members. We performed 11q13 microsatellite haplotype analysis in 8 living and 1 deceased members of a MEN1 Italian family without any MEN1 mutation. The application of ancient DNA forensic techniques, in association with modern molecular biology approaches, allowed to identify the familial disease-associated haplotype and to identify two young asymptomatic subjects as non-carriers of the disease haplotype and thus not at risk of develop MEN1 syndrome. The multiplex ligation-dependent probe amplification (MPLA) allows to identify large deletions within the gene using at least two generations of clinically affect subjects and non-affected relatives. Using this technique we identified a large deletion within MEN1 gene in a MEN1 family, identifying two mutated members, confirming the clinical diagnosis and enabling the early genetic test for the young, still unaffected, generations of this pedigree. A negative genetic test can also be due to a phenocopy, such as MEN4, caused by mutations in CDKI gene. Genetic screening of CDKI gene is strongly recommended in MEN1 negative subjects. We analyzed 7 suspected MEN4 patients for mutations of the CDKI gene, failing to find any mutation in all of them. In conclusion, MEN1 genetic test is strongly recommended for all the members (symptomatic and asymptomatic) of a family in which a germline MEN1 mutation has been previously identified, since it allows to identify asymptomatic mutant gene carriers leading to perform earlier and more frequent biochemical and imaging screenings for MEN1 tumors and helping to reduce morbidity and mortality. 1. Lemos MC, Thakker RV. Hum Mutat 29(1):22-32, 2008

P14

A Case series of three patients with multiple endocrine neoplasia type 1: molecular diagnosis, treatment and follow up.

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Introduction: We present the clinical profile, treatment, follow up and molecular diagnosis of 3 patients with MEN 1. Clinical Profile: All the patients were females and the age at the development of first symptom were 5years (patient 1), 20 years (patient 2) and 33 years (patient 3) respectively. The components at the presentation were insulinoma and prolactinoma respectively in patient 1 and 2, whereas patient 3 presented with both prolactinoma and insulinoma. Patient 1 subsequently developed prolactinoma (22 yrs) and hyperparathyroidism (HPT) (27 yrs); patient 2 developed gastrinoma (25 yrs) and HPT (30 yrs) and patient 3

developed HPT (35 yrs). All the patients had microprolactinoma. None of the patients had positive family history. Treatment: Microprolactinoma is contained with cabergoline in all patients (0.25-0.5 mg/week). Patient 1 and 3 underwent 3.5 parathyroidectomy, whereas patient 2 underwent total parathyroidectomy with implantation of the gland in sternocleidomastoid. For insulinoma, patient 1 has undergone Whipples Procedure and distal Panareactosplenctomy (due to presence of multiple PNETS in heads and tail) and patient 3 underwent distal Pancreatectomy and splenectomy. In patient 2, gastrinoma is symptomatically treated with PPIs. Follow up: Patient 1 and 2 have developed hypoparathyroidism whereas patient 3 developed recurrence of HPT after 3 years of surgery. Molecular studies: PCR based DNA sequencing was done for menin gene (exon 2-10). MEN 1 mutation is positive in all the patients (reported mutation in patients 1 and 2, novel mutation in patient 3). Patient 1 has point mutation in exon 4 [codon 220 TGG(Trp) > TAG(Stop)]. Patient 2 has point mutation in exon 2 [codon 42 GGC(Gly) > GAC(Asp)]. Patient 3 has deletion exon 3 [codon 156 c.506delG].

P15

Incidental Sarcomatoid Carcinoma of the Kidney in a Young MEN1 Patient. Case report and genetic profile.

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Herein we report a case, unique to our knowledge, of renal sarcomatoid carcinoma with concomitant omolateral non-secreting adrenal adenoma occurring in a young male MEN1 patient, previously operated on for hyperparathyroidism and multiple pancreatic neuroendocrine neoplasms with suspected liver metastasis: three duodenal gastrinomas, 25 non-secreting pancreating NETs with 5 metastatic pancreatic-duodenal lymph nodes. The hepatic lesion resulted Focal Nodular Hyperplasia. The following year he had undergone total parathyroidectomy and tymectomy with parathyroid graft in the right forearm. The patient is also affected by pituitary prolactinoma, recently increased in size, and microcytic anemia. MEN1 genetic analysis had evidenced the presence of the germinal missense Leu413Pro mutation in the exon 9 of the gene. In July 2010 the patient underwent surgical intervention. A lumbar incision was performed with removal of left twelfth rib, isolation of the kidney with the adipose capsule and identification of the tumor, which had a diameter of 3 cm; it appeared at the center of the kidney and deep, reaching to the pelvis. Above it there was an adrenal nodule about 1,5 cm in diameter. A nephro-adrenalectomy was chosen. Double ligation of the renal vessels, section of ureter, spermatic and phrenic vessels. An intraoperative ultrasonography showed no nodules in the distal pancreas. Macroscopic examination described a macroscopic renal lesion of 2.8 x 3 cm; adrenal gland of 7 x 3 x 1 cm with a lesion of 1.5 x 1.2 cm. After a proper request for consent to the patient, multiple biopsies were made on fresh surgical specimen, and immediately sent to our laboratory for genetic study. The somatic alterations found on biopsies were then compared with germline mutations detected in a blood sample. The histopathological diagnosis was as follows: malignant biphasic cortico-medullary renal neoplasm with tubulo-papillary epithelial component and mesenchymal components osteo-sarcomatous and giant-cell, 3 cm in greatest dimension. Morphological picture referring to carcino-sarcoma with heterologous differentiation. In the sections examined, no evidence of tumor in perirenal adipose tissue and in renal vessels. Capsulated cortico-adrenal tumor, maximum diameter 1.5 cm. No evidence of capsular and vascular invasion. Absence of necrosis and atypical mitotic figures. Proliferative fraction with Ki-67 below 1%. Histological and immunohistochemical findings suggestive for an adrenocortical adenoma. Molecular analysis in the MEN1 locus at 11q13 showed loss of eterozigosity (LOH) in the adrenal lesion, while the kidney tumor resulted unrelated to MEN1 syndrome. No chemotherapy was performed. The patient is disease-free after 2 years of follow up.

P16

Different phenotypes of multiple endocrine neoplasia type 1 (MEN1) : a case report of an Italian family.

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INTRODUCTION: MEN1 is a rare autosomal dominant familial cancer syndrome characterized by involvement of parathyroid glands, enteropancreatic endocrine tissues and anterior pituitary gland. This disease is linked to germline mutations in the MEN1 gene (more than 460 described), whose identification allows the familial genetic counselling. We describe here the clinical correlates of a germinal mutation in exon 10 of the MEN1 gene identified in an Italian family CASE REPORT: A 61-year-old woman was admitted to our Unit of Endocrinology because of recurrent kidney stones, osteoporosis, upper abdominal pain. She reported previous surgical removal of multiple lipomas, hysterectomy for uterine leyomiomas, hyperprolactinemia since the age of 30. Multiple cases of endocrine

and non endocrine tumors occurred in both maternal and paternal side of the family. Interestingly, one cousin and her four offspring were affected by familial isolated primary hyperparathyroidism. Proband's laboratory examinations showed PHPT, mild hypergastrinemia, high CgA and hyperprolactinemia. Neck US showed multinodular goiter and a voluminous parathyroid enlargement, while bone densitometry disclosed severe osteoporosis. Therefore, she underwent subtotal parathyroidectomy; histological diagnosis was diffuse cells hyperplasia. Abdomen MRI showed pancreatic cyst, undetermined pelvic lesions and left adrenal lesion. MRI with gadolinium disclosed a pituitary microadenoma. The proband's son (40 yr-old), reported a history of recurrent kidney stones already treated with laser lithotripsy, sexual dysfunction, pseudo-gynecomastia and multiple lipomas. Laboratory data revealed PHPT, severe hyperprolactinemia, hypogonadotropic hypogonadism. Neck US exploration disclosed multinodular goiter; (99m)Tc-sestamibi parathyroid scintigraphy was negative. The MRI showed a pituitary macroadenoma invading the right cavernous sinus. In all members with clinical diagnosis of MEN1 or FIHP the genetic testing identified a MEN1 germline heterozygous deletion of 1bp in exon 10, p.K564RfsX3 (c.1691delA), reference-sequence: ENST00000377313, resulting in a frameshift generating a premature termination codon. CONCLUSION: It has been well established that the penetrance for all clinical features of MEN1 syndrome is age-related and PHPT is the earliest (above 95% by age 40). In our family there is a different phenotypic expression of the same mutation in MEN1 gene: classical MEN1 and FIHP. We cannot exclude that other unknown molecular events may have influenced this different clinical expression. Further studies will be needed to confirm this hypothesis.

P17

Rare disease associated with multiple endocrine neoplasia type1 ?

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We investigated rare disease associated multiple endocrine neoplasia type1. There were 582 patients registered as MEN1from the data of MEN Consortium of Japan. 76 patients (13%) had complications without thymic tumor, adrenal tumor, facial angiofibroma, and lipoma. The average age was 48 years old (11~79) which is rather older than average age (47 years old) of all 582 patients of MEN1. Of 76 patients, 21 were male and 54 were female and 1 was unknown. Forty-one patients (53.9%) had malignant disease and 31(40.8%) had benign disease and 4 were unknown. The average age of complications was 49.9 years old for malignant disease and 43.3 for benign disease. Of malignant complication, 13 (17.1%) had thyroid neoplasia (1: medullary thyroid carcinoma & 12: papillary thyroid carcinoma) and 2 had colon cancer and another 2 patients had lung cancer. For benign complication, 13 (17.1%) had myoma uteri and 9 (11.8%) had thyroid adenoma or denomatous goiter of the thyroid. It is interesting that frequency of papillary thyroid cancer in MEN1 patients is rather high.

P18

CLINICAL CASE OF MULTIPLE ENDOCRINE NEOPLASIA (MEN) TYPE 1

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The age-related penetrance for all features of MEN type 1 rises above 50% by age 20 years and above 95% by age 40 years. Aim: to describe the clinical course of a patient with MEN type 1 developing sequentially endocrine tumors. Case report: A 32 y.o. woman presented with three main components of MEN type 1 and tumors in both adrenals. Disease had manifested at the age of 16 years with headaches and oligomenorrhea then progressed to amenorrhea. The CT scan and hormonal tests revealed a prolactin-secreting macroadenomа and absence of macroprolactinaemia. Long-term medical treatment with high doses of dopamine agonists didn't result in normalization of prolactin levels. At age of 22 years she underwent a transnasal adenomectomy without remission. Cabergoline was continued in escalating doses from 2.5 to 10 mg/week without positive effect on prolactin levels, which were normalized only after second transnasal adenomectomy at the age of 30 years. Primary hyperparathyroidism was diagnosed at age 28 years caused by 1.4cm adenoma of the right inferior parathyroid gland, hyperplasia of right inferior and left superior parathyroid glands and successfully cured by total parathyroidectomy with autotransplantation of the

least changed gland in the left forearm. Genetic tests were performed at age of 29 years and revealed R415X mutation in MEN1. Nonfunctioning adenomas in both adrenals were found at age of 30 years. One year later the symptoms of hypoglycemia appeared (anxiety, behavior changes, weight gain, headache, dizziness, hunger, tremor blood sugar levels 1.8-2.8 mmol/l). The 72-hour fasting test was positive stopped at 9 hours due to hypoglycemia with normal levels of insulin and C-peptide, extremely increased levels of proinsulin. On endoscopic ultrasound, CT with contrast and MRI two lesions were visualized in the head and the tail of the pancreas. Celiac arteriography, mesenterial arteriography, angiography of hepatic vein - showed an increase in insulin and C-peptide levels only in distal parts of the pancreas and allowed to perform a successful organ saving operation - resection of the pancreas tail, lymphadenectomy. At the last follow-up (2 years later) the patient has normal levels of prolactin, PTH, calcium, glucose, insulin, C-peptide, marginally increased proinsulin. Conclusion: This case demonstrates multiple formations of endocrine organs step-by-step appearing during 17 years. Starting with macroprolactonoma resistant to dopamine agonists therapy other endocrine disorders developed gradually eventually leading to life threatening extreme hypoglycemia caused by multiple pancreas lesions, one of which produced proinsulin in high levels.

P19

Proposal of diagnostic flow charts for MEN1 and MEN2

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The early diagnosis of MEN and disease-related endocrine disorders is expected to reduce morbidity and mortality of affected patients. However, many patients with MEN likely remain undiagnosed or are diagnosed only after a significant time has elapsed. For example, previous studies reported that in average, 10-17 years elapse from the appearance of first manifestation to the diagnosis of MEN1. There are some possible reasons for this delay. Most of clinical symptoms in MEN patients are nonspecific (such as urolithiasis, peptic ulcer, amenorrhea, hypertension etc.), and are commonly seen by other causes. Also, due to insufficient awareness of MEN in clinicians, diagnosis of one endocrine tumor does not always proceed to a work-up of other MEN-related tumors. For general physicians or specialists other than experienced endocrinologists, it may not be clear which patients are at higher risk of having MEN and should be further evaluated. To effectively select patients at high risk of having MEN and perform appropriate work-up procedure, we here propose diagnostic flow charts for MEN1 and MEN2. They consist of 6 and 2 flow charts for MEN1 and MEN2, respectively. Each flow chart starts from a single endocrine disorder (hyperparathyroidism, gastrinoma, insulinoma, other functioning pancreas NET, nonfunctioning pancreas NET and pituitary tumor for MEN1, and thyroid tumor and pheochromocytoma for MEN2). Although consistency with the previously published guidelines is maintained, we reflected some data obtained from analysis of the multicenter database established by MEN Consortium of Japan. This database includes 560 MEN1 cases and 505 MEN2 cases. For example, we unconditionally categorized patients with insulinoma diagnosed before 20 years as “suspected as MEN1” and recommended to offer them MEN1 genetic testing. These charts are now under peer-review by committee members of Japan Endocrine Society, and will be released after public hearing and subsequent revision. Although sensitivity and specificity of these flow charts need to be validated in future, they are expected to lead to early diagnosis and appropriate intervention for patients with MEN and to improve prognosis of those patients.

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A STUDY OF THE SPANISH NATIONAL REGISTER OF MULTIPLE ENDOCRINE NEOPLASIA TYPE 1

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Introduction: multiple endocrine neoplasia type 1 (MEN 1) is an autosomal dominant disorder that is due to mutations in the tumor suppressor gene MEN 1. MEN 1 syndrome is characterized by the occurrence of parathyroid (HPT) (82-100%), gastroenteropancreatic (GEP) (60-80%) and anterior pituitary tumors (PIT) (30-50%). Some patients may also develop carcinoid tumors, adrenocortical tumors, meningiomas, facial angiofibromas, collagenomas, and lipomas. Objective: the aim of this study was to analyse the clinical and genetic characteristics of Spanish MEN 1 patients. Methods: The Spanish Group for the Study of Multiple Endocrine Neoplasia designed an online national database, which was launched on January 2009. Data were collected from January 2009 to December 2011. Results: there were data on 70 genetic carriers from 26 unrelated families. 65 patients were diagnosed with any MEN 1 component at a mean age of 38.47±14.82 (14.62-71.8) years, and 5 subjects were asymptomatic genetic carriers at 13.6 ±4.03 years. HPT was diagnosed in 64 patients (91.42%) at 39.73 ± 13.61 (17-75) years. 34 GEPs were detected in 32 (45.71%) patients at 49.00±15.47 (30-75) years. As a whole, 17 (5%) patients developed gastrinomas, 9 (26.47%) non-functioning tumors, 3 (8.8%) PPomas, 2 (5.88%) insulinomas, 1 (2.9%) glucagonoma, 1 (2.9%) somastostatinoma, and 1 (2.9%) gastric carcinoid. At the time of diagnosis 6 patients had distant metastases. 26 (37.14%) patients developed a PIT at 36.84±11.71 (16-60) years. 15 (57.69%) patients had a prolactinoma, 9 (34.26%) non-functioning adenomas, 1 (3.84%) somatolactotrophinoma, and 1 (3.84%) corticotropinoma. Only 5 (19.23%) of out 26 were macroadenomas. 6 patients had adrenal tumors, 3 patients had lipomas, 2 patients were diagnosed of bronchopulmonary carcinoids, and 2 patients had angiofibromas. We have identified 13 different mutations in the coding region of the MEN1 gene, and in the splicing sites in 22 (84.61%) families. Of these identified mutations, 4 were in exon 10 (30.76%), 4 in exon 2 (30.76%), 3 in exon 3 (23.07%) and 1 in exon 7, and 1 in intron 3. One mutation has been found in 7 unrelated families. Most of the mutations are nonsense, or frameshift deletions or insertions that are likely to result in a functional loss of the menin protein. Conclusions: compared to the literature the clinical and genetic characteristics of Spanish MEN 1 patients are similar to those previously published, although the prevalence of pituitary macroadenomas is surprisingly lower than those reported in the literature.

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The Negative Feedback-Loop Between the Oncomir Mir-24-1 and Menin Modulates the MEN1 Tumorigenesis. Ettore Luzi, Francesca Marini, Francesca Giusti, Gianna Galli, Loredana Cavalli and Maria Luisa Brandi Regional Center on Endocrine Hereditary Tumors of the “Regione Toscana”, Department of Internal Medicine, University of Florence, Florence, Italy

Multiple endocrine neoplasia type 1 (MEN1) syndrome is a rare hereditary cancer disorder caused by mutation of MEN1 gene, a tumor suppressor gene, encodes menin protein. Loss of heterozygosity at 11q13 is typical of MEN1 tumors, in agreement with the Knudson's two-hit hypothesis. In silico analysis with Target Scan, Miranda and Pictar-Vert softwares for the prediction of miRNA targets indicated miR-24-1 as capable to bind to the 3'UTR of MEN1 mRNA. We investigated the relationship between MEN1 mRNA, miR-24-1 and menin protein, by analysis of miR-24-1 expression profiles in parathyroid adenomatous tissues 1) from patients bearing a MEN1 gene mutation, in which the main difference was the MEN1 LOH or the maintenance of the wild type MEN1 allele, 2) from parathyroid sporadic non-MEN1 adenomas, and 3) from normal parathyroid tissue. While parathyroid adenomas from patients that lost both MEN1 alleles showed no expression of MEN1 mRNA, of menin, or of miR-24-1, MEN1 parathyroid adenomas from patients that maintain one wild type copy of the MEN1 gene showed, as expected, a reduced expression of MEN1 mRNA, but no expression of menin, while miR-24-1 was expressed. Conversely, non-MEN1 adenomas and normal parathyroid showed the expression of MEN1 mRNA and menin but not of miR-24-1. Thus, the induction of miR-24-1 seems to depend on transcriptional expression of MEN1 mRNA and miR-24-1 expression contributes to the inhibition of menin expression. The ChIP study demonstrated the association of menin with the miR-24-1 promoter, suggesting that the pri-miR-24-1 transcription was regulated by menin. Therefore, the MEN1 tumorigenesis seems to be under the control of a "negative feedback loop" between miR-24-1 and menin protein, that mimics the second hit of Knudson's hypothesis and that could buffer the effect of the stochastic factors that contribute to the onset and progression of this disease. Our data show an alternative way to MEN1 tumorigenesis and, probably, to the “two-hit dogma”. The functional significance of this regulatory mechanism in MEN1 tumorigenesis is also the basis for opening future developments of RNA antagomir(s)-based strategies in the in vivo control of tumorigenesis in MEN1 carriers.

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IMMUNOEXPRESSION OF CELL CYCLE-ASSOCIATED PROTEINS IN MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 PARATHYROID GLANDS

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Introduction: the genetic basis for Multiple Endocrine Neoplasia type 1 (MEN 1) is the homozygous inactivation of MEN 1 gene. However, clinical and molecular findings suggest the involvement of other genetic changes. Objective: there are several reports about the role of p27kip1, PRAD1/ cyclin D1 and p53 in parathyroid adenomas. However, to our knowledge, there were no reports studying the expression of these proteins in a large panel of MEN 1 parathyroid glands, thus we undertook this study in order to contribute to the understanding of the molecular Materials and methods: we examined the immunoexpression of PRAD1/cyclin D1, p27, and Ki-67 in paraffin-embedded specific tumor samples of biopsies of 30 parathyroid glands from 8 genetic confirmed MEN 1 patients from a unique family, all of them carry a nonsense mutation in the exon 10 of the MEN 1 gen, W471X. In order to compare we studied 11 parathyroid adenomas and 7 associated glands obtained from patients with sporadic primary hyperparathyroidism. Results: Nuclear staining of Ki-67 protein was identified with the MIB-1 antibody in 13 of 30 (43.33%) MEN 1 parathyroid glands, in 9 of 11 (81.81%) parathyroid adenomas. No positive cells were identified in the associated glands. Ki-67 labelling index (LI) was higher in sporadic parathyroid adenomas (5.40 ± 4.57) than in MEN 1 parathyroid hyperplasias (2.07 ± 4.54) (p = 0.01). We identified p27kip1 positive cells in 3 out of 7 (42.85 %) associated parathyroid glands, with a p27kip1 LI: 1, 1 and 3. In MEN 1 parathyroid glands and parathyroid adenomas we identified positive cells in all of them. MEN 1 parathyroid hyperplasias had similar p27kip1 expression (LI: 33.66 ± 28.75, range 1.0 to 90) than parathyroid adenomas (LI: 38.72 ± 40.88, range 1.0 to 100). Statistically significant correlation between the immunoexpression of p27kip1 and that of Ki-67 was observed in both parathyroid adenomas (p =0.006) and MEN 1 parathyroid hyperplasias (p= 0.029). PRAD1/Cyclin D1 was overexpressed in 2 out of 11 (18%) adenomas, but in none of the MEN 1 hyperplasias. p53 immunostaining was negative in MEN 1 parathyroid glands, in parathyroid adenomas and in associated glands. Conclusions: these findings suggest that MEN 1 parathyroid glands have a low growth fraction; p27 protein would have a role in controlling parathyroid cell proliferation, PRAD1/cyclin D1 rearrangement and mutations in the p53 gene probably do not have a pivotal role in MEN 1 parathyroid tumorogenesis.

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Activity of novel water soluble epirubicin substituted polymer against small intestinal neuroendocrine tumor and medullary thyroid carcinoma cell lines

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Background: Small intestinal neuroendocrine tumors (SI-NETs) represent a group of rare neoplasms derived from neuroendocrine cells, mainly enterochromaffine cells. Medullary thyroid carcinoma (MTC) is a calcitonin-producing neuroendocrine tumor arising from the parafollicular C-Cells of the thyroid gland. It is noteworthy that SI-NETs as well as MTCs are known for their poor response to standard chemotherapy and radiotherapy and surgical intervention remains the only curative treatment. Therefore, there is a substantial need to establish new therapeutic options in the clinical treatment of these tumors. Aim: Earlier studies have demonstrated antiproliferative and tumoristic effects of epirubicin in neuroendocrine cells. Here we report on the effects of a novel water soluble epirubicin substituted polymer in small intestinal neuroendocrine tumors and medullary thyroid carcinomas. Therefore, the SI-NET cell lines, KRJ-I, P-STS, L-STS, H-STS and the MTC cell line MTC-SK were incubated for 24, 48 and 72 hours at different concentrations of epirubicin substituted polymer and the non substituted polymer, and cells were analysed using cell counting, WST-1 cytotoxicity assay, DAPI staining and caspase assays. Normal human fibroblats HF-SAR served as control cells. Results: Treatment with the epirubicin substituted polymer resulted in antiproliferative effects in SI-NET cell lines, KRJ-I, P-STS, L-STS, H-STS and the MTC cell line MTC-SK. An inhibition of cell proliferation, as well as a decrease in cell viability was noted, while human fibroblasts HF-SAR treated in the same way showed no significant alterations. Treatment of cells with non- substituted polymer did not show antiproliferative effects. Conclusions: Novel epirubicin substituted polymers could be a new option in the treatment of chemoresistant and radioresistant neuroendocrine tumors.

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Multiple microvascular alterations in the pancreatic neuroendocrine tumors of the MEN1 mouse model

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Background: Vascularization in each tumor type operates its distinct mechanism that involves a signaling network of angiogenic factors. Pancreatic neuroendocrine tumors (PNETs), the second most prevalent type of tumors in the multiple endocrine neoplasia 1 (MEN1) syndrome, have a highly developed vasculature. This study aimed to characterize the structural and functional features of vasculature in PNETs, and to investigate the underlying molecular mechanisms of angiogenesis. Methods: In this study a conventional MEN1 knock-out mouse model (4 months and >12 months) was used. We analyzed the vessel density (by CD31 immunoreactivity) and pericyte distribution (by NG2 immunoreactivity) within the endocrine pancreas. mRNA expression of angiogenic factors in VEGF, FGF, PDGF and angiopoietins signaling pathways was quantified by qPCR, using isolated islets and adenomas cultured in normoxic and hypoxic conditions. The vascular reactivity of arterioles supporting isolated islets and tumors, in response to D-glucose and L-nitro-arginine methyl ester (L-NAME), was documented by a single-islet/tumor perfusion technique as an indication of a functional vessel-adaptation. Results: A significant increase of microvascular density was observed within PNETs and Men1+/- islets (>12 months) compared to age-matched Men1+/+ islets. The distribution of the pericytes was considerably altered in the endocrine pancreas of Men1+/- mice as compared to their controls. Following hypoxic challenge, islets and adenomas isolated from mice older than12 months revealed elevated VEGF-A mRNA levels. In adenomas VEGFR2 mRNA expression was significantly higher than in islets. Hypoxia promoted increased mRNA levels of PlGF, FGF2 and FGFR1 in islets isolated from 4 month-old Men1+/- mice as well as in adenomas isolated from 12 month-old Men1+/- mice. In young mice, PDGF-BB and PDGFR-β mRNA expression levels were significantly higher in Men1+/- islets than in Men1+/+ islets. We also found a dramatic induction of their expressions in adenomas compared to islets (12 months) potentiated by hypoxia. A significant increase of mRNA expression of Ang2 and Tie2 was shown in adenomas compared to the age-matched islets. Perfusion with 17 mmol/l D-glucose caused vasodilation of arterioles supporting Men1+/+ and Men1+/- islets and tumors, while 0.1 mmol/l L-NAME caused their constriction. PNET's arterioles exhibited the slightest vasodilation (2%) and the maximal constriction (17%). Conclusion: The PNETs developed in the MEN1 mouse model were characterized by disturbed morphology, vascular reactivity and differential activation of several angiogenic molecular mechanisms. Upon validation in humans these findings may be exploited to improve cancer treatment.

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Pancreatic NETs with aggressive behaviour as the only manifestation of Multiple Endocrine Neoplasia Syndrome type 1 associated with a novel mutation of MEN1 gene in a large Italian family.

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Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant syndrome caused by germline mutation of MEN1 gene, located at chromosome 11q13. The classic features of this syndrome are related to parathyroid, pituitary tumor/hyperplasia and pancreatic neuroendocrine tumors (P-NETs). Our case report deals with a large Italian family affected by MEN1 caused by a novel germline mutation of the MEN1 gene. The proband, a 39-yr old female, was referred to our Department after incidental finding of a 2 cm non-functioning NET in the tail of the pancreas at abdomen MR. Surprisingly, during surgical exploration multiple p-NETs in the pancreatic head and tail were found. She subsequently underwent total pancreasectomy with splenectomy and lymph node dissection. The histological picture was consistent with multiple NETs G1, Ki67: 2% (WHO 2010) with perineural invasion and 18/30 lymph node metastases. Although parathyroid and pituitary involvement had been excluded by endocrinological evaluation and pituitary MR, the molecular test for MEN1 mutations was performed on a blood specimen, given the young age of the patient and the multifocality of neoplastic lesions. The sequencing analysis of the coding regions of MEN1 identified a non-synonymous mutation (P390R) affecting the exon 8 of the gene. This heterozygous mutation has not been described up to now and it is predicted to impact protein function because it affects a conserved aminoacidic domain that is involved in diverse protein functional interactions. At 1 yr follow up an abdomen MR showed retroperitoneal lymph node metastases whereas Ga-PET-DOTANOC and FDG-PET were negative. Genetic testing was extended to the other components of the family and gave positive results in 8 members (mother, 2 sisters, 2 brothers, 3 nephews). One asymptomatic 33 yr-old brother was diagnosed with multiple p-NETs with one peripancreatic lymph node metastases at MR and Ga-PET-DOTATOC, whereas pituitary MR and parathyroid function were negative. The

other brother and 2 sisters had normal parathyroid and pituitary function and negative abdomen MR but slightly elevated gastrin and Chromogranin A level in 2 out of 3. The other positive members are still under investigation. Two recent case reports (Raef H Clin Endocrinol 2011; 1365-2265; Hasani-Ranjbar S Fam Cancer. 2011; 343–8), described families with a high penetrance of malignant p-NETs, but with other classic features. Both these families carried germline mutations that completely abolish menin function. The only manifestation of this new mutation here described seems to be a predominant and aggressive involvement of the pancreas.

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SURGERY FOR MULTIPLE ENDOCRINE NEOPLASIA TYPE 1-INSULINOMA

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Background. Insulinoma is after gastrinoma the most frequent functioning pancreatic endocrine tumours in MEN-1, occurring in about 15-20% of affected patients. Since the usual presence of concomitant multiple pancreatic neuroendocrine lesions, the surgical management of these patients is troublesome. Objective. Aim of our study has been to evaluate the diagnostic and surgical approaches in MEN-1 insulinoma. Patients and Methods. Ten genetically confirmed MEN-1 patients were referred to our Surgical Unit (1992–2012) and were operated on for hypoglycemic crisis, associated with nonfunctioning pancreatic endocrine neoplasias in all patients and Zollinger-Ellison syndrome in two cases. Resection of the most affected pancreatic regions and enucleation of the nodules eventually found in least affected ones were performed. An accurate histopathological analysis of the specimen and the immunohistochemistry for neuroendocrine hormones antibodies were performed. The intraoperative insulin/glycemia ratio was evaluated in all patients. Results. Three pylorus-preserving pancreatoduodenectomies and seven distal pancreatectomies were performed. Enucleation of nodules in least affected pancreas was necessary in 8 pts. Spleen and splenic vessels were preserved in all but 2 cases. There was no postoperative mortality; three patients developed complications (1 pancreatic fistula and 2 acute pancreatitis). Pathological analysis showed a mean of 6.5 macrotumours (range 1-14) and of 19.8 microlesions (range 1-60). Immunohistochemistry confirmed the presence of insulinoma in all patients: four patients had 2 insulinomas for a total of 14 insulin positive lesions with a mean diameter of 1.26 centimeter (range 0.3-2.4 cm). In two cases insulinoma had a diameter ≤ 4 mm The IOUS proved a sensitivity of 87.6% for detecting the insulin positive neuroendocrine tumors. The I/G ratio decreased over 40% from higher pre-excision value at 30 minutes from resection and over 50% at 60 minutes. At a mean follow-up of 90 months, all patients were normoglycemic with no evidence of disease recurrence. Conclusion. MEN-1 insulinomas should be considered a surgically curable diseases. Choosing the type of pancreatic resection should depend on the localization of the pancreatic endocrine neoplasias, their relationship with Wirsung duct and the presence of other duodenal lesions. Because of the possibility of having multiple insulinomas or lesions less than 0.5 cm in diameter and the acceptable morbidity of the procedure a pancreatic resection associated to enucleation of endocrine tumors of the residual pancreas is preferable to a less radical surgical approach to ensure higher cure rates. Intraoperative insulin and glycemia assay with calculation of I/G ratio is of value in assessing the outcome of surgery.

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Case report: MEN1 patient with malignant pancreatic neuroendcrine tumor and multiple liver metastases surviving for 8 years.

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59 year-old man visited our hospital with right low back pain 8 years ago. Ultrasonography and MRI revealed pancreatic tumor and metastatic tumors in liver.

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Malignant Vipoma (a case report).

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Introduction: Vasoactive intestinal peptide producing tumour (VIPoma) or Verner-Morrison syndrome is a very rare neuroendocine tumour. VIPoma affect the

pancreas in 90% of the cases. Vasoactive intestinal peptide stimulates cAMP production by the intestinal tract resulting in profuse diarrhea manifesting as water and electrolyte loss especially potassium, this syndrome is also known as WDHA: Watery Diarrhea Hypokalemia Achlorhydria syndrome. In 5 to 15% of the cases Vipoma is associated with a multiple endocrine neoplasia type 1 (NEM 1). **Case presentation:** A 49-year old women admitted in an intensive-care unit for diarrhea, dehydration with electrolyte loss, septic shock after suppurative thrombophlebitis. The diagnosis of vipoma was based on a large volume diarrhea up to 7 at 8 l per day, a severe Hypokalemia under 1,40 mmol/l and a weight loss of 25 Kg, high Level of VIP up to 200pg/ml (normal<60pg/ml), other investigation including : serotonin, carcinoembryonic antigen (CEA), CA 19-9, alpha-foetoprotein (AFP), neuron specific enolase (NSE), and the 24 hour urine hydroxyindoleacetic acid (5 HIAA) were at the normal range. Clinical features and biochemical tests for multiple endocrine neoplasia type 1 were negative. Endoscopic-ultrasonography revealed a 2 cm mass arising from the body and tail of the pancreas. Abdominal scan and MRI showed a suspicious lesion measuring 5cm in the segment 7 of the liver, the biopsy and immunochemistry stained for chromogranin and synaptophysin were positive, the results implied the presence of a neuroendocrine tumour, the proliferation index cell Ki67 was at 3%.The somatostatin receptor scintigraphy with octreotide scan showed an intense uptake in both pancreas and liver mass. Preoperatively somatostatin analogue treatment was administrated to the patient with improvement of dehydration and diarrhea. The patient underwent a left splenectomy, a right liver resection with dissection of lymph nodes in the celiac and liver region. Histopathology diagnosed a liver metastasis of the pancreatic tumour, and the immunochemistry confirmed that the tumour cell expressed VIP, chromogranin and synaptophysin, proliferation index cell (Ki67) was about 4%. Postoperatively sandostatine treatment was stopped and there was no evidence of disease recurrence.**Conclusion:** despite malignancy in 50 à 60 % of the cases with liver or lymph nodes metastasis at diagnosis, vipoma can be treated successfully with surgery if the resection of both vipoma and metastasis is realized, somatostatin analogue before surgery, or when the tumour is unresectable improved symptoms and quality of life.

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Metastatic digestive neuroendocrine tumors Interest of serum choromogranin A

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Chromogranin A (CgA), is a member of the chromogranin family that comprises several proteins, is a hydrophilic acidic one-chain peptide containing 439 amino acids (49KDa). Chromogranin A occurs in chromaffin granules of neuroendocrine cells. Interest of serum CgA, initially demonstrated in pheochromocytoma, was quickly extended to other endocrine cancers. Serum levels of CgA are particularly high in the intestinal carcinoid and pancreatic endocrine tumors. Objective •Assess the value of serum CgA in the diagnosis and monitoring of metastatic gastrointestinal neuroendocrine tumors. •Comparison of CgA level between our population and control group (patients in advanced stage for other tumors) Materials and Methods This is a prospective study of 18 patients with digestive neuroendocrine tumor stage liver metastasis or peritoneal carcinomatosis, referred to the service of Medical Oncology at CPMC-Algiers for therapeutic management. The determination of CgA was carried out with the reagent CGA-RIACT (CIS bio). This type assay IRMA uses as tracer a monoclonal anti-CgA labeled with iodine 125. Detection limit: 1.5ng/ml Serum CgA in considered pathological values 100 ng/ml Results: Our work shows that: CgA serum is a very specific parameter of neuroendocrine tumors, which justifies its indication in the staging of gastrointestinal neoplasm in advanced, especially before the negativity of conventional tumor markers . Changes in CgA serum is parallel to the evolution of the tumor mass, hence the interest of its assay in monitoring therapeutic The sensitivity of the CgA serum depends on the volume of the tumor mass and the degree of differentiation and secretion of tumor. Significant difference in rates of CgA between our group of patients and the control group. Conclusion: View its specificity, serum CGA is a relevant parameter in the diagnostic workup and monitoring of neuroendocrine tumors in advanced stages, although its clinical sensitivity depends on the volume and character-secreting tumor.

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The thyrogastric syndrome: an under diagnosed etiology for acquired gastric neuroendocrine tumors. A Case Report

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Background: The thyrogastric syndrome is the autoimmune association of Hashimoto thyroiditis and atrophic gastritis. In man, atrophic gastritis leads to micronutriments malabsorption, chronic hypergastrinemia, subsequent enterochromaffin cells hyperplasia and eventually the development of a gastric neuroendocrine tumor. Case Report: Among a series of 410 patients with autoimmune disorders (AITD), we have identified 56 patients (14%) with autoimmune gastritis (1). The aim of this case report is to describe the first of such patients in which high levels of gastrine and chromogranine lead to the suspicion of a de novo neuroendocrine gastric tumor. In 2009 a 61 years old woman with Hashimoto Thyroiditis complains about bilateral tunnel carpal syndrome. Vitamin B12 is 58 pg/ml (>200), Hb=11.7 ng/L, mean corpuscular volume: 78 µ3 (84-94), ferritin 7 ng/ml (22-112), gastrin is 1705 ng/ml. Gastric auto antibodies and intrinsic factor antibodies are positive, whereas serology for Helicobacter Pylori is negative. A carpal tunnel electromyogram confirms bilateral low sensitive and motor conductance. Gastroscopy and biopsies show chronic atrophic gastritis with signs of intestinal metaplasia. Cyanocobalamin treatment ameliorates carpal tunnel syndrome and gastric follow-up is recommended. In 2010, an endoscopy with biopsies is performed again, showing enterochomaffin cells hyperplasia. In 2012, gastrin levels are increased at 2800 pg/ml and chromogranin is 212 ng/ml. Another gastroscopy is performed: a neuroendocrine tumor grade II, pT1 is found at the lesser gastric curvature and successfully resected. Discussion: The thyrogastric syndrome is prevalent but under diagnosed. Pernicious anemia, gastric carcinoid and stomach cancer are the primary complications, justifying a gastric screening. If unrecognized, pernicious anemia led to neurological and hematological potentially reversible complications. Early gastroscopy ameliorates the prognosis of gastric neoplasias. In some series (2) gastric carcinoids develop in up to 10% of patients with pernicious anemia, after a mean follow-up of 8 years. Nearly 76% of our patients with autoimmune gastritis have signs of metaplasia and enterochromaffin cells hyperplasia. The present case is our first gastric carcinoid prospectively diagnosed, which represent less than 3% (1/33) of patients with thyrogastric syndrome. References 1-H. Valdes Socin & al. Auto-immune gastritis characteristics in a large series of patients with auto-immune thyroiditis. XXIVth. Belgian Week of Gastroenterology 2012. 2-Kokkola & al. The risk of gastric carcinoma and carcinoid tumours in patients with pernicious anaemia. A prospective follow-up study Scand J Gastroenterol. 1998.

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VITAMIN D PATHWAY IN SPORADIC AND MEN1-RELATED NEUROENDOCRINE TUMORS

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Background & Aim: Vitamin D exerts anti-proliferative effects by binding to the vitamin D receptor (VDR), an intracellular nuclear receptor. There is evidence on the association between cancer and specific polymorphisms of VDR. Whit regard to neuroendocrine tumors (NETs), in murine and human insulinoma cell lines the 1-25(OH)2D has been shown to inhibit cell proliferation, induce apoptosis, inhibit insulin secretion, down-regulate the expression of insulin gene and up-regulate the expression of VDR. The aim of this study was to investigate expression and prognostic role of the vitamin D pathway in patients with NETs. Patients & Methods: 33 patients with NETs (15 sporadic and 18 hereditary MEN1-associated; 14 with recurrent/progressive disease and 19 with remission/stable disease) were evaluated and compared to 33 age- and sex-matched controls. Serum 25(OH)D levels were measured by chemiluminescence (CLIA) with dosing limits in the range 4-150 ng/ml. VDR polymorphisms evaluated included Fok-1 and Taq-1. Results: Mean serum 25(OH)D levels were significantly lower in patients with NETs (19.9+/-2.0 ng/ml) than in controls (30.4+/-1.9 ng/ml). Allelic variants of the VDR polymorphisms, Fok-1 and Taq-1, were similarly distributed in patients and controls (p=NS). Serum 25(OH)D levels were significantly lower in patients with recurrent/progressive disease than in those with remission/stable disease (16.2+/-2.0 vs 23.3+/-2.8 ng/ml). No significant differences were observed in the distribution of allelic variants of the VDR polymorphisms between sporadic and hereditary MEN1-associated NETs and between patients with recurrent/progressive disease and those with remission/stable disease. Conclusions: VDR polymorphisms, Fox-1 and Taq-1, do not differ between sporadic and hereditary NETs and do non influence the prognosis of these tumors. Serum 25(OH)D levels are significantly lower in patients with poor prognosis NETs, suggesting a potential therapeutic use of vitamin D in these patients.

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PREOPERATIVE DIAGNOSTICS: THE NEUROENDOCRINE TUMORS OF PANCREAS

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The purpose of research: to optimize modern possibilities of early diagnostics neuroendocrine tumors of pancreas (NETP) for substantiation choice of surgical treatment variant.The results of examination and treatment of 124 patients with NETP are analysed. Organic hyperinsulinism (OGI) diagnosed in 63 patients, gastrinoma – in 43, rare forms of tumors (carcinoid, glucagonoma, vipoma) – in 13 cases. The multiple tumors in patients with insulinoma diagnosed in 5,8%, with gastrinoma – in 26,9% cases. MEN-I syndrome recognized in 8,1% NETP patients. Checkup are realized with using hormone containing (immune reactive insulin, C-peptide, gastrin, chromogranin A), functional (hunger – probe, gastric secretion estimation), instrumental (US, CT, MRI, PET, angiography) and morphological researchs (light- and electronic microscopy, immunohistochemistry). The right diagnosis among OGI patients was established during first year in 42,5% cases only. Patients with gastrinoma repeatedly operated by complicated ulcerous. Hypoglycemia in OGI patients by complete rest observed in 50%, by hunger – probe in 89%. Complex using of laboratory research methods allowed to verify OGI in 93% cases. Hypersecretion in gastrinoma patients diagnosed in 67%, gastrin blood level research increased effectiveness preoperative diagnostics of disease till 96%. Ultrasonography sensibility in NETP diagnostics was 66%, CT – 64%, MRI – 62%. The most self-descriptiveness in topical diagnostics established by CT and PET with sensibility 75% and 80% accordingly. Intraoperative US allowed to localize tumor in pancreas with sensibility 93%. Tumor enucleation performed in 37% cases, distal pancreas resection with tumor – in 39%, marginal excision of pancreas – in 21%, pancreaticoduodenal resection – in 3%. So, modern methods of laboratory and instrumental diagnostics allow diagnosing NETP in proper time and determining selection of rational variants of operations for improvement patients surgical treatment results and quality of life.

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Multiple Neuroendocrinopathy type 2 (NEM 2)

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Introduction: the family CMT is characterized by the mutation of RET proto-oncogene. The objective of work is to distinguish between sporadic and familial forms of a particular support early surgical cases related Material and Methods: From 2003 to 2010, 70 index cases of CMT have been identified whose10 cases of MEN2 (MEN2A and 01 NEM2b 09) including 8 women and 2 men, the mean age was34 years. All have benefited from an ultrasound confirming the cervical or thyroid nodules. The biological euthyroidism was in all our patients, the TCT measured in 7 patients was pathological income. Cytoponction performed in 8 patients, was malignant in 4 cases, suspicious in 2 cases and mild in two others. T he metanephrines are high among 5cas/10 income. Abdominal CT scan showed an adrenal bilateral mass in 4 cases and unilateral in another, as to the adrenal MIBG scintigraphy performed only in 2 patients showed a bilateral setting. One patient had in addition to the CMT and pheochromocytoma, parathyroid nodule (P3) which established the biological and MIBI. Pheochromocytoma was operated initially in 3 cases and second time in two others. All patients underwent total thyroidectomy them with central neck dissection, lateral and bilateral ablation of a parathyroid nodule (P3) in a patient. Results: The histological and immunohistochemical studies confirmed the CMT and the parathyroid adenoma in the single case of pheochromocytoma in 5 cases showing two EOD. The genetic study done systematically in Algeria since 2003 found 10 mutations in 70 index cases (7%): eight in codon 634, one at codon 918 and a variant C515D Conclusion: Since 2003 The management of NEM 2 and it has evolved considerably through molecular analysis of the RET gene, genetic marker became available in Algeria.

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Two Brazilian kindreds with the rare p.Ser891Arg RET mutation

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The clinical behavior of some rare RET mutations causing MTC is not completely documented, and description of such kindreds may help to establish clinical protocols. The mutation p.Ser891Arg accounts for less than 5 % of the RET mutations and it is considered level A in the risk stratification of the American Thyroid Association guidelines. From the first description of two cases in 1989, up to 80 reports are found in the literature. Here we report two Brazilian kindreds with the p.Ser891Arg mutation. Kindred 1 - The index case is a 54-year-old woman who had been evaluated for a 2.5 cm thyroid nodule. FNA biopsy was suggestive of follicular neoplasm and she underwent total thyroidectomy and central compartment neck dissection. Histopathological analysis was consistent with MTC. Three months later, a bilateral neck dissection was performed and bilateral lymph node metastases were confirmed. Then she was referred to our center. Her family history was positive for cancer - her father had died at the age of 33 years with bone metastases from an unknown cancer. Post-operative serum calcitonin (sCT) levels were elevated and a neck ultrasound (US) revealed recurrent lesions in the thyroid bed. Screening for primary hyperparathyroidism was negative and the patient is under investigation for pheochromocytoma. RET genetic testing revealed a p.Ser891Arg mutation and analysis of 9 family members revealed the mutation in one son, one daughter and two nieces, all with ages between 20-27 years. sCT in all affected relatives was elevated (29-250 pg/mL) and they are now under scheduling to prophylactic thyroidectomy. Kindred 2 - A 47 year woman presented to another clinic with a history of thyroid enlargement over the past 5 years. Although such 3.5 cm thyroid nodule was classified as benign in the cytologic study, she underwent surgery due to compressive symptoms. Histopathology revealed a 3.5 cm MTC. She was later submitted to two ipsilateral cervical lymph node dissections, but still has persistent cervical disease and elevated levels of sCT. Her family history was unremarkable. RET sequencing revealed a p.Ser891Arg mutation. Family investigation uncovered the mutation in her 55-year old brother, who has no evidence of disease so far, and prophylactic thyroidectomy was recommended. Conclusion: Patients with the p.Ser891Arg RET mutation seem to have higher rate of biochemical cure when early thyroidectomy is performed.

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MEN 2A AND OTHER ENDOCRINE NEOPLASIAS: BEYOND MEN 2A?

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Multiple Endocrine Neoplasia Syndrome type 2A (MEN 2A) is a genetic, hereditary complex disorder characterized by the presence of medullary thyroid cancer (MTC), pheochromocytoma and primary hyperparathyroidism. MEN 2A has two rare variants, one associated to Hirschprung's disease and another to cutaneous lichen planus amyloidosis. MEN 2A is caused by missense germline mutations on RET protooncogen (10q11.2) which encodes the RET tyrosine kinase receptor, present on neuroendocrine cells of neural crest origin. RET is a developmentally important gene, playing a significant role on growth and cell differentiation. Exceptionally MEN 2 coexists with other syndromes resulting in combined phenotypes, like MEN 1 and 2. There are few reports where MEN 2A shows an unexpected phenotype, for example, V804M mutation with papillary thyroid carcinoma, medullary thyroid cancer and hyperparathyroidism. We report a family with MEN 2A by C634Y mutation. Their members developed expected neoplasias but also additional endocrine tumors and showed cutaneous stigmata typical of other multiple endocrine neoplasia syndromes. Two of the eight members with C634Y mutation had multiple café-au-lait spots, another one a liver carcinoid. One of the subjects who refused performing a genetic test had a pinealoma (See Figure 1). Association with other endocrine neoplasias and cutaneous stigmata characteristic of other MEN syndromes (Carney Complex, McCune-Albright Syndrome) makes us thinking about "amplified" forms of MEN2A in which RET mutation is expressed in other ectodermal tissues like skin and some endocrine cells, which derive from the neural crest too.

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Discrepant genotype-phenotype correlation in MEN 2 patients: meticulous screening for double RET germline mutation and discovery the largest kindreds with p.C634Y/p.Y791 RET double mutations

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BACKGROUND. Although a strong genotype-phenotype correlation is observed in MEN 2 syndrome, lack of correlation has been observed in few families. We have previously identified a four-generation kindred with p.Y791F RET mutation and more aggressive clinical phenotype. Due to the fact that double germline RET mutation may occur in patients with uncommon clinical manifestation and that it may have a certain impact on the prognosis and treatment of the patients, we here re-examined whether an additional mutation within the RET gene could be in

linkage disequilibrium with the p.Y791F variant. METHODS. We re-screened the entire coding region of the RET gene from the index-case using different sets of primers. In addition, in silico analysis was performed to investigate the probable effect of substitutions in codon 791. RESULTS. A p.C634Y substitution was found in cis with p.Y791F substitution. We additionally identified 4 families harbouring the p.C634Y/p.Y791F double mutation, comprising the largest case series with such genotype in the literature. Most patients have clinical and/or biochemical evidence of MTC. Hyperparathyroidism was found in three carriers of the same family. Interestingly, cutaneous lichen amyloidosis was reported in two families. Finally, we here report a family with p.Y791N substitution alone. Using the Protein Data Bank and the RET 3D resolved structure, we investigated in silico the effect of residue Asparagine substitution at codon 791, suggesting that this aminoacid change could be less pathogenic than the substitution for Phenilalanine. CONCLUSIONS. Double-mutation in RET gene may contribute to elucidating divergent genotype-phenotype correlation, what calls out for careful screening and re-screening when necessary in those patients. More studies are necessary to establish the potential pathogenic of the aminoacid changes in codon 791 other than phenylalanine and, thus, clarify the genetic couselling to the affected families, avoiding overtreatment.

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Frequent methylation of the first exon of orexin receptor type 2 in endometrial cancer

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Several DNA methylation epigenetic biomarkers have already been reported in endometrioid endometrial carcinoma (EEC) which is the most frequent gynecological cancer. A new genome-wide microarray analysis led to the identification of the frequent methylation of the first exon of the orexin receptor type 2 gene (OX2R) in EEC. The bisulfite sequencing method after bacterial cloning allowed mapping of the local DNA methylation changes in a cohort of 34 cancer patients and 18 control endometrium. high methylation percentages were demonstrated in grade 1 and 2 EEC whereas grade 3 had lower values. The distribution of OX2R was next evaluated thorough the normal menstrual cycle and in the EEC samples tested for the methylation status. Our data provide for the first time the evidence of the expression of the OX2R protein in normal endometrial epithelia and its frequent loss in EEC. In order to establish correlation between OX2R methylation and the protein expression MFE280, ECC1 and Ishikawa cell lines were used. In ECC1 and Ishikawa, methylation correlated with weak or absent transcription. Interestingly for the MFE280 cells, completely unmethylated and methylated alleles were present concomitantly leading to a hemizygous locus permissive for OX2R expression. Additionally, in vitro treatment of the three EEC cell lines with orexin did not result in proliferation change. The exact role of OX2R in the endometrial cancer progression remains to be elucidated. Altogether our data provide evidence for the hypermethylation of OX2R first exon in EEC leading to complete or partial loss of the protein in tumoral tissues.

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The case of severe congenital hyperinsulinism and undermasculinization in a boy

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Background. Congenital hyperinsulinism (HI) represents a heterogeneous group of insulin regulation disorders. Genetic defects in four common genes have been identified: SUR1, KIR6.2, GK and GDH HI. The causes of both HI and disorders of sex development (DSD) are presented by mutations in specific genes, however, the combination of DSD and HI was not found by authors. We report patient C. was born from the 3rd pregnancy and the second delivery (1 abortion in the anamnesis) at 40 weeks of gestation. The birth weight was 4500g, length. In 5 months weight was 10500g, length - 66 cm, BMI>95 percentile. Heart and liver were increased. The features of undermasculinization included micropenis, mild hypospadias, hypoplasia of scrotum and bilateral abdominal criptorchidism. Karyotype was 46XY. Persistent hypoglycemia in the newborn was revealed during the first five hours of life: the lowest glucose level was 0,7 mmol/L, the highest insulin level was 2512,0 pmol/L. Hypoglycemia was accompanied by trembling, irritability, hypodynamia, diffuse cyanosis, seizures. No disease causing mutation in KCNJ11 and the ABCC8 genes was observed. Glucose blood level 2,0±0,5 mmol/l was maintained by intravenous infusion of 20% glucose solution with the rate of infusion 15-20 mg/kg/h. At the age of 1,5 months treatment with octreotide

by dose 23 mkg/kg/d by 4 times a day was started. However, severe hypoglycemia was repeated with the risk of developmental delay and neurological damage. At the age of 4 months open near-total pancreatectomy was made. In histologic examination the diffuse subtype of congenital HI was confirmed. Conclusions. The undermasculinization in the boy with congenital HI can be related by increase of aromatase activity in the HI-binding too much fatty tissue, and, therefore, by decrease of level and activity of testosterone in fetus. A rare type of Beckwith-Viedemann syndrome be dscussed. The GLP-1 receptor may be a useful therapeutic target for the management of patients with hyperinsulinemic hypoglycemia.

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CLINICAL CHARACTERISTICS OF FAMILIAL AND SPORADIC MTC'S DURING RECENT YEARS IN A SINGLE CENTRE IN GREECE

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Background: MTC has varying clinical course. Sporadic disease is believed to have more aggressive course as familial cases are usually diagnosed earlier. The purpose of this study was to assess the differences in the clinical features between familial and sporadic cases in a single centre in Greece. Methods: 179 MTC patients (36.9% males) were followed up for 0.9-34 years (mean 6.16±6.1 yrs, median 4.0 yrs). Total thyroidectomy was performed in all patients. 48.1% had familial disease. 57 patients (31.8%) were diagnosed between 1977-2000 and 122 patients between 2001-2011 (recent period). Extent of disease at diagnosis and during follow-up, and pre- and postoperative calcitonin levels were recorded. Results: Median age at diagnosis for familial MTC was 33.11±15.41 (range 5-72 years) and for sporadic: 53.53±12.58 (range 32-78, $p < 0.001$). The prevalence of familial disease was significantly higher in women than in men (72% vs 51.8%, $p = 0.014$, linear by linear association). When the whole period of observation was considered, patients with familial MTC had more frequently remission of disease (64.7% vs 47.5%) and less frequently progressive disease (8.8% vs 31.3%, $p = 0.003$) at follow-up. They also had more frequently multifocality and c-cell hyperplasia ($p < 0.001$), smaller tumour size (1.24±0.8 vs 1.94±1.59 cm, $p=0.001$), less frequently capsular invasion (33.3% vs 50.7%, $p=0.042$) and distant metastasis at diagnosis (4.5% vs 14.5%, $p=0.045$) and at follow-up (11.8% vs 34.9%, $p=0.005$). No significant differences in lymph node invasion, microcarcinoma prevalence and stage at diagnosis were found between groups. Significantly worse clinical features (outcome of disease, stage at diagnosis) were noted in the sporadic cases only in the first period of observation, while no such differences were observed during the more recent period. No significant differences in pre- and postoperative calcitonin levels were found between sporadic and familial cases. Conclusions: Currently diagnosed sporadic and familial MTC cases do not differ significantly concerning clinical features as opposed to worse characteristics observed in sporadic cases during the previous decades. Women may have increased awareness for genetic screening in familial disease.

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Interest of genetics in the management of familial medullary thyroid cancer

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Introduction: The CMT is a tumor of parafollicular cells of the thyroid. It represents 3.95 to 6.35% of thyroid cancers in Algeria. The gene responsible for familial MTC is the RET proto-oncogene, specific genetic marker. Purpose of the work: Research familial forms, to allow early management of genetically related cases at risk. Materials and methods: CMT 70 (index case) had a genetic study which has found 13 familial forms, with the following mutations: nine at codon 634, one in codon 918, one in codon 804, one at codon 768 and one at codon 891. 42 cases related to these 13 families had a genetic screening and finding 16 cases (RET +) carrying the same mutation as their parents. Among these 16 cases, 9 underwent total thyroidectomy, which immunohistochemical examination had found: 4 cases of micro cancers and 3 cases of C-cell hyperplasia and 2 cases were histologically healthy thyroid. Recovery was in 9 cases, confirmed by a postoperative pentagastrin test normal. . Conclusion: Through molecular analysis of the RET gene, genetic marker became available in Algeria Since 2003 we have moved from curative surgery to prophylactic surgery.

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Medullary thyroid cancer: last 5-year experience of the Portuguese Oncology Institute of Oporto

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Introduction: Medullary thyroid cancer (MTC) can be challenging as it usually presents as advanced disease and has a poor response to conventional treatments. Additionally, its low incidence makes it difficult to obtain extensive experience in treatment and follow-up. This study aims to review our experience with MTC during the last 5 years. Methods: Retrospective study of all MTC patients (n= 52) followed in the Portuguese Institute of Oncology of Oporto between 2006 and 2011. Information on demographics, genetics, disease staging and progression was obtained. Data were analyzed in SPSS. Results: Mean age at diagnosis was 52.3±15.5 years; 61.5% were female patients. The majority of patients presented with stage IV disease (63.8%), 4.3% in stage III, 8.5% in stage II and 23.4% in stage I. Mutation analysis of RET gene was done in 75.0% of the patients. Among index cases, a RET mutation was found in 12.9%. Three patients were diagnosed after genetic testing because of an affected relative. The group with hereditary disease had a higher proportion of female patients (71.4% vs 64.0%), stage IV disease (75.0% vs 52.0%) and lower mean age at diagnosis (34.3±7.8 vs 52.3±15.7 years), but differences were not statistically significant. The initial therapy was thyroidectomy and lateral neck dissection in 59.6% (and also radiotherapy in 5.8%); total thyroidectomy in 21.2%; hemithyroidectomy in 7.7% (42.9% of the patients were initially treated in another hospital). After this, 45.2% were submitted to other treatments: 38.1% reoperation; 4.8% radiotherapy; 4.8% chemotherapy; 2.4% 131I-MIBG; 2.4% tyrosine kinase inhibitor and 2.4% somatostatin analogue. Biochemical evidence of persistent/recurrent disease was found in 53.2%, but recidive or metastases were identified only in 42.6% (lymph nodes 14.9%; lung 12.8%; bone 12.8%; liver 10.6%). Overall survival was 80.8%; 2-year survival 91.8% and 5-year survival 81.7% (all dead patients presented stage IV disease at diagnosis; overall survival of this stage was 70.0%). Conclusions: Most MTC cases were diagnosed in advanced stages, which are associated with lower survival. We found a lower proportion of inherited MTC than is described in the literature (20-25%). The small sample size limited analysis of the differences between the sporadic and inherited groups. Surgery was the main treatment option; experience with new treatments is still limited.

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MIXED MEDULLARY AND PAPILLARY CARCINOMA OF THE THYROID PRESENTED WITH THYROTOXICOSIS

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The coexistence of a papillary and medullary carcinoma in the thyroid gland has been reported extremely rarely. For now in the literature there are 35 reports for a total of 46 cases. Most of these reports described the simultaneous occurrence of a papillary and medullary carcinoma as two distinct tumors, and only 15 cases of true mixed tumors (both components in the same lesion) were found. The malignancy may be rarely found in hyperfunctioning “hot” nodules. Aim: to describe a rare case of mixed thyroid carcinoma with thyrotoxicosis. Case report: A 61-year old woman presented to our department with increasing breathing distress syndrome and dysphagia. The patient has been treated succesfully with antithyroid drugs for hyperthyroidism (TSH=0,27 mcg/mL and fT3-fT4 within the normal range) during the last two years. A large swelling of the left side of the neck was revealed by physical examination. An ultrasound confirmed a 30x35x45 mm hypoechoic nodule of the left lobe, with multiple nodules in the right lobe and another nodule (around 1 cm) in the isthmus. Thyroid scintigraphy with I-131 showed a cold area corresponding to the nodule in the left lobe and a hot area in the isthmus. Serum level of calcitonin was within the normal range (1 pg/mL). A fine needle aspiration showed the benign behaviour of the left nodule. Because of multinodular structure and thyrotoxicosis the patient underwent a total thyroidectomy. The histology demonstrated a mixed medullary and papillary cell carcinoma in the nodule of the isthmus with coexistent multinodular goiter in both lobes of thyroid. Conclusion: while mixed medullary and papillary carcinomas are extremely rare, especially in hyperfunctioning nodules, these cases are clinically challenging because it's components apart require different approaches on treatment and follow up.

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FMTC and prolactinoma: a casual association or a new genetic syndrome?

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Medullary thyroid carcinoma (MTC) is a rare thyroid malignancy of C-cell derivation representing approximately 5% to 10% of all thyroid cancers. Twenty-five to 30% of MTC are inheritable, associated with multiple endocrine neoplasia (MEN) 2A, MEN 2B or with the familiar medullary thyroid carcinoma syndrome (FMTC), due to autosomal dominant mutations of the RET proto-oncogene. In MEN 2A and 2B MTC can be associated to other endocrine neoplasia (pheochromocytoma, hyperparathyroidism) but the association with pituitary adenomas has been rarely described. We report the association of MTC and prolactinoma in two FMTC families. Family 1. A 49-years-old male patient underwent thyroidectomy at the age of 31 for MTC with cervical lymphnode and liver metastases. He was followed up for the succeeding 15 years when, for sexual dysfunction, a prolactin-secreting pituitary adenoma was diagnosed and surgically resected. The RET gene was analyzed by direct DNA sequencing. The proband was found to have the c.2711C>T exon 15 mutation leading to a substitution of serine 904 with either a phenylalanine or a leucine (p.S904F/L). This is a rare mutation, first described in 2007 in a case of FMTC. The only first degree alive relative, his 53 years-old sister, underwent clinical and biochemical evaluation with evidence of MTC. Total thyroidectomy was performed. The same mutation in the RET gene was found. Prolactin was normal. Family 2. FMTC family with three affected members: a 55 years-old female diagnosed with MTC in 2011, her 54 years-old sister with an history of PRL-secreting pituitary microadenoma treated with cabergoline diagnosed with MTC in 2011 and their 52 years-old brother with recently diagnosed mild hypercalcitoninemia waiting for thyroidectomy. The RET gene was analyzed by direct DNA sequencing and the c. 2410G>A exon 14 mutation (p.V804M) was found in all affected members. This mutation, first described in 1996, is associated with FMTC and MEN 2A. Moreover the second proband was found to have a pancreatic (body-tail junction) 6 mm lesion with high levels of gastrin and chromogranin A waiting for diagnostic evaluation. Conclusions: we report the association between FMTC and prolactinoma in two families with different RET mutations. Reviewing the literature only one case of MEN 2A and prolactinoma has been described (Bertrand JH et al, Clin Endocrinol 1987). It’d be interesting to understand the possible role of RET mutations in the pathogenesis of prolactinomas so as prolactinemia could become a screening assay in patients affected by inheritable MTC.

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Aggressive medullary thyroid cancer and follicular thyroid cancer in a patient with the rare germline RET variant p.Val648Ile

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A 65 year-old woman was evaluated for medullary thyroid cancer (MTC) and follicular thyroid cancer (FTC). She had undergone total thyroidectomy in another medical center at the age of 46 years due to a thyroid nodule. Pathological study revealed a 2.0-cm MTC in the left lobe and a 1.3-cm moderately differentiated follicular carcinoma in the right lobe, invading surrounding soft tissue. She denied having received radioactive iodine therapy and, apparently, she was never under suppressive therapy with levothyroxine.

Her initial evaluation at our center revealed elevated serum thyroglobulin levels under rhTSH stimulation (Tg 52.8 ng/mL), low calcitonin levels (<2 pg/mL), and left cervical lymphadenopathy. Whole body scan with I131 was positive in the anterior neck area. A neck computed tomography revealed a 3.5cm-solid lesion from the level of the sternal notch to the hyoid bone, in close contact with the trachea and the esophagus, possibly infiltrating their wall, and infiltrating the adjacent muscle and the cricoid and thyroid cartilages. FNA biopsy of such lesion and the left lymph nodes was suggestive of MTC metastasis, and FNA biopsy of a 2.5cm lymph node in the central compartment was suggestive of neoplasia from follicular origin. *RET* genetic testing was positive for the variant p.Val648Ile. Her family is under investigation and, so far, her 3 children who tested positive for the same *RET* variant do not show evidence of disease. In conclusion, we report a case of a patient with a rare germline *RET* variant – p.Val648Ile – with an aggressive MTC and an FTC, and whose relatives known to carry the variant are not affected so far. Whether this variant has a pathogenic role is unclear and the relatives carrying this variant must be closely followed-up.

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Expression pattern of matrix metalloproteases in human medullary thyroid carcinoma cell lines

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Objectives: Medullary carcinoma of the thyroid (MTC) is a rare carcinoma that originates in the parafollicular C cells of the thyroid gland having a high metastasis potential. Over the last decade, several findings indicate that matrix metalloproteases (MMPs) are involved in tumourigenesis and metastasis. Their expression is related to the progression of several types of cancer. However the impact of MMPs in the formation and metastasis of MTC is not investigated. Materials and Methods: In this study we verified the expression pattern of the 23 MMPs, presently known in humans, in well characterized human medullary carcinoma cell lines. In total, 9 MTC-cell lines (BOJO, GRS-IV, GRS-V, MTC-SK, RARE, SHER-I, SINJ, OEE-III and TT) were examined. The expression of matrix metalloproteases was analysed by RT-PCR. Western blot analyses were performed on selected MMPs. Results and conclusion: All MTC cell lines constitutively express a wide variety of MMPs at mRNA and protein levels. In our study MMP-12, -20, -25, -26 and -27 have not been detected at mRNA levels in any of the 9 cell lines analysed, but MMP-2, -9 and -14 in most of them. Some of MMPs had a rather diverse expression pattern. We conclude that MTC cell lines can serve as promising candidates for future investigation of the role of MMPs in tumourigenesis and metastasis of medullary thyroid carcinoma.

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Establishment and Characterization of the Cell Line OEE-III derived from Sporadic Medullary Thyroid Carcinoma with RET mutation

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Background: Medullary thyroid carcinomas (MTC) are associated with mutations in the RET proto-oncogene located on chromosome 10q11.2. RET-Mutations are mainly found in hereditary MTC forms but also in about 50% of sporadic cases. Representative MTC cell lines are rare due to technical difficulty. Aims: Our objective is the establishment of MTC cell lines and their biologic and genetic characterization. Here we present the novel cell line OEE-III which was derived from a lymph node metastasis of a 50 year-old female; tumor stage at time of surgery pT2aN1bM1. Results: The cell line grows adherently and forms clusters of triangular or spindle shaped cells. The cells maintained neuroendocrine markers, such as calcitonin and contain a large amount of neuroendocrine granules. Distinctive chromosomal abnormalities were found by conventional chromosome analyses, multiplex FISH and array-CGH. Sequence analysis revealed a M918 mutation (ATG>ACG) in the RET proto-oncogene. In early passages (p18), the mutation was detected in both allels, later passages (P43) were heterozygous. Several molecular markers for NETs could be identified by RT PCR. OEE-III cells express mRNA for the neuroendocrine markers achaete-scute complex homolog (ASCL1), Homo sapiens cut-like homeobox 1 (CUX1), chromogranin A (CHGA), secretogranin II (SCG2), secretogranin III (SCG3), secretogranin V (SCG5), chromogranin B (CHGB), synaptophysin (SYP), highly similar to Galectin-3-binding protein (M2BP), Enolase 1 (ENO1) and Enolase 2 (ENO2). Tumorigenicity was evaluated by subcutaneous transplantation of OEE-III cells in severe combined immunodeficiency (SCID)–mice. Resulting nodules had comparable cytogenetic and molecular properties as cultured cells. Conclusion: As MTC is resistant to chemo- and radiation therapy, the OEE-III cellline may serve as a promising model for novel therapies.

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Prevalence and clinical significance of macroprolactinemia in patients with prolactinomas

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Background: Presence of high-molecular prolactin isoform is generally suspected in cases of mild hyperprolactinemia presenting with no typical hyperprolactinemia-related symptoms and negative pituitary imaging. Objective: The aim of this observational case-control study was to assess the prevalence and clinical significance of macroprolactinemia among patients with prolactinomas. Subjects with non tumoral hyperprolactinemia were not recruited. Methods: The study population consisted of 239 subjects: 131 patients and 108 sex-, age- and ethnicity– matched healthy controls. Macroprolactinemia was defined by a PRL recovery after PEG precipitation of < 40%. Results: Macroprolactinemia was equally prevalent in newly diagnosed prolactinoma patients and healthy controls (3.5 vs. 3.7%; p=1.000). Significant association between serum levels of the monomeric form and macroprolactin and disruptions of ovarian function was found not only in subjects with true hyperprolactinemia but also in macroprolactinemic patients. Conclusions: In few cases, the presence of typical hyperprolactinemia-related clinical symptoms and their disappearance after treatment with DA suggests biological activity of macroprolactin comparable with that of monomeric prolactin isoform. Decrease of macroprolactin levels after dopamine agonist (DA) treatment suggests tumoral origin of the high-molecular isoform in these rare cases. An individualized approach to the management of patients with macroprolactinemia should be applied. Pituitary imaging, DA treatment, and prolonged follow-up may be necessary in certain cases.

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THE COEXISTENCE OF A PITUITARY ADENOMA AND PHEOCHROMOCYTOMA (a case report)

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For now 29 cases of the coexistence of a pituitary adenoma and pheochromocytoma have been reported in the literature. Most of these cases presented with GH-secreting pituitary tumors and rarely prolactinomas. On our knowledge pituitary adenoma with mixed secretion has not been described so far in association with pheochromocytoma. Aim: to describe a rare association of pheochromocytoma with a prolactinoma with probably GH cosecretion. Case report: A 49-year old man, who had been operated for a pheochromocytoma 15 years ago with complete normalization of blood pressure and levels of cathecolamines, presented to our department with gynecomastia and hyperprolactinemia (PRL=1.372 mUI/L). The biological tests confirmed the absence of macroprolactinaemia and the IRM showed a invasive into the right cavernous sinus pituitary adenoma (7,5x4,7x6,3 mm). He was succesfully treated with dopamine agonist (PRL=120 mUI/L) without pituitary tumor growth during the whole follow-up. Three years after the patient developed again episodic headache, elevated blood pressure and flushing face. The urinary test revealed the elevated levels of normetanephrine (521 mcg/24h) and normal level of metanephrine (155 mcg/24h) that could be evident for the recurrence of pheochromocytoma. Moreover in the hormonal tests the elevated level of IGF1 was noticed (244 ng/mL, normal range till 221 ng/mL) with normal basal GH (0,19 ng/mL) but without the suppression during the OGTT, in the meantime the levels of PRL were within the normal range (60mUI/L) on the treatment with cabergoline (0.25 mg/week). According to the clinical examination the patient didn't present any signs of acromegaly. The size of pituitary adenoma was stable (5mm) with extension into right cavernous sinus. No mutations in pituitary adenoma and adrenal tumors predisposing genes were found neither in direct sequencing nor in MLPA. Conclusion: the associations of pheochromocythoma and pituitary adenoma could represent a new syndrome and might be caused by other than known predisposing to these tumors genes.

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Successful Temozolomide treatment in a MEN1-related aggressive resistant prolactinoma

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Prolactinomas (PA) in Multiple Endocrine Neoplasia type 1 (MEN1) tend to be more aggressive and resistant to dopamine-agonist drugs (DA) than sporadic cases. Temozolomide (TMZ), an alkylating agent, has become the drug of choice for highly aggressive PA and carcinomas, but experience in MEN1 patients is limited. Case-report. In 1983, an apparently sporadic non-invasive macroprolactinoma was diagnosed in a 23 yrs-old woman and successfully removed by transphenoidal surgery (TS). Fifteen years later, a recurrence developed, with an increasing resistance to DA (bromocriptine up to 30 mg/d, cabergoline up to 8.0

mg/w y) requiring repeated TS surgery (1998,2004,2008) and conformational radiotherapy (2004), followed by a transient reduction in PRL secretion (from 2000 ng/ml to 130 ng/ml) and tumor volume (~30%). In 2004 she also developed primary hyperparathyroidism (2004) and a germline heterozygous truncating MEN1 gene mutation was found. Since 2007, progressive tumour regrowth was observed, and in 2009, the patient developed a severe right cavernous sinus syndrome (ophtamoplegia, palpebral ptosis, invalidating headache and facial pain) with increasing plasma PRL levels (2500 ng/ml), aggressive local tumour growth, no evidence of cranio-spinal or distant metastasis. TMZ was started in February 2010 (200 mg/m2/d, 5 days every 28 days) and continued for 24 months with a rapid clinical improvement, progressive PRL normalization and marked tumour reduction with large cystic areas in the residual mass. Two months after TMZ withdrawal plasma PRL remains &lt5 ng/ml on a 1.0 mg weekly CAB dose. Noteworthy, methylation-specific PCR study of the MGMT promoter on tumor DNA showed no evidence of methylation, and diffuse MGMT expression was found by immunohistochemistry. Conclusion. TMZ appears as a suitable option in aggressive prolactinomas regardless of MEN1 mutation. In our patient, a rapid therapeutic response was observed despite unfavourable MGMT status and correlated with the long-term response, supporting the view that MGMT expression should not be considered as an exclusion criteria for TMZ treatment.

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Increased prevalence of the GCM2 polymorphism, Y282D, in three Italian primary hyperparathyroidism cohorts: results of a meta-analysis

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The protein product of the GCM2 gene is a key participant in the organogenesis of the parathyroid glands. Inactivating mutations, either recessive or dominant negative, are a known cause of congenital isolated hypoparathyroidism. The continued expression of GCM2 in the adult parathyroid raises the possibility that overactive forms could play a role in the evolution of parathyroid hyperactivity or tumorigenesis. One single nucleotide polymorphism (SNP) – a c.844T>G transversion encoding a Y282D missense variant – has been identified by several investigators, but its association with primary hyperparathyroidism has not been tested. We undertook a genotyping study of a large southern Italian cohort (San Giovanni Rotondo, SGR) and then included smaller cohorts from Pisa and Milan, subjecting the three independent results to a meta-analysis. For all samples, genotyping was performed by PCR amplification and enzymatic digestion with Hph1. In the SGR cohort, the variant allele frequency (f) was significantly higher in 310 primary hyperparathyroid subjects (PHPT, f=0.066) than in 433 controls (C, f=0.029) by Fisher exact test (p=.0008). The Y282D SNP was also more frequent in PHPT cases from Pisa and Milan, compared to their controls, but the differences were not significant. Meta-analysis (PHPT=510; C=665), yielded a likelihood ratio of 2.27 (95% CI 1.50 – 3.42) for the presence of PHPT in subjects carrying at least one copy of the Y282D mutation

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THE CLINICAL CHARACTERISTICS OF PRIMARY HYPERPARATHYROIDISM (PHPT) IN PATIENTS WITH MULTIPLE ENDOCRINE NEOPLASIA (MEN) TYPE 1.

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Rare cases of PHPT could be a component of some genetic syndromes, most frequently in patients with MEN 1 (2-4.5%). Little is known about clinical differences between MEN1 related and sporadic PHPT. Aim: to compare the clinical features of PHPT in MEN type 1 cases and sporadic PHPT. Materials and methods: Data were obtained in 442 patients: 62 sharing MEN1 phenotype presented with PHPT in association with pituitary adenomas and/or gastroenteropancreatic neuroendocrine tumors (Group-I) and 380 with apparently sporadic PHPT (Group-II). Serum Ca, intact PTH, urine Ca, 25-OH-VitD, osteocalcin, β-CTx) and BMD Z-scores measurments, renal ultrasonography,

visualization of parathyroid glands were performed in both groups. The additional diagnostic tests (prolactin levels, ultrasound of pancreas and adrenals) were performed in patients to exclude or evaluate other than PHPT endocrine disorders. Results: The age at diagnosis was younger in Group I (39.5yr. [28;51]) than in Group II (57yr. [48;64]), p&lt0.001. In 50% of Group I PHPT debuted before age of 40 years, whereas in Group II in 70% of patients the diagnosis was made after age of 50yr. The distribution of males/females was 1:2,6 in Group I and 2:10 in Group II. In 93% of Group II PHPT was caused by a single parathyroid adenoma, in Group I in 59,6 % an enlargement of multiple parathyroid glands was observed. In 64% of iPTH was higher than upper normal range less than 2,5 times, whereas in Group II 54% was more than 3 times higher than normal limits. In the meantime the levels of Са in both groups weren't significantly different (p=0.28). The mild PHPT was observed in Group I more frequently than in Group II (33,8% vs 19%). PHPT presented with osteoporosis with the same prevalence in both groups, but with more frequent renal manifestations in Group II. In Group I the decrease in BMD at all sites and nephrolithiasis developed independently of the degree of PTH elevation, as opposed to Group II. Multifactorial analysis revealed the combination of moderately elevated PTH and the age at diagnosis younger than 40 years to increase the risk of MEN1-related PHPT in these patients independently of their gender and the mild course of PHPT. Conclusion: The patients with MEN1-related PHPT are younger at the diagnosis of PHPT, have more frequently multiple enlarged parathyroid glands and show mostly mild course of disease (especially in young age) and less increased PTH compared with those with sporadic PHPT.

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ABSENCE OF PRIMARY HYPERPARATHYROIDISM IN MEN 2A

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Background: Hyperparathyroidism is defined by an inappropriately high PTH secretion for the degree of calcemia. Primary hyperparathyroidism (PHTP) is caused by autonomization of one or more parathyroid glands. PHPT is the least common component of Multiple Endocrine Neoplasia Syndrome type 2A (MEN 2A), that is characterized by the presence of medullary thyroid cancer, pheochromocytoma and primary hyperparathyroidism. PHTP occurs in 15-30% of cases and is related to certain mutations in protooncogen RET. Surveillance for PHPT should include annual corrected or ionized calcium with or without PTH. In carriers of RET mutations in codons 630 and 634 it should begin by 8 years old and continue throughout life. Patients and Methods: We present eight members from the same family with MEN2A by C634Y mutation and absence of PHTP. Results: None of our patients showed PHTP at diagnosis neither during follow-up, which lasted between 10 and 23 years. Case 5 was submitted to excision of some enlarged parathyroid glands during thyroid surgery. This conditioned a permanent hypoparathyroidism with high requirements of calcium and vitamin D. Comments: PHTP in MEN 2A is infrequent and often asymptomatic, being the last component of the syndrome to develop, with a median age at diagnosis of 38. In 75%-80% of patients the diagnosis of PHPT occurs synchronously with the diagnosis of medullary thyroid cancer or pheochromocytoma, in the remainder ones PHPT is detected years after thyroidectomy. Initial lesion appears to be an asymmetric hyperplasia of parathyroid glands, which does not cause hypercalcemia. It explains that some patients show normal PTH values although pathologic parathyroid glands are found at surgery. In spite of surgery indications are the same of sporadic PHTP is common to remove macroscopically enlarged parathyroid glands, generating permanent hypoparathyroidism in 30% of cases. Conclusions: PHPT in MEN 2A has some peculiarities that make us to reconsider the suitability of an exhaustive and aggressive management. The removal of macroscopically enlarged parathyroid glands in normocalcemic patients carries significant morbidity without any clinical benefit.

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Clinical and genetic studies in patients with Pituitary/ Parathyroid variant of MEN1 without MEN1 gene mutation: the french GENEM collaborative study.

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Background : We have previously identified some patients harboring only a pituitary and a parathyroid(s) adenoma(s) without MEN1 or CDKN1 gene mutations (1,2). Most of these were sporadic patients with a predominant female sex ratio. A pituitary GH adenoma was the most frequent phenotype whereas a

pituitary adenoma was usually diagnosed before the parathyroid tumor. None other typical MEN1 associated features were observed after long term follow-up. Aim: To enlarge our study in collaboration with the French GENEM database and to compare clinical characteristics of these patients (group I) to those with germline MEN1 confirmed mutations (group II). Results : We identified 35 patients (sex ratio 29F/6M) presenting the double association of pituitary and primary hyperparathyroidism without germline MEN1 mutations. There were 30 sporadic and 5 familial cases. Pituitary adenoma was diagnosed at a mean age of 57 years whereas parathyroid adenoma was found later, at 60 years (p>0.05). Among the pituitary phenotypes there were 23 somatotrophynomas (2 micro/21 macro), 3 mixed GH/PRL (all macro), 7 prolactinomas (all micro) and 2 non secreting adenomas (both macro). Cervicotomy identified one parathyroid adenoma in 10 cases and multiple parathyroid adenomas/hyperplasia in 10 cases. Long term follow- up did not showed other MEN1 features in Group I. Clinical characteristics of group I will be compared to 30 patients of group II. Patients will be matched by age and pituitary phenotype adenoma. Conclusions: Pituitary-parathyroid adenoma MEN1 variant seems, from clinical grounds, different from MEN1 syndrome. The pituitary and parathyroid adenoma tumorigenesis might involve other mechanisms than either MEN1 or CDKN1B mutations. 1-H Valdes-Socin & al. for the GENEM. Association acromégaly –primary hyperparathyroïdism : MEN 1 or a different entity ?. Abstract. In Annales d'Endocrinologie 2006. 2-L Rostomyan & al. The Pilot Study on clinical presentation of pituitary adenoma in patients with multiple endocrine neoplasia type 1 phenotype with and without MEN1 mutation. Abstract ICE/ECE 2012.

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Identification and functional characterization of CDC73 gene mutations in HPT-JT families

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Hyperparathyroidism Jaw-Tumour (HPT-JT) syndrome is characterized by primary hyperparathyroidism (PHPT), including parathyroid carcinoma in ~15% of cases, and maxillary/mandible ossifying fibromas. Inactivating mutations of the CDC73/HRPT2 gene cause HPT-JT, but are also a major determinant in sporadic parathyroid carcinoma. The CDC73 gene is rarely inactivated in the atypical or typical parathyroid adenomas seen in sporadic or familial isolated PHPT. Here, we report the molecular analysis of the CDC73/HRPT2 gene in affected patients of three HPT-JT families. We identified 4 CDC73 mutations: 3 germline (c. 679_680delAG, p.Val85_Val86del, and p.Glu81_Pro84del), and 1 somatic (p.Arg77Pro). Three of the mutations are within the 76-92 region designated as a nucleolar localization signal sequence while the 125-139 region was previously identified as the nuclear localization signal sequence. Constructs encoding Flag-tagged wild-type (WT) and the three mutant (delVV, delENIP, R77P) CDC73/parafibromin proteins were transiently transfected in HEK293 cells, and whole cell expression, nuclear localization, and cell viability/proliferation assessed. All three mutant proteins were expressed at greatly reduced levels relative to WT, and the proteasome inhibitor MG132 was ineffective in restoring the mutant protein levels to those of WT. Indirect immunofluorescence of HEK293 cells transfected with Flag-tagged WT and mutant constructs demonstrated that WT was absent in the cytoplasm but localized to the nucleus with punctate staining indicating concentration in nucleolae. In contrast, neither the delVV nor the R77P mutants were found in the nucleus but were present in the cytoplasm. Cells expressing the delENIP mutant demonstrated both cytoplasmic and some nuclear staining. Thus, all mutants exhibited mislocalization in comparison to WT. Cells transfected with any of the three mutant parafibromin constructs demonstrated higher values relative to WT in the MTT viability assay at 48 and 72 h after transfection. In conclusion, we report the identification of three novel CDC73 mutations in HPT-JT families. The mislocalization and loss of cellular growth control of the mutants emphasizes the importance of nuclear/nucleolar location for normal tumour suppressor actions of the CDC73/parafibromin protein.