

20th ENS@T Scientific Symposium

Host: UniversitätsSpital Zürich Clinic of Endocrinology, Diabetology and Clinical Nutrition



30th of September- 2nd of October 2021, e- Congress





Welcome

Zürich, 15th of July 2021

Dear colleagues and friends,

it is our greatest pleasure to welcome you to the 20th Scientific Meeting of the European Network for the Study of Adrenal Tumors (ENSAT). The second time as an online meeting, it will be the first to be combined with the International Adrenal Cancer Symposium.

Following its tradition, the meeting is divided into oral presentations according to the four working groups: Pheochromocytoma/Paraganglioma (PCC/PGL), Aldosterone producing adenoma (APA) and Non-Aldosterone producing adenoma (NAPACA). To allow for a close interaction with the international ACC community, the forth disease topic (ACC) will be embedded into the program of the International Adrenal Cancer Symposium.

Based on the submitted abstracts we believe that also the current ENSAT symposium will continue to be - together with the International Adrenal Cancer Symposium - the prime event to cover all aspects of adrenal tumor related research. We appreciate your support and interest and wish you three meeting days filled with stimulating research topics.

For the local organizing committee,

Felix Beuschlein and Constanze Hantel



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Program outline

Thursday, 30.09.2021

09.00	Welcome	
09.15	NAPACA oral communications I	
10.30	Coffee break	
10.45	NAPACA oral communications II	
12.00	NAPACA flash talks	
12.30	Lunch	
13.30	Start ACC symposium	

Friday, 01.10.2021

09.00	PPGL oral communications I	
10.15	Coffee break	
10.30	PPGL oral communications II	
11.45	ENSAT general assembly (+/- coffee break)	
12.45	PPGL flash talks	
13.35	Lunch	
14.00	Start ACC symposium	

Saturday, 02.10.2021

09.00	APA oral communications I	
10.00	Coffee break	
10.15	APA flash talks II	
10.35	ENSAT award session	
11.00	PPGL working group meetings (+ Lunch) https://uzh.zoom.us/j/65018213627?pwd=cEZ5bVFDdHVkUWIrSWJxZ0FETzJ0UT09	
12.00	NAPACA working group meetings (+ Lunch) https://uzh.zoom.us/j/64167106358?pwd=VWk5QkZuZFArSVcwaVoxeUVYRXNEUT09	
13.00	APA working group meetings (+ Lunch) https://uzh.zoom.us/j/68643507591?pwd=QTBicEJsWnIJUjh4MjZJNkJjNFFDUT09	
14.00	Start ACC symposium	





Registration and Connection guidelines

The WEB CONFERENCE will be organized on ZOOM platform and the link, to be used on the day of the live broadcast, will be sent the day before the event. Prerequisite for the link acquisition is the registration to the ENSAT Scientific Symposium via the following link:

https://www.ensat2021.com/registration/

and for the International Adrenal Cancer Symposium via the link:

https://www.acc2021brescia.com/home/8th-international-adrenalcancer-symposium/registration/

The program will take place as per the timetables attached.

All the speakers and chairpersons are invited to connect 30 min before the start of the session and be present throughout their session and always coordinated by the technicians.

The chairperson will introduce live speakers / presentations and the technicians will automatically start the recordings of the presentations previously made. At the end of the session, the part of discussion begins, and chairperson and speakers go live and interact with each other. The event will always be coordinated by a technical direction that will support the meeting throughout the day.

The webinar has been structured with live parts and parts in recording. The introductions of the moderators of each session and the faculty discussions will be broadcast live. All speeches will be pre-recorded.



Local Organizing Committee

Felix Beuschlein	Constanze Hantel
Head of Department	Head of the Endocrine Research Lab
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Scientific Committee

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Marcus Quinkler, MD

EndoKrinologiepraxis am Stuttgarter Platz Berlin, Germany



Conference Program



Thursday, September 30th 2021

09.00 Welcome and online meeting instructions Felix Beuschlein and Constanze Hantel, Zürich Guillaume Assié, Paris Martin Fassnacht, Würzburg

09.15NAPACA Communications I [10 + 5 minutes each]Chairs:Cristina Ronchi, Birmingham
Martin Fassnacht, Würzburg

OC1: Inactivation of the tumor suppressor gene ARMC5 leads to alteration of cell redox response, adrenal steroidogenesis and ferroptosis.

<u>Isadora P Cavalcante</u>, Marthe Rizk-Rabin, Christopher Ribes, Karine Perlemoine, Jérôme Bertherat, Bruno Ragazzon

OC2: Integrated genomic characterization of primary bilateral macronodular adrenal hyperplasia (PBMAH) reveals molecular groups and identify KDM1A as a new gene responsible for food-dependant Cushing.

<u>Anna Vaczlavik,</u> Lucas Bouys, Florian Violon, Gaetan Giannone, Anne Jouinot, Roberta Armignacco, Isadora P. Cavalcante, Annabel Berthon, Eric Letouzé, Patricia Vaduva, Maxime Barat, Fidéline Bonnet, Karine Perlemoine, Christopher Ribes, Mathilde Sibony, Marie-Odile North, Stéphanie Espiard, Philippe Emy, Magalie Haissaguerre, Igor Tauveron, Laurence Guignat, Lionel Groussin, Bertrand Dousset, Martin Reincke, Maria C. Fragoso, Constantine A. Stratakis, Eric Pasmant, Rossella Libé, Guillaume Assié Bruno Ragazzon, Jérôme Bertherat

OC3: Risk of Mental and Sleep Disorders after the Diagnosis of Adrenal Adenomas: A Population-Based Cohort Study

<u>Dingfeng Li</u>, Sumitabh Singh, Catherine D. Zhang, Ravinder Jeet Kaur, Andreas Ebbehoj, Elizabeth J. Atkinson, Sara J. Achenbach, Nikki H. Stricker, Michelle Mielke, J Michael Bostwick, Walter Rocca, Irina Bancos

OC4: Generation and characterization of a novel inducible Zona Fasciculata-specific Cre mouse model (Cyp11b1-Cre) as a tool for zone specific adrenal studies. <u>Christina Bothou</u>, David Penton, Mirko Peitzsch, Felix Beuschlein

OC5: Should the 1mg-overnight dexamethasone suppression test be repeated in patients with benign adrenal incidentalomas and no overt hormone excess? Lakshmi Narayanan Rengarayan, G.Knowles, M.Asia, Y.S.Elhassan, W.Arlt, C.L.Ronchi, A.Prete



10.30 Coffee break

10.45NAPACA Communications II [10 + 5 minutes each]Chairs:Wiebke Arlt, Birmingham
Iacopo Chiodini, Milan

OC6: Characterization of transcriptional and miRNA based dysregulations in Cushing syndrome.

<u>Sharmilee Vetrivel</u>, Ru Zhang, Andrea Osswald, Mareen Engel, Felix Beuschlein, Alon Chen, Silviu Sbiera, Martin Reincke, Anna Riester

OC7: Steroid profiling using liquid chromatography mass spectrometry during adrenal vein sampling in patients with primary bilateral macronodular adrenocortical hyperplasia.

<u>Ru Zhang</u>, G. Rubinstein, S. Vetrivel, F. Vogel, M. Kroiss, S. Deniz, A. Osswald, T. Knösel, S. Kunz, M. Bidlingmaier, S. Sbiera, M. Reincke, A. Riester

OC8: Impaired cognition in patients with mild autonomous cortisol secretion. <u>Rohic Nathani,</u> K. Thangamuthu, S. Singh, C. Zhang, M. Suresh, A. Ebbehoj, M. Thomas, I. Bancos

OC9: Plasma steroid profiling in patients with adrenal incidentaloma.

<u>Kristina Berke.</u> G. Constantinescu, J. Masjkur, O. Kimpel, U. Dischinger, M. Peitzsch, A. Kwapiszewska, P. Dobrowolski, S. Nölting, M. Reincke, F. Beuschlein, S.R. Bornstein, A. Prejbisz, J.W.M. Lenders, M.Fassnacht, G. Eisenhofer

OC10: Method Comparison and Threshold Evaluation for Cortisol measured with CLIA and LC-MS/MS in Dexamethasone Suppression Test.

Nora Vogg, M. Kurlbaum, H. Raff, M. Fassnacht, M. Kroiß

12.00 Flash talks NAPACA [3min audio narration + 2 minutes live discussion]

Chairs: Darko Kastelan, Zagreb

FT1 A conserved role for prolactin signalling in the regulation of the sexually dimorphic adrenocortical function: insights from mouse models and clinical studies.

C. Ruggiero, B. Altieri, E. Arnold, L. Siqueiros-Marquez, M. Doghman-Bouguerra, M. Detomas, N. Durand, M. Jarjat, M. Kurlbaum, T. Deutschbein, C. Clapp, <u>E. Lalli</u>

FT2 Depression: another cortisol-related comorbidity in patients with adrenal incidentalomas and (possible) autonomous cortisol secretion.

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<u>ŠOJAT AS</u>, Dunjić-Kostić B, Marina Lj. V, Ivović M, Radonjić N.V, Kendereški A, Ćirković A, Tančić-Gajić M, Arizanović Z, Mihajlović S, Vujović S

FT3 Circadian rhythm of salivary cortisol and cortisone in adrenocortical tumors.

L. Tucci, C. Lena, M. Magagnoli, M. Mezzullo, V. Vicennati, J. Newell-Price, U. Pagotto, F. Fanelli, <u>G. Di Dalmazi</u>

FT4 Attenuation value in adrenal incidentalomas: a longitudinal study

<u>Filippo Ceccato,</u> Irene Tizianel, Giacomo Voltan, Gianmarco Maggetto, Emilio Quaia, Mattia Barbot, Filippo Crimì, Carla Scaroni

FT5 Sleep impairment in patients with mild autonomous cortisol secretion is associated with lower mood and decreased quality of life.

Karthik Thangamuthu, R. Nathani, C. Zhang, S. Singh, A. Ebbehø, M. Suresh, M. Thomas, I. Bancos

FT6 Clinical course of benign adrenal cysts – a single center retrospective study of 56 patients.

Prerna Dogra, Irina Bancos

- 12.30 Lunch break
- 13.30 Start of the ACC Symposium



Friday, October 1st 2021

09.00PPGL Communications I [10 + 5 minutes each]Chairs:Judith Favier, Paris
Rodrigo Toledo, Barcelona

OC11: Deciphering the genomic and immune profile of metastatic pheochromocytoma. <u>Bruna Calsina,</u> E. Piñeiro-Yáñez, ÁM. Martínez-Montes, C. Fustero-Torre, M. Monteagudo, E. Gil, R. Letón, S. García- Martín, N. Bechmanno, E. Rapizzi, M. Quinkler, HJ. Timmers, M. Mannelli, G. Eisenhofer, C. Montero- Conde, G. Gómez-López, A. Cascón, J. Leandro-García, F. Al-Shahrour, M. Robledo

OC12: Extracellular matrix reprogramming promotes a metastatic phenotype in SDHBdependent PPGL.

<u>Judith Goncalves</u>, Sophie Moog, Mélanie Menara, Catherine Monnot, Stéphane Germain, Anne-Paule Gimenez-Roqueplo, Judith Favier

OC13: Preclinical studies for the evaluation of targeted therapies and the identification of early non-invasive biomarkers of tumor response in Sdhb-mutated tumors.

<u>Sophie Moog.</u> Betty Salgues, Thomas Viel, Yasmin Braik-Djellas, Daniel Balvay, Gwennhael Autret, Anne- Paule Gimenez-Roqueplo, Bertrand Tavitian, Charlotte Lussey-Lepoutre, Judith Favier

OC14: ANALYSIS OF TELOMERE MAINTENANCE RELATED GENES REVEALS NOP10 AS A NEW METASTATIC-RISK MARKER IN PPGL.

Maria Monteagudo, P. Martínez, L.J. Leandro-García, A.M. Martínez-Montes, B. Calsina, A.Díaz-Talavera, R. Letón, E. Gil, M. Pérez, D. Megías, R. Torres-Pérez, S. Rodriguez-Perales, P. González, E. Caleiras, S. Jiménez-Villa, G. Roncador, C. Álvarez-Escolá, R. Regojo, M. Calatayud, S. Guadalix, M. Currás- Freixes, E. Rapizzi, N. Svenja, H. Remde, M. Fassnacht, N. Bechmann, G. Eisenhofer, M. Mannelli, F. Beuschlein, M. Quinkler, A. Cascón, M.A. Blasco, C. Montero-Conde, M. Robledo

OC15: Exploring the molecular mechanisms behind the pathogenicity of DLST variants in PPGLs.

<u>Sara Mellid.</u> J.M. Roldan-Romero, B. Calsina, M. Santos, M. Monteagudo, L.J. Leandro-Garcia, A. Diaz, A. Martinez- Montes, F. Garcia, J. Muñoz, A. Cerezo, R. Campos- Olivas, M. Robledo, A. Cascon

10.15 Coffee break

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10.30PPGL Communications II [10 + 5 minutes each]Chairs:Henri Timmers, NijmegenAnne-Paule Gimenez-Rogueplo, Paris

OC16: Indicators of disease-specific survival in patients with pheochromocytomas and paragangliomas.

<u>Christina Pamporaki,</u> T. Prodanov, L. Meuter, A.M.A. Berends, N. Bechmann, G. Constantinescu, F. Beuschlein, Martin Fassnacht, A. Prejbisz, M.N. Kerstens, H.J.L.M. Timmers, D. Taieb, M.Robledo, J.W.M. Lenders, K. Pacak, G. Eisenhofer

OC17: Development and internal validation of SGAP-score, a predictive model for postsurgical recurrence of pheochromocytoma.

<u>Fabio Bioletto,</u> M. Parasiliti-Caprino, C. Lopez, F. Maletta, M. Caputo, V. Gasco, A. La Grotta, P. Limone, G. Borretta, M. Volante, M. Papotti, M. Terzolo, M. Morino, B. Pasini, F. Veglio, E. Ghigo, E. Arvat, M. Maccario

OC18: Immune cell infiltration of phaeochromocytoma and paraganglioma's tumour microenvironment.

Nicola Tufton, J.P. Chapple, S.A. Akker

OC19: Targeting the redox balance pathway using ascorbic acid.

Margo Dona, Svenja Rohde, Maaike Lamers, Marnix Gorissen, Henri J.L.M. Timmers

OC20: Phenotypic characterization of head and neck paragangliomas: focus on tumor location.

<u>Susan Richter</u>, B. Qiu, M. Ghering, C. Kunath, C. Luth, G. Constantinescu, C. Pamporaki, N. Bechmann, L. Meuter, A. Kwapiszewska, T. Deutschbein, S. Nölting, M. Peitzsch, M. Robledo, A. Prejbisz, K. Pacak, V. Gudziol, H. J.L.M. Timmers, G. Eisenhofer

11.45 ENSAT General Assembly (+/- coffee break)

12.45 Flash talks PPGL [3min audio narration + 2 minutes live discussion]

Chairs: Elena Rapizzi, Florence

FT7 SDHB-SDHD variant type impacts phenotype and malignancy in pheochromocytoma-paraganglioma.

<u>Jean-Pierre Bayley,</u> B. Bausch, J.C. Jansen, E.F. Hensen, K. van der Tuin E.P.M. Corssmit, P.Devilee, H.P.H. Neumann

FT8 Improved Diagnostic Accuracy of Clonidine Suppression-Testing using an agerelated cut-off for Plasma Normetanephrine.

Hanna Remde, Pamporaki C, Fuß CT, Quinkler M, Masjkur J, Prejbisz A, Nölting S, Lenders J, 7, Fassnacht M, Eisenhofer G, Deutschbein T

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FT9 Expanding the tool-box to study tumorigenesis in adult heterozygous sdhb mutant zebrafish.

<u>Margo Dona</u>, Andor Veltien, Ine van Raaij, Andre Olthaar, Benno Kusters, Antonius E van Herwaarden, Tom W J Scheenen, Aswin Menke, Marnix Gorissen, Henri J L M Timmers

FT10 Differences in clinical presentation and management between pre- and postsurgical diagnoses of urinary bladder paraganglioma: is there clinical relevance? a systematic review.

Minghao Li, X. Xu, N. Bechmann, C. Pamporaki, J. Jiang, S. Pröpping, L. Liu, H. Langenhuijsen, K. Pacak, G. Eisenhofer, J. Lenders

FT11 Characterization of outgrowth processes in pheochromocytoma spheroids.

Serena Martinelli, T. Mello, F. Amore, M. Mannelli, M. Maggi, E. Rapizzi

FT12 NOVEL GERMLINE PHD2 VARIANT IN A METASTATIC PHEOCHROMOCYTOMA PATIENT IN THE ABSENCE OF POLYCYTHEMIA.

A Provenzano, M Chetta, G De Filpo, G Cantini, A La Barbera, G Nesi, R Santi, S Martinelli, E Rapizzi, M Luconi, M Maggi, M Mannelli, T Ercolinoç, <u>Letizia Canu</u>

FT13 Clinical spectrum of bladder paraganglioma: results from 53 patients.

Kai Yu, W. Young, I. Bancos

FT14 In-patient versus out-patient testing and other preanalytical considerations for use of plasma metanephrines for diagnosis of pheochromocytoma.

<u>Georg Pommer</u>, C. Pamporaki, M. Peitzsch, K. Langton, G. Eisenhofer, M. Fassnacht, H. Remde, T. Deutschbein, S. Nölting, J. Lender, H. J.L.M. Timmers, J. Deinum, L. Müller, L. Braun, S. Gruber, A. Pecori, P. Mulatero, E. Davies, A. Stell, G. P. Rossi, F. Ceccato, P. Dobrowolski, A. Januszewicz, A. Prejbisz, M. Zennaro

FT15 Safe observation of early-recurrence of genetically-determined pheochromocytomas.

Marie Puerto, M. Haissaguerre, M.-L. Nunes, H. Najah, A. Tabarin

FT16 Succinate dehydrogenase loss in a familial Von Hippel-Lindau inherited Pheochromocytoma.

<u>Aglaia Kyrilli</u>, J-L. Van Laethem, M. Pezzullo, C. Mathey, V. Lucidi, D. Germanova, M. Van Eycken, I. Vandernoot, L. Ghisdal, N. Driessens

13.35 Lunch break

14.00 Start of the ACC Symposium



Saturday, October 2nd 2021

09.00APA Communications [10 + 5 minutes each]Chairs:Maria-Christina Zennaro, Paris
Mitsuhide Naruse, Kyoto

OC21: Development of an adrenocortical cell model of calcium signalling modulation to decipher the molecular mechanisms responsible for primary aldosteronism.

<u>Bakhta Fedlaoui,</u> T. Cosentino, I. Giscos-Douriez, L. Borowski, FL. Fernandes-Rosa, C. Magnus, SE. Sternson, MC. Zennaro, S. Boulkroun

OC22: Interaction Between Wnt/β-Catenin and ACTH Signaling Pathways and Paracrine Regulation in Aldosterone Producing Adenoma.

<u>Alaa Abdellatif,</u> Kelly De Sousa, Isabelle Giscos- Douriez, Tchao Meatchi, Laurence Amar, Fabio Luiz Fernandes-Rosa, Sheerazed Boulkroun, Maria- Christina Zennaro

OC23: Zona glomerulosa derived Klotho regulates potassium-stimulated aldosterone synthesis.

Cong Tang. Y. Xie, A. Scapin, D. Lofing, D. Breault, J. Lofing, F. Beuschlein

OC24: A conserved adrenal super-enhancer encompasses blood pressure-associated intergenic SNPs in the KCNK3 locus.

C. Ruggiero, M. Doghman-Bouguerra, N. Durand, M. Jarjat, F. Chatonnet, <u>E. Lalli</u>

10.00 Coffee break

10.15 Flash talks APA [3min audio narration + 2 minutes live

discussion]

Chairs: Jacques Lenders, Nijmegen

FT17 Integration of artificial intelligence with mass spectrometry-based plasma steroid profiling: application to primary aldosteronism.

<u>Georgiana Constantinescu</u>, M. Schulze, M. Peitzsch, U. Scholl, S.R. Bornstein, J. Lenders, G. Eisenhofer

FT18 Prevalence of primary aldosteronism in patients with acute stroke: a prospective study.

Van Nguyen, Tian Ming Tu, Marlie Jane, Jovan Lai, Meifen Zhang, Troy Puar



FT19 Sublethal hyperthermia in combination with Heat Shock Protein Inhibitors as an adrenal sparing, targeted disruption of hyper functional nodules in APA's.

<u>Nathan Mullen</u>, Sarah Feely, Padraig Donlon, Grazia Cappiello, Anna Bottiglieri, Laura Farina, Matthew T. Basal, Martin O'Halloran, Punit Prakash, Michael C Dennedy

FT20 Japan Endocrine Society Clinical Practice Guideline for the Diagnosis and Management of Primary Aldosteronism 2021.

<u>Mitsuhide Naruse</u>, Takuyuki Katabami, Hirotaka Shibata, Masakatsu Sone, Katsutoshi Takahashi, Akiyo Tanabe and Members of Japan Endocrine Society Task Force of PA Guideline 2021

10.35 ENSAT Award Session

11.00 Working Group Meetings and Lunch break

- 11.00 PPGL working group meetings
- 12.00 NAPACA working group meetings
- 13.00 APA working group meetings

14.00 Start of the ACC Symposium



Abstracts: Oral Communications



OC1:

Inactivation of the tumor suppressor gene ARMC5 leads to alteration of cell redox response, adrenal steroidogenesis and ferroptosis.

<u>Isadora P Cavalcante,</u> Marthe Rizk-Rabin, Christopher Ribes, Karine Perlemoine, Jérôme Bertherat and Bruno Ragazzon

Institut Cochin, INSERM U1016, Paris, France

Background: ARMC5 is the most frequently altered gene in primary bilateral macronodular adrenal hyperplasia (PBMAH) and is considered as a tumor suppressor gene regulating cell apoptosis and adrenal steroidogenesis by mechanisms still unknown. Moreover, tumor suppressor genes play an important role in modifying intracellular redox response, which in turn regulate diverse cell signaling pathways, such as p38. The objective of this study was to investigate ARMC5 implication in cell redox response, p38 pathway and adrenocortical steroidogenesis.

Methods: In order to investigate ARMC5 levels in response to stress, H295R cells were treated with Menadione. Redox response actors, ARMC5 protein and p38 phosphorylation were investigated by western blotting and steroidogenic enzymes expression was investigated by real time-PCR. Lipid peroxidation was investigated by flow cytometry using Bodipy and cell viability was assessed with crystal violet staining.

Results: In this study we observed that ARMC5 levels are decreased by high concentrations of menadione (p<0.001). ARMC5 inactivation with siRNA modifies cell redox response by increasing superoxide dismutase 1 and peroxiredoxin 1 (p<0.05), as well as NRF2 luciferase reporter activity (p<0.001) in H295R cells. Moreover, ARMC5 inactivation decreases CYP11A1 and StAR expression (p<0.001) partially through the activation of p38 pathway.

Finally, ARMC5 inactivation decreases lipid peroxidation and cell sensitivity to ferroptosis upon inhibition of GPX4 (p<0.001), increasing cell viability.

Conclusion: Altogether, this study describes a new function of ARMC5 as regulator of the redox homeostasis in adrenocortical cells, controlling steroidogenesis and cell survival, opening new perspectives to understand adrenal Cushing pathophysiology.

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OC2:

Integrated genomic characterization of primary bilateral macronodular adrenal hyperplasia (PBMAH) reveals molecular groups and identify KDM1A as a new gene responsible for food-dependent Cushing.

<u>Anna Vaczlavik1,2,</u>Lucas Bouys1, Florian Violon1,3, Gaetan Giannone1, Anne Jouinot1,2,4, Roberta Armignacco1, Isadora P. Cavalcante1, Annabel Berthon1, Eric Letouzé5, Patricia Vaduva1,6, Maxime Barat1,7, Fidéline Bonnet1,8, Karine Perlemoine1, Christopher Ribes1, Mathilde Sibony1,3, Marie-Odile North9, Stéphanie Espiard1,10, Philippe Emy11, Magalie Haissaguerre12, Igor Tauveron13, Laurence Guignat2, Lionel Groussin1,2, Bertrand Dousset14, Martin Reincke15, Maria C. Fragoso16, Constantine A. Stratakis17, Eric Pasmant1,9, Rossella Libé1,2, Guillaume Assié1,2 Bruno Ragazzon1, Jérôme Bertherat1,2

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9 Unit of Oncogenetics, Hôpital Cochin, Assistance PubliqueHôpitaux de Paris, F-75014, Paris, France

10 CHU Lille, Department of Endocrinology, Diabetology, Metabolism and Nutrition, F-59000 Lille, France

11 Department of Endocrinology, CHR Orleans, F-45100, Orléans, France

12 Department of Endocrinology, University of Bordeaux and CHU Bordeaux, F-33000, Bordeaux, France

13 Department of Endocrinology, CHU Clermont Ferrand, F- 63000, Clermont-Ferrand, France

14 Department of Digestive and Endocrine Surgery, Hôpital Cochin, Assistance PubliqueHôpitaux de Paris, F-75014, Paris, France

15 Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, München, Germany

16 Department of Endocrinology, Adrenal Unit, University of Sao Paulo, Sao Paulo, Brazil

17 Section on Endocrinology & Genetics, Eunice Kennedy Shriver National Institute of Child Health & Human Development, National Institutes of Health (NIH), 20892, Bethesda, Maryland, USAInstitut

Introduction: PBMAH is an heterogenous disease responsible for a large spectrum of cortisol dysregulation from very mild form to overt-Cushing. ARMC5 germline genetic alterations

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currently explain 20 to 25 % of PBMAH patients, usually with a significant cortisol excess, and have never been reported in food-dependant Cushing.

Methods: RNAseq, miRNA study, SNP analysis, methylome and exome sequencing have been performed in 52 PBMAH nodules (COMETE network biobank) from 36 operated patients to understand the PBMAH heterogeneity.

Results: Integrated genomics identify 3 groups with different gene expression profiles : Gr1: ARMC5 mutated group (n=16). Gr2: PBMAH with aberrant overexpression of the GIP receptor responsible for food-dependant Cushing (n=6).G2 patients present a significantly lower fasting cortisol [p<0,01] and pathological analysis shows that eosinophil cells are more abundant in G2 PBMAH [p<0,01]. Gr3: 14 patients with less severe cortisol excess. Exome sequencing in G2 reveals KDM1A (Lysine Specific Demethylase 1) germline inactivating mutations constantly associated with a somatic loss of the KDM1A wild-type allele on 1p, leading to a loss of KDM1A expression both at mRNA and protein levels. Subsequently, KDM1A pathogenic variants were identified in 4/4 additional index cases with food-dependant Cushing.

Conclusion: This integrated genomics analysis reveals 3 PBMAH molecular groups with clinical and pathological specific features. KDM1A is identified for the first time as a new tumor suppressor gene causing adrenal tumors and is mutated in 9/10 of the food-dependant Cushing index cases of this study. This now expend the possibility of genetic screening already done with ARMC5 by the sequencing of KDM1A in PBMAH patients.



OC3:

Risk of Mental and Sleep Disorders after the Diagnosis of Adrenal Adenomas: A Population-Based Cohort Study

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Objective: Adrenal adenomas are commonly encountered in clinical practice. To date, population-based data on its impact on mental health and sleep is still lacking. Our objective was to determine the prevalence and incidence of mental and sleep disorders in patients with adrenal adenomas.

Design: Population-based cohort study, Olmsted County, MN, 1995-2017.

Methods: Patients diagnosed with adrenal adenoma were 1:1 matched with referent subject by age and sex. Patients with overt hormone excess were excluded. Main outcomes measures were prevalence and incidence of mental and sleep disorders.

Results: Of 1004 patients with adrenal adenomas, 582 (58%) were women, and median age at diagnosis was 63 years. At the time of diagnosis of adenoma, the prevalence was 32% in depression, 24% in anxiety, 12% in insomnia, and 12% in sleep-related breathing disorders. After adjusting for age, sex, BMI, smoking status, and education level, patients with adenoma demonstrated a higher risk of prevalent depression (aOR: 1.3, Cl 95% 1.1–1.6), anxiety (aOR: 1.4, Cl 95% 1.1–1.8), substance abuse disorders (aOR: 2.4, Cl 95% 1.7–3.4) than age- and sex-matched referent subjects.

Median duration of follow-up was 6.8 years (range: 0-21.9 years). During follow-up, after adjusting for confounders, patients demonstrated a higher risk of developing new depression (aHR: 1.7, CI 95% 1.4–2.2), anxiety (aHR: 1.4, CI 95% 1.1–1.7), and schizophrenia (aHR: 1.5, CI 95% 1.03–2.1). Risk of new sleep disorders was also high in insomnia (aHR: 1.4, CI 95%

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1.1–1.9), sleep-related breathing disorders (aHR: 1.4, CI 95% 1.1–1.8), parasomnias (aHR: 2.1, CI 95% 1.03– 4.2), and sleep-related movement disorders (aHR: 1.5, CI 95% 1.1–2.2).

Conclusions: Patients with adenomas are at increased risk for mental and sleep disorders, possibly due to the underlying subtle cortisol secretion. They should undergo baseline hormonal workup at the time of diagnosis, and be screened for mental well-being as an integrated part of the management.



OC4:

Generation and characterization of a novel inducible Zona Fasciculata-specific Cre mouse model (Cyp11b1-Cre) as a tool for zone specific adrenal studies.

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Hormone secreting or non-secreting adrenocortical adenomas (AA) have been found to carry somatic mutations in different genes. However, up to date, the exact molecular pathogenetic mechanism of AA is not well understood and no mouse model recapitulating the disease phenotype is available. One of the reasons is the difficulty of achieving zone-specific and temporal control of gene activation/inactivation, which is, though, a key issue in the understanding of mechanisms involved in normal and pathological development and in tumorigenesis. Here, we aimed to generate and characterize a transgenic mouse model bearing a tamoxifen-dependent Cre recombinase (Cre-ERT2) gene in the genetic locus for Cyp11b1, to facilitate targeted Zona Fasciculata-specific somatic recombination in adrenal cells. For the design, a similar strategy with the previously generated Zona Glomerulosaspecific Cre model (AS-Cre) has been followed and, consequently, introduction of the Cre-ERT2 gene results in the knock-out of the Cyp11b1 gene. Indeed, zone-specific GFP presence using a reporter mouse (mTmG) was detected only in adrenals of tamoxifen induced Cyp11b1-CreERT2 animals vs. uninduced controls. Homozygous Cyp11b1- CreERT2 (Cyp11b1-knockout) mice displayed, in accordance with previous studies, a congenital adrenal hyperplasia phenotype characterized by low survival rates, adrenal enlargement and infertility among female mice. As expected, in homozygous mice Cyp11b1 expression was absent. LC-MS/MS data showed absence of 18-oxo-cortisol, corticosterone and 18-OH-corticosterone in contrast to extremely high 11-deoxycorticosterone production (45 times vs. WT mice). On the other hand, heterozygous Cyp11b1- CreERT2 compared to WT mice displayed slightly reduced Cyp11b1 expression, reduced corticosterone production only among males (male: 36.8±10.3 vs. WT: 149.0±18.1 ng/mL, p=0.002; female: 251.3±22.8 vs. WT: 223.4±34.9 ng/mL, p=0.074) and responded sufficiently upon synacthen administration. Further characterization and tracing experiments are ongoing. Overall, our preliminary findings support the successful generation of a time and zone- specific adrenocortical mouse model, which can be used in developmental and pathophysiological adrenal studies.



OC5:

Should the 1mg-overnight dexamethasone suppression test be repeated in patients with benign adrenal incidentalomas and no overt hormone excess?

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Introduction:All patients with adrenal incidentalomas(AI) undergo 1mg- overnight dexamethasone suppression test (1mg-DST) to exclude cortisol excess (non-functioning adrenal tumours, NFAT; serum cortisol ≤50 nmol/L) or diagnose possible mild autonomous cortisol secretion (MACS; serum cortisol >50 nmol/L. Current guidelines discourage repeating hormonal work-up in patients with benign AI. However, data underpinning this recommendation are scarce.

Aim: To determine the proportion of AI patients who develop incident changes in 1mg-DST results.

Methods: Retrospective single-centre study including benign AI cases with no clinical evidence of steroid excess and at least one 1mg-DST repeated during follow-up. Patients treated with glucocorticoids or strong CYP3A4 inducers were excluded. Mann Whitney and Fisher tests were used for statistical analysis.

Results: 177 patients were included (median follow-up 21 months [range 2-44]). At baseline, 99 patients were classified as NFAT; 22 (22%) developed an abnormal 1mg-DST during follow-up. Patients converting from NFAT to MACS had higher 1mg-DST results at baseline (median cortisol 42 nmol/L [IQR 37-46] vs. 33 nmol/L [26-40], p<0.001), lower DHEAS at baseline (median 1.4 µmol/L [0.8-2.1] vs. 2.2 [1.0- 4.3], p=0.046), and lower DHEAS during follow-up than patients who remained classified as NFAT. At baseline, 78 patients were classified as MACS; 14 (18%) developed a normal 1mg-DST during follow-up. Patients converting from MACS to NFAT had smaller adrenal tumours (median diameter 20 mm [12-26] vs. 28 [22-34]), higher baseline ACTH (median 18.8 ng/L [12.5-23.5] vs. 5.3 [2.5-10.9], p<0.001), higher baseline DHEAS (median 2.9 µmol/L [1.9- 3.2] vs. 1.0 [0.6-1.9], p=0.010), and higher ACTH and DHEAS during follow-up than patients with persistently abnormal 1mg-DST.

Conclusion: Based on 1mg-DST, 20% of patients with benign AI changed their functional status during follow-up. 1mg-DST repetition may therefore be warranted and tumour size, 1mg-DST, ACTH, and DHEAS results can guide this decision.

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OC6:

Characterization of transcriptional and miRNA based dysregulations in Cushing syndrome.

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Introduction: Transcriptional regulation of gene expression by miRNAs is critical for the finetuning of adrenal stress response. However, its role in hypercortisolism has not been explored well. The study addresses this gap using adrenal samples of 3 patient groups from the German Cushing registry: Cortisol-Producing-Adenoma (CPA, n=8), Primary Bilateral Adrenal Hyperplasia (PBMAH, n=10) and controls (adrenal samples of patients with pheochromocytoma (n=8).

Methods: Next generation sequencing based miRNA profiling and associated target analyses by QPCR were performed. Transcriptomic data of RNA-Seq were analysed and validated by QPCR. For pathway mapping bioinformatic tools (R, String, KEGG, Gprofiler) were used.

Results: miRNA based NGS revealed 23 miRNAs to be differentially expressed between Cushing (PBMAH and CPA) and Controls. Of these, significantly upregulated miRNAs (n=6) were used for validation. Upregulated expression of hsa- miR-139-3p (l2fc>1.4), hsa-miR-1247-5p (l2fc>2.5) and hsa- miR-150-5p (l2fc>1.9) in PBMAH and CPA (vs Controls) could be confirmed by QPCR (p<0.05). Next, the experimentally validated targets of the individual miRNAs were selected from miRWALK and majority of the selected genes were found to be involved in steroid biosynthesis (Alox15, Cyp2b6, Cybrd1). In-vitro and QPCR analyses of the targets are in process. In case of RNA seq, PBMAH was found to have the most dysregulated genes compared to Controls and CPA (n=1248). Pathway mapping using the significantly altered genes in PBMAH gave neuronal synaptic signalling as top hits. Specifically, there was an increased expression (l2fc >5; p< 0.05) of dopamine (Drd2) and glutamate receptors (Gria4, Grin2a) in PBMAH. Validation of the pathway analysis is on-going.

Conclusion: This study identifies a miRNA-target gene network in possible steroid biosynthesis dysfunctions in adrenals of patients with Cushing's syndrome. Additionally, potential changes in neuronal synaptic pathways in PBMAH were identified.



OC7:

Steroid profiling using liquid chromatography mass spectrometry during adrenal vein sampling in patients with primary bilateral macronodular adrenocortical hyperplasia.

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Background Primary bilateral macronodular adrenocortical hyperplasia (PBMAH) is a rare cause of ACTH-independent Cushing's syndrome. The biochemical characterization of adrenal vein sampling (AVS) of patients with PBMAH hasn't been studied previously.

Objectives To characterize biochemical differences between the adrenals in patients with PBMAH and to correlate biochemical findings with clinical symptoms.

Methods For this retrospective analysis, we included 17 patients with PBMAH from the German Cushing's registry who underwent AVS. 15 steroids were quantified in the AVS samples and the peripheral blood samples using LC-MS/MS. All data were evaluated by Microsoft Excel 365, SPSS 26.0 and Graphpad prism 8.0.

Results Cortisol and DHEAS were the most dominant metabolites in AVS and in peripheral blood samples. In correlation analysis between peripheral steroid concentrations and clinical data, DHEAS showed highly significant correlation with urine free cortisol (r=0.82, p=0.001). The steroid profile of the AVS LC-MS/MS data using unsupervised hierarchical cluster analysis revealed huge interindividual differences in patients with PBMAH. Selectivity index (SI) was calculated for all steroids. DHEA and androstenedione were found to be good reference hormones with high selectivity index and without correlation to cortisol in the adrenal vein samples. After correction, adrenals were grouped based on radiological asymmetry and adrenal volume for further analyses. Corrected conversion (metabolite/ its precursor) ratios were also calculated for the different groups to identify possible differences in the steroidogenesis. Aldosterone/corticosterone ratio (mediated by the enzyme CYP11B2) was higher in smaller adrenals (p<0.05), whereas androstenedione /DHEA ratio (mediated by the enzyme HSD3B2) showed the opposite result (p<0.05).

Conclusions This study characterized the biochemical output of adrenals in AVS samples of patients with PBMAH. The analysis helped in identify key steps in steroidogenesis that are dysregulated in PBMAH.

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OC8:

Impaired cognition in patients with mild autonomous cortisol secretion.

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Background: Limited data describe cognitive deficits in patients with Cushing syndrome (CS). The impact of mild autonomous cortisol secretion (MACS) on cognition is unknown.

Aim: To determine the impact of MACS on cognitive function.

Methods: Single-center cross-sectional study of adults with MACS and age and sex-matched volunteers. MACS was defined as cortisol >1.8 mcg/dL following the dexamethasone suppression test (DST) in patients with an adrenal incidentaloma and no features of CS. Cognitive function was measured through the NIH toolbox cognition battery (7 standardized tests of attention, episodic and working memory, language, executive function, and processing speed). T-scores corrected for age, sex, education, and race were used for analysis. All patients completed the SF-36 questionnaire. The frailty index was calculated.

Results: Participants included 50 patients with MACS and 50 volunteers (Table). Compared to volunteers, patients with MACS performed worse in the domains of attention and executive function (effective allocation of one's limited capabilities in an abundance of stimuli) (median T-score of 50 vs. 46, p=0.03), cognitive flexibility (tested as the capacity to plan and monitor goal-directed activities) (median T- score of 61 vs. 55, p=0.01). The fluid composite score (includes executive function, episodic memory, working memory, and processing speed) was lower in patients with MACS (mean of 53.2 vs. 48.9, p=0.03). The total composite score was lower in patients with MACS when compared to volunteers (median T score of 50 vs. 54, p=0.06). The total composite cognitive scores in patients of MACS were associated with the Frailty Index (R2 = 0.13, p = 0.025) and SF-36 scores (R2 = 0.08, p = 0.05), but not post-DST cortisol. Conclusion: Compared to volunteers, patients with MACS demonstrate cognitive impairment, particularly in the executive domain, which closely mimics changes observed with aging. These deficits do not correlate with the degree of cortisol excess.



OC9:

Plasma steroid profiling in pafients with adrenal incidentaloma.

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Most patients with adrenal incidentaloma have non-functional lesions that do not require treatment, while others have functional or malignant tumors that require intervention. The plasma steroid metabolome may be useful to assess therapeutic need. This retrospective multicenter cross-sectional study examined the utility of plasma steroid profiling combined with metanephrines and adrenal tumor size for differential diagnosis of patients with adrenal incidentaloma. The study involved 577 patients with adrenal incidentaloma, including 19, 77, respective patients with adrenocortical carcinoma and 65. 104 312 (ACC), pheochromocytoma, primary aldosteronism (PA), autonomous cortisol secretion (ACS) and non-functional adrenal incidentaloma (NFAI). Diagnostic performance of plasma LC-MS/MS measured steroids and metanephrines were assessed for discriminating different subgroups of patients with adrenal incidentaloma. Patients with ACC were characterized by elevated plasma concentrations of 11- deoxycortisol, 11-deoxycorticosterone, 17- hydroxprogesterone, androstenedione and dehydroepiandrosterone-sulfate, whereas patients with PA had elevations of aldosterone, 18-oxocortisol and 18- hydroxycortisol. A selection of those 8 steroids, combined with 3 others (cortisol, corticosterone, and dehydroepiandrosterone) and plasma metanephrines, proved optimal for identifying patients with ACC, PA and pheochromocytoma at respective sensitivities of 83.3[66.1-100]%, 90.8[83.7-97.8]% and 94.8[89.8-99.8]% and

specificities of 98.0[96-9-99.2]%, 92.0[89.6-94.3]% and 98.6[97.6-99.6]%. With addition of tumor size, discrimination improved further, particularly for ACC (100[100-100]% sensitivity, 99.5[98.9-100]% specificity). In contrast, discrimination of ACS and NFAI remained suboptimal (70-71% sensitivity, 89-90% specificity). In conclusion, among patients with adrenal incidentaloma, the combination of plasma steroid metabolomics with routinely available plasma free metanephrines and data from imaging studies may facilitate identification of almost all clinically relevant adrenal tumors.

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OC10:

Method Comparison and Threshold Evaluation for Cortisol measured with CLIA and

LC-MS/MS in Dexamethasone Suppression Test

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BACKGROUND: The low dose dexamethasone (Dex) suppression test (DST) is the first-tier test recommended by guidelines to evaluate autonomous cortisol secretion in adrenal incidentalomas. After 1 mg oral Dex, suppression of serum cortisol below 1.8 μ g/dL (50 nmol/L) is considered to exclude hypercortisolism. While having excellent clinical sensitivity for hypercortisolism, specificity is moderate. We have shown that 100% test sensitivity can be retained while specificity is improved when adequate Dex exposure can be demonstrated (>1.8 ng/mL) and cortisol is suppressed below 2.4 μ g/dL (66 nmol/L) in a liquid chromatography tandem mass spectrometry assay.

OBJECTIVE: To compare cortisol concentrations in DST serum samples measured by automated chemiluminescence immunoassay (CLIA) vs. LC-MS/MS and to evaluate the cortisol cutoff concentration for the diagnosis of Cushing's syndrome (CS) in a large sample set.

METHODS: Cortisol was measured by CLIA (Immulite 2000 XPi, Siemens) and LC-MS/MS in 400 DST serum samples, including 100 CS patients. The optimum cutoff concentration for cortisol measurements by CLIA was evaluated with ROC analysis and Youden index in samples with CS or exclusion of CS based on standard endocrine workup.





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p<0.001). Higher cortisol concentrations were found with CLIA compared to LC-MS/MS, especially in samples with high serum cortisol. In patients with Dex >1.8 ng/mL, the optimal cortisol cutoff concentration for diagnosing CS was found to be at 2.7 μ g/dL (Youden Index: 0.973) measured by CLIA.

CONCLUSIONS: The awareness of higher cortisol measured by CLIA compared to LC-MS/MS is important for DST interpretation, as the results should be interpreted considering method- specific thresholds. By adapting the cortisol threshold from 1.8 μ g/dL to 2.7 μ g/dL cortisol for CLIA measurements, clinical test specificity for CS can be increased significantly while retaining excellent sensitivity.



OC11:

Deciphering the genomic and immune profile of metastatic pheochromocytoma

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The highly variable clinical behavior of pheochromocytomas and paragangliomas (PPGLs) hinders the management and treatment of metastatic disease. The detailed molecular characterization of metastatic PPGLs (mPPGLs) is a prerequisite to understanding the mechanisms driving metastatic behavior and propose a personalized therapeutic strategy. We performed whole-exome-sequencing and RNA-Seq in 87 paired germline-tumor and 118 tumor samples, respectively, from metastatic patients mainly. In addition, the TCGA cohort was added to all analyses. After integration of both platforms, we aimed to define the mutational, copy-number alteration (CNA), transcriptomic and immune landscapes of the entire cohort. We identified a higher mutational load, microsatellite instability and CNA burden in metastatic tumors, events also associated with TERT and ATRX alterations, as well as with progression free-survival. Even revealing a large inter- tumor heterogeneity in terms of mutated genes, we delineated an enrichment of genes related to extracellular matrix organization, cell adhesion and neuronal projection/morphogenesis. Otherwise, some recurrent arm- level CNA alterations were also identified. Transcriptional analyses exposed several biological processes deregulated in mPPGLs linked to metastatic behavior. One of them is an immunosuppressive phenotype, supported by multiple signaling cascades related to T cell activation, interferon signaling and other cytokines with anti- tumor properties, as well as, the results of an extensive characterization by deconvolution of RNA-Seq data and subsequent validation by IHC. Moreover, the classification of tumors into different subtypes according to their tumor microenvironment identified a subgroup of PPGLs characterized by a higher lymphocytic infiltration and PD-L1 expression. Although our study provides evidence of a heterogeneous nature at the mutational level of mPPGLs, it uncovers new biological processes commonly altered at the CNA and transcriptional level, such as the immunosuppressive phenotype. Moreover, the resulting data from this study are proposed to serve as a tool for future targeted genomic studies to further advance in the field of mPPGLs.

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OC12:

Extracellular matrix reprogramming promotes a metastafic phenotype in SDHB-dependent PPGL.

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Background: Pheochromocytoma and Paraganglioma (PPGL) are rare neuroendocrine tumors, characterized by a strong genetic component. Among the 20 PPGL susceptibility genes, SDHB mutations are associated with an increased metastatic risk. The extracellular matrix (ECM) is a dynamic structure of the microenvironment, mostly constituted of collagens. ECM remodeling is involved in the metastatic progression of cancer. However, its role in the metastatic potential of SDHB-dependent PPGL remains unknown.

Objective: To evaluate the role played by the ECM in the metastatic phenotype of Sdhbdeficient cells compared to Sdhd-deficient cells and WT cells.

Material and method: We analyzed the transcriptomic data from 188 PPGL included in the COMETE cohort and from immortalized murine chromaffin cells (imCC) invalidated for either Sdhb or Sdhd genes and their wild-type (WT) counterpart. We also performed matrisome experiments to analyze the composition of the ECM secreted in vitro, as well as matrix-swaps experiments followed by adhesion and migration assays. The role of pseudo-hypoxia was also studied after inactivation of HIF2a in Sdhb-/- imCC.

Results: Transcriptome analysis revealed that SDHB- mutated PPGL and Sdhb-/- imCC both display a significant overexpression of ECM genes and especially collagens, which was in accordance with the higher collagen levels displayed in the ECM secreted in vitro by Sdhb-/- cells in comparison to Sdhd-/- and WT imCC. Functional studies showed that the ECM secreted by Sdhb-/- imCC increased the adhesive and migratory properties of Sdhd-/- and WT imCC, suggesting its involvement in the metastatic phenotype associated with SDHB. Inactivation of HIF2a had a very moderate effect in the remodeling of the ECM secreted by Sdhb-/- imCC.

Conclusion: Altogether, these data highlight how SDHB- deficiency leads to a major ECM reprogramming and strongly suggest its involvement in the metastatic phenotype of SDHB-related PPGL. Unexpectedly, we also reveal that unlike other 2OG-dependent dioxygenases (HIFs PHDs or TET DNA demethylases) collagen hydroxylases are not inhibited in SDHB-mutated PPGL.



OC13:

Preclinical studies for the evaluation of targeted therapies and the identification of early non-invasive biomarkers of tumor response in Sdhb-mutated tumors

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Rationale: New targeted therapies and identification of early non-invasive biomarkers of response are urgently needed in metastatic SDHB-dependent pheochromocytoma and paraganglioma (PPGL) whose prognosis remains reserved.

Methods: We characterized by Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) and 18FDG-PET an in vivo allograft model of spontaneously immortalized murine chromaffin cells (imCC) with inactivation of the Sdhb gene. The response to several therapies (IACS (mitochondrial respiratory chain complex I inhibitor), Sunitinib (tyrosine kinase inhibitor with anti-angiogenic activity), Temozolomide (alkylant agnent) and Talazoparib (PARP inhibitor) and pharmacological inhibitors of HIF2 α (PT2385 and PT2977)) and inactivation of HIF2 α (imCC Sdhb-/- shHIF2 α) was evaluated. Multimodal images were performed, including magnetic resonance spectroscopy (1H- MRS) to monitor the level of succinate in vivo.

Results: Allografted model of Sdhb-/- imCC mimics SDHB- deficient tumors, with a highly vascularized pattern secondary to increased neoangiogenesis and a particular avidity for 18FDG. After 14 days of treatment, IACS, Sunitinib, Temozolomide and Talazoparib at high doses allow a significant reduction of tumor volume. In contrast to the tumor growth inhibition observed in Sdhb-/- shHIF2 α imCC tumors, pharmacological inhibitors of HIF2 α (PT2385 and PT2977) show no anti-tumor action in this model, alone or in combination with Sunitinib. 1H-MRS, but not DCE-MRI, enables monitoring response to Sunitinib, which is the best treatment in this model, promoting a decrease in succinate levels detected in vivo.

Conclusion: This study paves the way for new therapeutic options and reveals a potential new early biomarker of response to treatment in metastatic SDHB-dependent PPGL.



OC14:

ANALYSIS OF TELOMERE MAINTENANCE RELATED GENES REVEALS NOP10 AS A NEW METASTATIC-RISK MARKER IN PPGL

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One of the main problems we face in PPGL is the lack of molecular markers capable of predicting the development of metastases in patients. Telomere related genes such as TERT and ATRX have been recently described in PPGL supporting the association between the activation of immortalization mechanisms and disease progression. However, the contribution of other genes involved telomere preservation machinery has not been previously investigated. In this work, we aimed to analyze the prognostic value of a comprehensive set of genes involved in telomere maintenance. For this study, we collected 165 PPGL samples (97 non-metastatic/63 metastatic), genetically characterized, in which the expression of 29 genes of interest was studied by NGS. Three of the 29 genes studied, TERT, ATRX and NOP10, showed differential expression between metastatic and non-metastatic cases, and alterations in these genes were associated with a shorter time to progression, independent of SDHB-status. We studied telomere length by Q-FISH in patient samples, and in an in-vitro model. NOP10 overexpressing tumors displayed an intermediate-length telomere phenotype without ALT, and together with in-vitro results, we suggest that NOP10 has a role in telomerase-dependent telomere maintenance. In summary, we showed that NOP10 is a novel metastatic risk marker in PPGL that, in combination with alterations in TERT and ATRX. provides the strongest means of stratification in our series, independently of SDHB- mutation status. We propose to include the NOP10 immunostaining within the current battery of markers for stratifying PPGL patients to fine-tune their prognosis, thereby providing early detection of metastatic disease and ultimately better planning of treatment options.
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OC15:

Exploring the molecular mechanisms behind the pathogenicity of DLST variants in PPGLs

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Pheochromocytomas and paragangliomas, together known as PPGLs, are rare neuroendocrine tumors characterized by its high heritability. Up to 20 genes have been described to be involved in PPGL susceptibility so far, highlighting the remarkable diversity of biological pathways influencing the development of these tumors. Recently, our group identified one recurrent pathogenic mutation (p.Gly374Glu) and four additional variants of unknown significance in the DLST gene in patients with multiple PPGLs. DLST is the E2 subunit of the OGDH complex, which catalyzes the conversion of α - ketoglutarate to succinyl-CoA in the TCA cycle. Although TCA cycle enzymes are common targets of PPGL predisposing mutations, our previous study suggested that the mechanisms leading to tumorigenesis in this case could be different from the ones previously described. For this reason, the present work focuses on the study of the molecular mechanisms behind the pathogenicity of DLST variants in PPGLs. Using stable cell lines, we examined the potential consequences of mutated DLST in terms of its subcellular localization, function and affected molecular pathways. While DLST subcellular location was not altered, proteomics studies evidenced a significant decrease in the overall protein succinvlation levels in the presence of DLST alterations. Succinylation is a newly discovered post- translational modification (PTM) that consists in the transfer of the succinyl group in succinyl-CoA to protein lysine residues. This PTM provides significant chemical and structural changes to proteins, most likely influencing their function. Accordingly, the dysregulation of succinvlation observed in our cell model seems to have an impact in essential pathways within cellular metabolism. These results suggest a key role of DLST in protein succinylation and support the increasing number of publications disclosing the importance of this PTM in the development of different types of cancer.

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OC16:

Indicators of disease-specific survival in patients with pheochromocytomas and paragangliomas

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Pheochromocytomas and paragangliomas (PPGLs) exhibit an up to 20% malignancy rate. The reported 5-year overall survival rate in patients with PPGLs ranges from 65% to 85%. whereas among patients with metastatic disease it varies from 12% to 84%. Utilizing retrospective data from 989 patients with PPGLs, 312 with and 677 without metastatic disease, we examined potential clinical, biochemical and genetic predictors of survival. The mean duration of follow up was 6.8 years. Patients with metastatic disease were more often males (P<0.001) and presented more frequently with larger (P<0.001), extra-adrenal (P<0.001), multifocal (P<0.001), noradrenergic tumors (P<0.001), and at a younger age (P<0.001) compared to patients without metastatic disease. The 5-year disease-specific survival rate was 85% in patients with and 98% without metastatic disease. The median disease-specific survival was 44 years for patients without and 27 years for those with metastatic disease. Among all patients with PPGLs, multivariate analysis showed that the presence of metastatic disease (HR 14.2, 95%CI 6.981-29.081, P<0.001) was the most important independent factor of disease-specific survival, followed by extra-adrenal tumor location (HR 1.77, 95%CI 1.122-2.805, P=0.014) and younger age at time of initial tumor diagnosis (HR 1.370, 95%CI 2.097-7.393). Among patients with metastatic disease, multivariate analysis revealed that the presence of synchronous metastases (HR 8.369, 95%CI 4.077-17.224, P<0.001) was the most important independent factor of disease-specific survival, followed by older age at time of initial tumor diagnosis (HR 8.21, 95%CI 17.014, P<0.001), higher plasma methoxytyramine concentrations (HR 6.157, 95%CI 1.158-3.482, P=0.0013), presence of SDHB mutation (HR 2.012, 95%CI 1.151-3.159, P=0.014) and larger size of the initial tumor (1.748, 95%CI 1.017-3.005, P=0.040). In conclusion, differences in disease presentation among patients with

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PPGLs are important to consider and indicate the need for an individualized approach to the management and follow-up of patients with PPGLs.



OC17:

Development and internal validation of SGAP-score, a predictive model for postsurgical recurrence of pheochromocytoma

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Background. Various features have been identified as predictors of relapse after complete resection of pheochromocytoma, but a comprehensive multifactorial model for recurrence risk prediction is lacking. The aim of this study was to develop and internally validate an integrated predictive model for post-surgical recurrence of pheochromocytoma.

Methods. Baseline and follow-up data from 177 patients with radically operated pheochromocytomas were retrospectively collected from nine referral centers in north-western Italy. Supervised regression and machine-learning techniques were used for model development.

Results. Variables independently associated with recurrence were age at diagnosis (HR=0.97, 95%CI=0.94-0.99), tumor size (HR=1.01, 95%CI=1.00-1.02), positive genetic testing (HR=5.14, 95%CI=2.10-12.55) and PASS (HR=1.16, 95%CI=1.04-1.29). After the application of a dichotomization algorithm, optimal cut-points retrieved for continuous variables were \leq 35 years for age at diagnosis, \geq 51 mm for tumor size and \geq 3 for PASS. A novel prognostic score was thus created, on an 8-point scale, by assigning 3 points for positive genetic testing, 3 points for PASS \geq 3, 1 point for tumor size \geq 51 mm and 1 point for age at diagnosis \leq 35 years. The overall model showed a higher accuracy than any of the single predictors alone; internal validation was obtained by bootstrapping techniques, which estimated a modest optimistic bias of 7.9%. A 3-class clustering was proposed for clinical application: low-risk-class (0-2 points), with a recurrence-free-survival of 100% both at 5 years and 10 years; intermediate-

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risk-class (3-4 points), with a recurrence-free-survival of 89% at 5 years and 84% at 10 years; high-risk-class (5-8 points), with a recurrence-free- survival of 70% at 5 years and 37% at 10 years.

Conclusions. We proposed the first multifactorial model for post-surgical pheochromocytoma recurrence prediction, derived by the integration of genetic, histopathologic and clinical data. We developed a practical scoring system for recurrence risk stratification, which may be of value for a comprehensive tailoring of post-surgical follow-up.



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OC18:

Immune cell infiltration of phaeochromocytoma and paraganglioma's tumour microenvironment.

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Introduction: Solid tumours are complex tissues consisting not only of tumour cells but other surrounding cells, including various subtypes of immune cells. The combination of these tumour and stromal cells together comprise the tumour microenvironment (TME). Emerging evidence suggests that the TME plays a critical role in tumourigenesis, initiation, growth and metastasis. The TME appears to be highly specific to each tumour type and tumour-infiltrating immune cells have been identified in numerous solid tumours and found to correlate to clinical outcomes. Little is known about the TME in phaeochromocytomas and paragangliomas (PPGLs).

Method: Histopathological analysis of 65 PPGLs and 20 normal medulla tissue samples was undertaken to assess the infiltration of macrophages (CD68+, CD163+, HLADR3+), lymphocytes (CD3+) and neutrophils (neutrophil elastase+) and the association to other pathological and clinical factors.

Results: There was a higher infiltrate of macrophages, lymphocytes and neutrophils in both benign and malignant PPGLs compared to normal medulla, with a higher concentration of M2 macrophages in malignant PPGLs compared to benign ones. There was a positive correlation between Ki67 index and macrophage infiltration, as well as a positive correlation between macrophage and lymphocyte infiltration with increasing tumour size. There was a higher macrophage infiltration, but lymphocyte depletion in functional PPGLs compared to non-functional PPGLs. The immune cell infiltration did not differ based on the location of the PPGL or the underlying genetic diagnosis.

Conclusions: Despite the relatively small sample size and heterogenous tumour group, preliminary data suggests that the tumour immune signature differs dramatically from normal chromaffin tissue, suggesting PPGLs do recruit immune cells into their TME, and this may be influenced by hormone production. This data also suggests that there may differences in the immune signature between benign and malignant PPGLs which could potentially have implications for earlier diagnosis for tumours with malignant potential and new treatment options.

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OC19:

Targefing the redox balance pathway using ascorbic acid in sdhb zebrafish mutant larvae

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Background: Phaeochromocytomas or paragangliomas (PPGLs) caused by mutations in the β -subunit of the succinate dehydrogenase (SDHB) have the highest metastatic rate, for which effective systemic therapy is lacking. Therapy development is severely hampered by the limited availability of suitable in vivo models. We previously generated a sdhbrmc200 zebrafish mutant and revealed similar metabolic characteristics as observed in human SDHB tumours [1]. Biochemical analysis revealed decreased mitochondrial complex-II activity and significant succinate accumulation in homozygous sdhbrmc200 larvae1.Zebrafish models are widely recognized as powerful tools in compound screens and served to discover compounds that progressed into clinical trials.

Aim: Investigate the potential of the sdhbrmc200 zebrafish model to study SDHB-associated PPGLs using a drug screening approach.

Results: One possible molecular mechanism of SDHB- associated tumorigenesis originates in an overproduction of reactive oxygen species (ROS) due to mitochondrial dysfunction. Redox imbalance is one of the key features of cancer initiation and progression. Vitamin C has already been shown to act as anti-cancer agent in several clinical trials for various types of cancer. We identified increased basal ROS levels in homozygous sdhbrmc200 larvae. Using a semi high-throughput drug screening, the effectiveness of different dosages of antiand pro-oxidant vitamin C were assessed evaluating differences in phenotype, ROS levels, locomotor activity, and survival. Low-dosage levels of vitamin C induced a decrease of ROS levels and an increase in locomotor activity but no significant effects on lifespan. In contrast, high-dosage levels of vitamin C shortened the lifespan of the homozygous sdhbrmc200 larvae, while not affecting the lifespan of heterozygous and wild-type siblings.

Conclusion: We validated the sdhbrmc200 zebrafish model as a powerful drug screening tool to provide valuable insights into pathomechanisms, which may lead to novel therapeutic targets and therapy development in the future.

Funding: Paradifference foundation

1 Dona, M. et al. Loss of sdhb in zebrafish larvae recapitulates human paraganglioma characteristics. Endocr Relat Cancer, doi:10.1530/ERC-20-0308 (2020).

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OC20:

Phenotypic characterization of head and neck paragangliomas: focus on tumor location.

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Head and neck paragangliomas (HNPGLs) are tumors of parasympathetic origin and often associated with syndromic presentation due to germline mutations in one of the succinate dehydrogenase (SDHx) subunit genes or in assembly factor AF2. They can occur at different locations and occasionally produce catecholamines. In a multicenter study including 244 patients with and 71 patients without HNPGLs, we investigated whether HNPGL location is associated with specific phenotypic features, including catecholamine production and the presence of SDHx mutations. Plasma methoxytyramine was elevated in 25.2% of patients with HNPGL compared to 1.4% of patients without HNPGL. Diagnostic sensitivity increased to 29.4% with inclusion of plasma normetanephrine, while specificity dropped to 92.9%. Plasma normetanephrine was elevated in 8.8% of HNPGL patients. Increases in plasma metabolites did not relate to HNPGL location or SDHx mutation status, but were associated with tumor volume (plasma methoxytyramine, r squared=0.08, p=0.003). SDHx mutation and also sex showed complex relationships to locations of HNPGLs. In particular, 88% of jugulotympanic HNPGLs arose in women, among whom only 24% had SDHx mutations. In comparison, 55% of jugulotympanic HNPGLs in men were associated with SDHx mutations. Compared to other locations, jugulotympanic HNPGLs were rarely bilateral, had a smaller size, and were less often metastatic. Our findings confirm that increases in plasma methoxytyramine and/or normetanephrine occur in a significant proportion of patients with HNPGLs, but that this has no relevance to tumor location and SDHx status. More importantly, our findings support sexdependent differences in the development of SDHx-negative HNPGLs, and are relevant to the sex-specific diagnosis, management and outcomes of these tumors.



OC21:

Development of an adrenocortical cell model of calcium signalling modulation to decipher the molecular mechanisms responsible for primary aldosteronism.

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Primary aldosteronism (PA) is the most frequent form of secondary arterial hypertension. The identification of germline or somatic mutations in different genes coding for ion channels (KCNJ5, CACNA1D, CACNA1H and CLCN2) and ATPases (ATP1A1 and ATP2B3) defines PA as a channelopathy. These mutations promote increased intracellular calcium concentrations and activation of calcium signalling, the main trigger for aldosterone biosynthesis. The objective of our work was to elucidate the molecular mechanisms underlying the development of PA by modulating calcium signalling using chemogenetic tools. We have developed an adrenocortical H295R S2 cell line stably expressing a chimeric ion channel receptor generated by fusing the mutated extracellular ligand-binding domain of the α 7 nicotinic acetylcholine receptor to the ion pore domain of the serotonin receptor 5HT3a (a7-5HT3). The mutations introduced in the ligand-binding domain allow to use synthetic ligands (PSEM-817) to activate the receptor. This activation results in sodium entry into the cell. The cell line was characterized in terms of intracellular calcium concentrations, cell proliferation, aldosterone production and steroidogenic gene expression. Treatment of α 7-5HT3 expressing cells with increasing concentrations of PSEM-817 (from 10-9 to 10-5 M) induced a significant increase in intracellular calcium concentrations, greater than that observed in response to potassium treatment (12 mM). This stimulation of calcium signalling did not affect cell proliferation, but was responsible for an increase in CYP11B2 expression and aldosterone production after 24h of treatment. However, while increased intracellular calcium concentrations were observed from 10-8 M of PSEM, CYP11B2 expression and aldosterone production were only affected from 10-7 M, suggesting a dose dependent effect. These cell lines, in which we can modulate the intracellular calcium concentration «on demand», are a useful tool for a better understanding of the alterations of intracellular ion balance and calcium signalling in the pathophysiology of PA.



OC22:

Interaction Between Wnt/β -Catenin and ACTH Signaling Pathways and Paracrine Regulation in Aldosterone Producing Adenoma

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Primary aldosteronism (PA) is the most frequent form of secondary arterial hypertension and is caused in the majority of cases by an aldosterone producing adenoma (APA) or bilateral adrenal hyperplasia. Different somatic mutations have been identified in APA and in other aldosterone producing structures, which can be distinct within the same adrenal, suggesting multiple mechanisms underlying APA development. Also, APA show important cellular and molecular heterogeneity which may be due to interaction of different signaling pathways involved in adrenal cortex cell differentiation and function.

The aim of this study was to investigate the role of Wnt/ β - catenin and ACTH signaling as well as elements of paracrine regulation of aldosterone biosynthesis and vascularization in the development of APA and aldosterone producing cell clusters (APCC) and their relationship with intratumoral heterogeneity and mutational status.

We performed immunohistochemistry and multiplex immunofluorescence (CYP11B2, CYP17A1, β -catenin, MC2R, pCREB, Tryptase, S100, CD34) multispectral image analysis on 11 adrenals with APA and one with micronodular hyperplasia from patients with PA. Immunofluorescence revealed abundant mast cells and a dense vascular network in APA, independent of mutational status. Within APA, mast cells were localized in areas expressing CYP11B2 and were rarely co-localized with nerve fibers, suggesting that their degranulation is not controlled by innervation. In these same areas, β -catenin was activated, suggesting a zona glomerulosa cell identity. In heterogeneous APA with KCNJ5 mutations, MC2R and VEGFA expression was higher in areas expressing CYP11B2. A similar pattern was observed in aldosterone producing cell clusters (APCC), with high expression of CYP11B2, activated β -catenin, and numerous mast cells.

Our results suggest that aldosterone producing structures in adrenals with APA share common molecular characteristics and cellular environment, despite different mutation status, suggesting common developmental mechanisms

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OC23:

Zona glomerulosa derived Klotho regulates potassium- stimulated aldosterone synthesis

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Klotho (KI), initially identified as an antiaging gene, plays a critical role in the regulation of renal and adrenal dependent fluid homeostasis. A previous study reported that haplodeficiency of KI in mice resulted in increased aldosterone synthase (CYP11B2) expression, elevated plasma aldosterone and high blood pressure. This phenotype was presumed to result from diminished KI expression in zona glomerulosa (zG) of the adrenal. To examine whether KI expressed in zG is indeed a critical regulator of aldosterone synthesis, we generated a tamoxifen-inducible, zG-specific mouse model of KI deficiency by crossing KI-flox mice with Cyp11b2-CreERT mice (zG-KI-KO). Tamoxifen-treated Cyp11b2-CreERT animals (zG-Cre) served as controls. Rosa26-mTmG reporter mice were used for Cre-dependent lineagemarking. Two weeks after tamoxifen induction, the specificity of the zG- Cre line was verified using immunofluorescence analysis to show that EGFP expression was restricted to the zG. RNAScope[™] in situ hybridization revealed a 65% down- regulation of KI mRNA expression in zG of zG-KI-KO mice at 12-weeks of age compared to control mice. Despite this, zG-KI-KO mice under basal conditions exhibited no difference in adrenal mRNA expressions of Cvp11b2 and its relevant transcription factors compared to control mice independent of sex. Interestingly, primary adrenal cells of zG-KI-KO mice showed greater response to potassiumstimulated Cyp11b2 expression compare to that of zG-Cre mice. These results suggest that zG-derived KI is able to regulate potassium- stimulated instead of baseline aldosterone synthesis. Further studies are required to investigate the function of adrenal KI in potassiumstimulated aldosterone synthesis in vivo and the underlying mechanism.



OC24:

A conserved adrenal super-enhancer encompasses blood pressure-associated intergenic SNPs in the KCNK3 locus

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Enhancers are distal regulatory elements that control tissue- specific gene expression. More than 60% non-coding SNPs associated with human disease identified by genome-wide association studies (GWAS) are localized in enhancer regions. We performed H3K27ac epigenomic profiling by high-throughput sequencing (ChIP-seq) to map the distribution of active enhancers in mouse adrenal at E18.5 and at post-natal P1, P2 weeks and P12 weeks in both sexes. A subset among the identified enhancers has the features of super-enhancers (SE), which are strongly enriched with active chromatin markers. Super-enhancers are known to regulate developmental and tissue-specific programs and often encompass disease-associated genomic loci.

Biosynthesis of aldosterone, a key hormone produced by the adrenal cortex involved in salt excretion, plasma volume and blood pressure (BP) regulation, stands out as one of the most significantly enriched biochemical pathways associated to mouse and human adrenal SE. We intersected the lists of genes identified by GWAS studies to be implicated in the regulation of BP, genes with expression enriched in the adrenal gland and genes associated with adrenal SE. Four genes (CYP11B2, CYP17A1, KCNK3 and KCNQ1) were common among those three datasets. While no intergenic BP SNP was found associated with CYP11B2, CYP17A1 and KCNQ1, four intergenic SNPs were localized in a conserved SE upstream the KCNK3 gene, encoding the K2P3.1 (TASK-1) potassium channel: rs1275984, rs1275985, rs1275986 and rs1275988. Those SNPs are associated with very high significance to mean, systolic and diastolic BP values. Interestingly, rs1275988 is in high linkage disequilibrium with rs2586886, an intronic KCNK3 SNP significantly associated with plasma aldosterone levels in a multiethnic cohort (1). A strong link between Kcnk3 and adrenal aldosterone production has been validated in animal models (2, 3). These data suggest that transcriptional control of KCNK3 expression in the adrenal gland through its associated SE plays an important role in BP regulation.



Abstracts: Flash Talks



FT1:

A conserved role for prolactin signalling in the regulation of the sexually dimorphic adrenocortical function: insights from mouse models and clinical studies.

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Many reports exist about different adrenal functionality and propensity to develop disease in the two sexes. To get deeper insight into the mechanisms underlying the sexually dimorphic adrenal function, we performed an analysis of the mouse adrenal transcriptome by RNA-seq at different ages in both sexes. We highlighted a core sexually dimorphic gene expression program in the adult mouse adrenal gland, which is conserved in the rat. Among the genes with higher expression in the female adrenal, PRLR, encoding the receptor for the pituitary hormone prolactin (PRL), also has sexually dimorphic expression in the human adrenal gland. All Prir isoforms were up-regulated in the female adrenal compared to male at P12, but not P2 weeks of age. Adrenal gland weight was significantly reduced in adult female, but not male, Prlr -/- mice compared to wild-type. Female Prlr -/- mice also had significantly reduced circulating corticosterone levels and a trend toward an increased ACTH/corticosterone ratio. To assess the role of PRL in adrenal function in humans, we compared circulating adrenal steroid hormone levels in patients with PRL- secreting (PRLA) and non-functioning pituitary adenoma (NFPA) matched for sex, age, BMI, tumour size and with normal circulating ACTH levels. DHEAS levels were significantly and selectively higher in patients with PRLA compared to NFPA. In the PRLA group men had higher circulating PRL levels than women and the PRL/DHEAS ratio was significantly higher in men than in women. Therapy with dopamine agonists significantly reduced DHEAS levels in patients with PRLA. In conclusion, modulation of adrenal steroid secretion by PRL may contribute to the positive effects of physiological concentrations of this hormone on metabolic homeostasis in basal conditions and under stress, while its deregulation in hypo- and hyperprolactinemic states may play an important role in the clinical manifestations of PRL deficiency or excess.

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FT2:

Depression: another cortisol-related comorbidity in patients with adrenal incidentalomas and (possible) autonomous cortisol secretion.

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Purpose: Hypercortisolism is associated with a high prevalence of depression and impaired health-related quality of life (QoL). According to the available literature, studies examining the depression risk in patients with adrenal incidentalomas (AI), nonfunctioning and the ones with (possible) autonomous cortisol secretion ((P)ACS) are scarce. The aim of this observational, case–control study was to screen patients with nonfunctioning adrenal incidentalomas (NAI) and the ones with (P) ACS for depression and to assess their QoL.

Methods: The total studied group consisted of 92 subjects— 26 with NAI, 34 with (P)ACS and 32 age-matched healthy controls (HC). To screen for depression, we used the Beck Depression Inventory-II (BDI-II) and to assess the QoL, we used the Short-Form 36 Health Survey (SF-36).

Results: Patients with (P)ACS had significantly higher BDI-II scores and substantially lower QoL than patients with NAI or HC. Midnight cortisol level was the most significant predictor of BDI-II and SF-36 score. The receiver operating characteristic curve analysis demonstrated that a midnight cortisol value of 86.95 nmol/l had a high sensitivity (82.8%) and high specificity80%) for detection of mild depression in patients with (P)ACS (Figure 1).



Conclusion: Screening for depression and QoL assessment should become an integral part of clinical evaluation in patients with (P)ACS.

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FT3:

Circadian rhythm of salivary cortisol and cortisone in adrenocortical tumors

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Background. A few studies investigated the circadian rhythm of salivary cortisol and cortisone in patients with adrenal incidentalomas with autonomous cortisol secretion (ACS), providing contrasting results.

Aim. To investigate the circadian profiles of salivary cortisol and cortisone in patients with adrenal tumors.

Methods. We enrolled 79 subjects: consecutive patients with adrenal tumors (non-secreting, n=15 and with ACS, n=27), normal volunteers and subjects with hormonal tests excluding Cushing's syndrome (CS) or endocrine hypertension included as controls (n=28), 9 patients with CS. Saliva samples were collected at 10 time-points throughout a day (7:00, 7:15, 7:30, 10:00, 12:30, 14:00, 16:00, 19:30, 21:00, 23:00). Cortisol and cortisone were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a validated in-house method.

Results. Patients with CS showed higher levels of salivary cortisol and cortisone at each time point from 10:00. After age-adjustment, higher hormonal levels in ACS vs controls at 10:00 (cortisol P=0.048, cortisone P=0.017), 12:30 (cortisone P=0.008), 19:30 (cortisol P<0.001, cortisone P<0.001), 21:00 (cortisol P=0.009, cortisone P=0.001), and 23:00 (cortisone P=0.025) were detected. Cortisol and cortisone at 19:30 showed significant differences among groups (P<0.05 for all comparisons), and had the highest correlation with post-DST cortisol (B=0.668, P<0.001 and B=0.706, P<0.001, respectively) than hormonal levels at the remaining time points. ROC curve highlighted a good accuracy for cortisone at 19:30 in identifying ACS (area under the curve-AUC 0.818, P<0.001, sensitivity 81.5%, specificity 77.5%). AUC for salivary cortisone in the late afternoon-evening (16:00 to 23:00) showed significant differences among groups (CAR) at 15 and 30 minutes was not different among groups. Cosinor analysis highlighted a circadian rhythm for all groups except CS. Conclusion. Patients with ACS showed a circadian rhythm of salivary cortisol and cortisone, with higher exposure to cortisol during late afternoon-evening than NS and controls.



FT4:

Attenuation value in adrenal incidentalomas: a longitudinal study.

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Context: A tendency to grow has been reported in adrenal incidentalomas. However, long-term data regarding attenuation value, a measure of lipid content, are not available.

Objective: Describe diameter (mm) and attenuation value (Hounsfield units, HU) with computed tomography (CT) in adrenal incidentalomas during follow-up.

Design: Longitudinal from 2002 to 2020. Setting: Referral University-Hospital center.

Patients: 277 patients with 355 different cortical adenomas (baseline group), 181 patients with 234 adenomas (follow-up cohort, 12-175 months).

Main Outcome Measure: CT modification according to endocrine function: autonomous cortisol secretion (ACS) if cortisol >50 nmol/L after 1-mg dexamethasone test (DST). Results: At baseline CT diameter was 18.7 mm and attenuation value 0.8 HU (higher in ACS, 66 cases >10 HU), without modification in early imaging (12-36 months). The size increased over time (r=0.289), achieving the largest differences after at least 60-months of follow-up (diameter +2 mm and attenuation value -4 HU), combined with a reduction in the attenuation value (r=-0.195, especially in patients with ACS). Lipid-poor adenomas (>10 HU) presented a reduced cortisol suppression after 1-mg DST, an increase in size and the largest decrease in attenuation value during follow-up. Univariate analysis confirmed that larger adenomas presented reduced suppression after DST and increase in size during follow-up.

Conclusions: Growth is clinically modest if adrenal incidentaloma are selected with benign criteria: a 5-year CT follow-up is reasonable, especially in ACS. Mean density is increased in patients with ACS and reduces during follow-up, suggesting an increase in lipid content.



Figure: bar chart depicting Δ diameter (panel a) or Δ attenuation (panel b) in patients with nonfunctioning adrenal incidentaloma (light gray bar) or ACS (dark gray bar) according to follow-up short, intermediate or long-term (in months).



FT5:

Sleep impairment in patients with mild autonomous cortisol secretion is associated with lower mood and decreased quality of life.

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Background: Patients with mild autonomous cortisol secretion (MACS) may have impaired sleep due to nocturnal hypercortisolism, impacting the patients' mood and quality of life (QoL).

Objective: To determine the MACS on sleep, mood, and quality of life.

Methods: Single-center cross-sectional study of adults with MACS and sex- and age-matched volunteers without adrenal disorders. MACS diagnosis was made based on abnormal post-1 mg dexamethasone suppression test (DST) cortisol >1.8 mcg/dL in patients with an adrenal adenoma, and absence of overt features of hypercortisolism and other adrenal disorders. Mood was measured using Beck Depression Inventory (BDI), sleep was assessed using the Pittsburgh Sleep Quality Index (PSQI) survey, and QoL was assessed through the short form 36(SF 36) questionnaire. Results: BDI, SF-36, and PSQI surveys were administered in 51 consecutive patients with MACS and 51 volunteers (59% women, median age of 59.5, range 37-88 years). Compared to volunteers, patients had lower QoL (median total SF36 score of 46.9 (8.0-95.6) vs 90.6 (58.0-100), p<0.0001), lower mood (median BDI score of 10 (0-15) vs 2 (0-50), p<0.0001), and impaired sleep (median global PSQI of 9(2-20) vs 3(1), p=0.0001). Global PSQI score was associated with a higher BDI score (R2 0.38, p=0.0001) and a lower total SF36 score (R2 0.41, p<0.0001). In patients with MACS, post-DST cortisol was not associated with BDI, SF-36, and PSQI scores.

Conclusions: Patients with MACS have impairment in sleep that is associated with lower mood and QoL. As post-DST cortisol was not associated with the observed impairment in sleep, mood, and QoL, duration rather than the degree of MACS may play a more critical role.

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FT6:

Clinical course of benign adrenal cysts – a single center retrospective study of 56 patients.

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Introduction: Benign adrenal cysts (BACs) are rare lesions of the adrenal. Scarce data are available to guide management.

Objective: To describe the presentation and outcomes of BACs.

Design: Single-center retrospective study, 1995-2018.

Patients Adult patients with radiologically and/or histologically confirmed BACs.

Results: In 56 patients included in the study (median age of 43.9 years (range 15-83), 34 (61%) women), BACs were diagnosed incidentally in 34 (75%) and, in 11 (20%) due to mass effect. Median size was 5.1 cm (range 0.5-20) and only 1 patient had bilateral BACs. On imaging, most BACs were round/oval (89%) lesions without vascular enhancement (98%), with calcifications in 66%. During a median imaging follow up of 18.5 months, most BACs were stable in size (median delta size of 1 mm (-5 to 105), median growth rate of 0.01 mm/year (-8 to 1.5). When compared to BACs < 5 cm, BACs > 5 cm were more likely to present at younger age (36.5 vs 50.5 years, p = 0.01), but did not demonstrate any differences in enlargement during imaging follow up. Adrenalectomy was performed in 29(51%) patients (laparoscopic in 79% and open in 21%); 1 patient was treated with cystectomy. Younger patients were more likely to be treated with adrenalectomy (median age of 37 vs 50 years, p value= 0.02), however, no differences in surgical management were found in patients with larger BACs or the ones presenting with mass effect. Following adrenalectomy, none of the patients had cyst recurrence during follow up.

Conclusion: BACs present as large oval lesions without enhancement, and frequently with calcifications on imaging. Unlike other benign adrenal masses, the proportion of patients presenting with mass effect symptoms is higher, likely due to BACs large size. Younger patients are more likely to demonstrate large BACs and to be treated with adrenalectomy.

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FT7:

SDHB-SDHD variant type impacts phenotype and malignancy in pheochromocytomaparaganglioma

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Background: In a recent study we found strong and specific genotype-phenotype associations for SDHD variants. In the present study we zoom in on the genotype-phenotype associations of SDHB gene variants, considering the impact of individual gene variants on disease risk and risk of malignancy. We also discuss the implications for the biology and pathogenesis of PPGL/HNPGL.

Methods: We analysed two large independent datasets, including a total of 448 PPGL and HNPGL patients, and studied the association of missense or truncating SDHB variants with tumour incidence, age-of-onset and malignancy risk using binomial testing and Kaplan-Meier analysis.

Results: Truncating SDHB variants were significantly and consistently more common in PPGL patients compared to missense variants, by a 20 percentage point margin. Malignancy was also significantly more common in truncating vs. missense variant carriers. No overall differences in age of PPGL onset were noted between carriers of the two variant types, although some individual variants may differ in certain cases. Missense variants were marginally overrepresented amongst HNPGL patients, but the difference was not statistically significant.

Conclusions: SDHB truncating variants convey an elevated risk for development of both PPGL and malignancy compared to missense variants. These results further support earlier robust associations between truncating variants and PPGL, and also suggest that the two variant types differ in their impact on complex II function, with PPGL/HNPGL tissues displaying differing sensitivities to changes in complex II function.



FT8:

Improved Diagnostic Accuracy of Clonidine Suppression-Testing using an age-related cut-off for Plasma Normetanephrine.

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Context: Borderline elevations of plasma normetanephrine (NMN) are frequent among patients with suspected pheochromocytoma/paraganglioma (PPGL). Clonidine suppression testing (CST) is recommended to distinguish patients with from those without PPGL. Data on diagnostic accuracy of CST is scarce.

Objective: To evaluate diagnostic accuracy of CST in patients with borderline NMN elevations.

Design: Post-hoc analysis of prospectively collected data. Plasma NMN levels during CST were measured by liquid chromatography tandem mass spectrometry. Receiver operating characteristics (ROC) analysis was performed to identify optimal cut-off values.

Setting: Six European reference centers.

Patients: 89 patients with suspected PPGL and with borderline NMN elevations upon screening where included. During follow-up, PPGL was confirmed in 16 and excluded in 73 cases.

Interventions: Plasma NMN was measured before and 180 minutes after oral clonidine.

Main Outcome Measures: NMN levels and percentage decrease of plasma NMN at 180 minutes.

Results: More than half of the patients with positive screening results demonstrated normal plasma NMN levels at baseline. If published diagnostic criteria for CST (i.e., NMN ≥112 ng/l and NMN-suppression < 40%) were applied, sensitivity of 88% (CI 61-98%) and specificity of 97% (CI 90- 100%) were observed. An improved cut-off for plasma NMN 180 minutes after clonidine was established at 80% of the age-related upper limit of normal (ULN), resulting in





a sensitivity of 94%, and a specificity of 97%. False negative CST results occurred in two patients with small PPGL. Conclusions: This first large study in patients with suspected PPGL and slightly elevated NMN confirmed the diagnostic accuracy of CST. The application of an adapted cut-off further improved sensitivity.

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FT9:

Expanding the tool-box to study tumorigenesis in adult heterozygous sdhb mutant zebrafish

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Background: Patients with mutations in the β -subunit of the succinate dehydrogenase (SDHB) have the highest risk to develop incurable malignant phaeochromocytomas and paragangliomas (PPGL). Progress in clinical therapy development is hindered by limited possibilities to test new therapeutic strategies in vivo. We previously generated a sdhbrmc200 zebrafish mutant and discovered similar metabolic characteristics as observed in human SDHB tumours[1]. Biochemical analysis revealed decreased mitochondrial complex-II activity and significant succinate accumulation in homozygous sdhb larvae, although no tumour formation was identified in these 10 days-old larvae. Aim: To develop a tool box for tumour screening in adult heterozygous sdhb mutant zebrafish.

Results: Multiple functional read-outs were designed to monitor tumour development. First, we optimized pre- analytic conditions for LC-MS measurements of catecholamines and metanephrines in fish lysates and in the water in which fish were cultivated, the latter as a reflection of their urinary secretion, to serve as biomarkers for presence of PPGLs as in the human situation. Second, we developed a magnetic resonance imaging (MRI) protocol for in vivo imaging of adult zebrafish to localize tumours and monitor their growth over time. Third, we optimized histological preparation and analysis of adult zebrafish to screen for cell proliferation in relevant tissues and compare between heterozygous sdhb mutants and wild-type siblings. Conclusion: We have created a comprehensive toolbox for tumour detection in our sdhbrmc200 zebrafish model.

Heterozygous mutants are currently being screened to study whether they can serve as a model for SDHB-related PPGL.

Funding: Paradifference foundation

1 Dona, M. et al. Loss of sdhb in zebrafish larvae recapitulates human paraganglioma characteristics. Endocr Relat Cancer, doi:10.1530/ERC-20-0308 (2020).



FT10:

Differences in clinical presentation and management between pre- and postsurgical diagnoses of urinary bladder paraganglioma: is there clinical relevance? a systematic review

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Purpose: Paraganglioma of the urinary bladder (UBPGL) is a rare neuroendocrine tumor diagnosed in many patients only after surgery. We assessed clinical clues relevant to presurgical diagnosis and clinical consequences in patients with a missed presurgical diagnosis of UBPGL.

Materials and Methods: Case reports describing a UBPGL (published from 1-1-2001 and 13-1-2021) were identified in Pubmed. Two authors independently performed data extraction and assessed data quality according to the PRISMA guideline. Patients were divided into two groups: UBPGL diagnosis before and after surgery.

Results: We included 177 articles reporting 194 cases. In 90 (46.4%) patients the UBPGL was diagnosed before and in 104 (53.6%) after surgery. In presurgically diagnosed UBPGL, hypertension and catecholamine-associated symptoms were 2-3 fold (p<0.001) more frequent than in postsurgically diagnosed patients whereas hematuria was 2-fold (p=0.003) more prevalent in those with postsurgical diagnosis.

Hypertension was an independent factor for presurgical biochemical testing (OR 4.45, 95%Cl 1.66-11.94) while hematuria (OR 0.23, 95%Cl 0.09-0.60) was an independent factor for not performing presurgical biochemical testing. Most patients diagnosed after surgery were not pretreated with alpha-adrenoceptor blockade (95.2%), underwent more frequently transurethral resection instead of cystectomy (70.2% vs. 23.1%) and had more frequent peroperative complications, discontinued surgery and residual tumor mass.

Conclusions: In nearly half of all patients with a UBPGL the diagnosis was not established before surgery. Hypertension and hematuria contributed independently to a presurgical diagnosis. Hematuria should not be used to discard the possibility of a UBPGL. Postsurgical diagnosis, which was associated with suboptimal presurgical and surgical management, resulted in more peroperative complications and incomplete tumor resections.



FT11:

Characterization of outgrowth processes in pheochromocytoma spheroids

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Tumor is a complex issue comprising also non-tumor cells, such as cancer-associated fibroblasts (CAFs), which could influence tumor growth and progression.

We have previously demonstrated that conditioning 3D tumor spheroids with CAFs leaded to formation of filaments invading the surrounding matrix. Only spheroids silenced for the B subunit of succinate dehydrogenase (SDH) showed a collective migration type.

Our aim was to evaluate whether the filaments of the spheroids derived from the murine pheochromocytoma MTT cells, silenced for the SDHB or the SDHD subunits, differently express some proteins, found deregulated in several cancer types, compared to their nonsilenced counterpart (Wt). By confocal microscopy, we evaluated N-cadherin and vimentin, proteins involved in the epithelium-mesenchymal transition. We observed that all tumor spheroids expressed high levels of these proteins, without differences between the SDH subunits involved. Analysing vinculin, a focal adhesion protein, conditioned spheroids interestingly revealed homogeneous expression along the filaments, showing a great focal adhesion assembly and disassembly activity during the elongation process. Claudin-1 expression, a protein of the tight junctions, was increased in conditioned spheroids compared to the not-conditioned ones. Since Claudin-1 has role also in regulating intracellular signaling pathways, we hypothesize a possible modulation of the downstream cascade. Further evaluations are necessary to clarify this aspect. Evaluating MAP2 and Tau, markers of nervous cell microtubules, interestingly SDHB silenced spheroids showed a significantly lower percentage of MAP2 and Tau positive filaments compared to Wt and to those silenced for SDHD. Moreover, Western-Blot analysis revealed MAP2 protein downregulation in spheroids conditioned by CAFs, compared with not-conditioned ones, but only in SDHB silenced spheroids that decrease was significant, demonstrating a less differentiated profile according to a major aggressive SDHB phenotype. The proteins involved in the formation of filaments, used by cells to migrate, could represent new pharmacological targets for inhibiting tumor spread.



FT12:

NOVEL GERMLINE PHD2 VARIANT IN A METASTATIC PHEOCHROMOCYTOMA PATIENT IN THE ABSENCE OF POLYCYTHEMIA

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Background: Pheochromocytoma (Pheo) and paraganglioma (PGL) are rare tumours, mostly resulting from pathogenic variants of predisposing genes, with a genetic inheritance that is now approximately 70%, where germline variants account for 40%, while the remaining 30% is attributable to somatic variants.

Methods: Genetic analysis was performed by Next generation Sequencing (NGS). The analysis was initially carried out using a panel of genes known for tumour predisposition (EGLN1, EPAS1, FH, KIF1Bbeta, MAX, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, and VHL), followed by SNP-CGH array, to exclude the presence of pathogenic Copy Number Variant (CNV) and loss of heterozygosity (LOH), and by whole exome sequencing (WES) comparative sequence analysis of the DNA extracted from tumor samples and peripheral blood.

Results: We found a novel germline PHD2 (EGLN1) gene variant, c.153G>A, p.W51*, in a patient affected by metastatic Pheo and chronic myeloid leukemia (CML) in the absence of polycythemia. Computational analysis highlighted the possible involvement of peptide in two different mechanisms: a reduction in propensity of PHD2 to generate dimers and a straight interaction with Nuclear receptor corepressor 2 (NCoR2). Neoplastic cells were negative for PHD2 expression and Western Blot analysis showed a higher level of HIF2 α in the tumour tissue compared to healthy adrenal.

Conclusions: According to the latest guidelines, genetic analysis is mandatory in all Pheo/PGL cases regardless of phenotype. In patients with metastatic disease and no evidence of polycythemia, we propose testing for PHD2 (EGLN1) gene variants. A possible correlation between PHD2 (EGLN1) pathogenic variants and CML clinical course should be considered.

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FT13:

Clinical spectrum of bladder paraganglioma: results from 53 patients

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Context and Objective: Bladder paraganglioma (BPGLs) is a rare and easily misdiagnosed entity. We aimed to determine the presentation and outcomes of BPGLs.

Methods: We conducted a single-center retrospective study of consecutive patients with pathologically confirmed BPGLs evaluated between January 1966 and July 2021.

Results: BPGL was diagnosed in 53 patients (30, 57% women) at a median age of 48 years (IQR 39-60). Patients discovered by symptoms of mass effect (19, 36%), by typical symptoms of catecholamine excess (17, 32%), by other symptoms (4, 8%), or incidental in 13 (25%) cases. Micturition disturbances, pain, and hematuria were reported by 15 (28%) patients. At the time of initial diagnosis, tumor size was 3.0 (IQR 2.0-4.6) cm. Initial endocrine assessment was inadequate, of the 38 patients with available work up for catecholamine excess, 30 (79%) had functional BPGL (27 with noradrenergic phenotype). BPGL management included surgical resection (50, 94%), observation (1, 2%), others (3,6%). Multimodal therapy was administered in 2, 4% of patients. Of 22 patients treated with transurethral resection of BPGL, complete resection was not possible in 5 (in 4/5 patients, BPGL with muscularis propria invasion). Among 25 patients evaluated for a genetic association, 15 (60%) were found to have known pathogenic variant (succinate dehydrogenase, SDH B, C, D, and AF2 in 14 patients, and Von Hippel Lindau syndrome in 1 patient). Patients were followed for a median of 2 (IQR 0.5-7.6) years. Metastatic BPGL was diagnosed at baseline or during follow-up in 29 (55%) patients. All patients with SDHB genetic variant developed metastatic BPGL. Overall disease specific survival was 91%.

Conclusions: Patients with BPGL present with variable symptomatology that requires a high index of suspicion to make an accurate diagnosis. Association with SDH pathogenic variant is frequent. More than half of patients develop metastatic disease.



FT14:

In-patient versus out-patient testing and other preanalytical considerations for use of plasma metanephrines for diagnosis of pheochromocytoma

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Sampling of blood in the supine position for diagnosis of pheochromocytoma and paraganglioma (PPGL) results in lower rates of false-positive results for plasma normetanephrine than seated sampling. It is not completely clear how other preanalytical factors, particularly in-patient versus out-patient sampling, impact rates of false-positive results. To address this knowledge gap the present study utilized data on plasma metanephrines from 3178 patients (including 278 with PPGL) sampled under different conditions at 10 European centers. Patients were evaluated as in-patients at two centers and out-patients at others. Sampling was carried out in the supine position, though at two centers this involved the semi- rather than the fully recumbent position and at four centers intravenous (i.v.) lines were used instead of direct venipuncture. Multivariable analyses, also taking into account age and seasonal changes in temperate, indicated that sampling in the in- patient rather than out-patient setting resulted in 29% lower plasma concentrations of normetanephrine and an associated 70% reduction in false-positive results. Warmer conditions, a fully-recumbent position, and use of an indwelling i.v. for blood sampling also resulted in significant lowering of plasma concentrations and rates of false-positive results for plasma normetanephrine, though with lower impact than in-patient versus out-patient sampling. Preanalytical factors were without impact on plasma metanephrines in patients with PPGL. In conclusion, while in-patient rather than out-patient sampling of blood is largely impractical for routine diagnostics, this along with other pre- analytical precautions (e.g., use

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of an indwelling i.v. and warm testing conditions) may be useful for follow-up of isolated patients in whom it is dificult to distinguish true- from false-positive results. For all situations, blood sampling after at least 20 minutes in a fully supine position remains important.

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FT15:

Safe observation of early-recurrence of genetically- determined pheochromocytomas

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Controlateral recurrence of pheochromocytoma is frequent in genetically predisposed patients. Cortical sparing adrenalectomy is currently recommended in this situation, but conveys a risk of adrenal insufficiency up to 45 %. The appropriate timing of surgery and the possibility to postpone safely surgery remain debatable owing to scarse data concerning the natural history of these tumors. We report our experience of long-term follow up of nonoperated 17 pheochromocytomas in 10 MEN2 and 4 Von-Hippel-Lindau carriers. Initially, 13/14 patients were normotensive. 7/14 had normal metanephrines values and diagnosis was based on imaging studies (CT-scanner and/or nuclear imaging). 7/14 had 1.4 x ULN mean metanephrines levels (range : $1.1 - 4.0 \times ULN$). Tumor size at diagnosis was $12.6 \pm 2.6 \text{ mm}$. During follow- up (mean 7 years ; range : 2 - 18), growth was 1.6 ± 2.3 mm/year. Among the 7 "non-secreting" patients, metanephrine levels increased from 1.2 to 4.6 ULN in 4 patients and remained normal in 3. Among the initially "secreting" patients, metanephrines increased from 1.8 to 18.0 x ULN (mean 3.0 ULN). Tumor growth and metanephrine increment were correlated (r=0.82, p < 0.01). Hypertension occurred in 2 patients and was controlled with alpha-adrenergic blockade. No morbid cardiovascular event occurred. Surgery of 8/17 pheochromocytomas was performed after 2 to 10 years of follow-up of. Despite delaying surgery, parameters of per-operative hemodynamic instability were significantly milder than those of a control group of patients with hypertensive sporadic pheochromocytomas (p<0.05).

Conclusion: tumoral and secretory progression is slow in MEN2 and VHL pheochromocytomas. In early-stage, asymptomatic recurrence, a careful follow-up allows to postpone safely complementary surgery and its attendant risk of adrenal insufficiency.





FT16:

Succinate dehydrogenase loss in a familial Von Hippel-Lindau inherited pheochromocytoma

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We present the case of a young female patient, diagnosed as an heterozygous carrier of the germline c.313_314dupAC [p.(Gly106Argfs*54)] Von Hippel-Lindau (VHL) gene mutation with VHL disease through familial screening at the age of eighteen. Her medical history consists of idiopathic generalized epilepsy since childhood and a benign bone femoral lesion. Due to a left flank pain, an abdominal ultrasound was performed and revealed a voluminous (5cm) mass in the pancreatic tail. Abdominal MRI showed that this large lesion was actually originated from the left adrenal gland and revealed the existence of another 2cm lesion with identical characteristics in the right adrenal gland, compatible with bilateral pheochromytoma, as well as of a third 12mm lesion at the root of the mesenteric artery compatible with a pancreatic neuroendocrine tumor or with a Zuckerkandl organ's paraganglioma. All lesions were FDG-avid, while only the mesenteric lesion showed a DOTATATE uptake during PET imaging. Clinically, she was normotensive despite an elevation of normetanephrine and noradrenalin levels at respectively 14- and 7-fold the upper limit of the normal (ULN) range on repeated 24-hour urine collection. Chromogranine A was also 4-fold the ULN. After discussion with the French COMETE network and the patient, a laparoscopic left adrenalectomy with simultaneous removal of the mesenteric lesion was performed. Pathological examination concluded to a pheochromocytoma showing loss of SDH expression on immuno-staining (Ki67 3%) and to a grade 2 pancreatic TNE (Ki67 16%) respectively. VHL is an inherited multitumour disease that can affect the adrenal medulla and the autonomic ganglia of the peripheral nervous system (PPGL). VHL-related PPGL are less aggressive than SDH-deficient PPGL but at our best knowledge, there is little data about combination of germline VHL-mutated PPGL with simultaneous somatic SDH-expression loss and no particular guidelines for the follow-up in such cases.



FT17:

Integration of artificial intelligence with mass spectrometry-based plasma steroid profiling: application to primary aldosteronism

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Measurements of plasma aldosterone and renin represent current screening tests for identifying primary aldosteronism (PA). Mass spectrometry-based steroid profiling combined with machine learning (ML) utilizes computational algorithms to improve the diagnosis, but requires conveyance of results and interpretations to clinicians. Integration of machinelearning algorithms within the data- output ends of laboratory information management systems (LIMS) for clinical report generation. Our approach was established under a prospective multicenter research protocol for prospective validation of ML for diagnostic stratification of patients with PA. The approach involves integration of ML algorithms with commercially available mathematical programming software and a web-based LIMS prototype that allows for automated generation of patient reports. For display of clinical utility the approach has been applied to over 500 patients with suspicion of PA. Generated reports include plasma concentrations of steroids in relation to age- and sex specific reference intervals. Reports also include results of machine learning algorithms and automated narrative interpretations. These interpretations include probabilities of PA and if PA is predicted likelihood of bilateral disease versus unilateral disease and for the latter probabilities of KCNJ5 mutations. Under the hypothesis being tested we are investigating whether patients with KCNJ5 mutations and imaging findings of an adenoma might bypass confirmation studies and adrenal venous sampling to go directly to surgery. Preliminary results establish that patients with somatic mutations of KCNJ5 can be identified by ML. Our work brings novel data together on the integration of ML with LIMS in a clinical setting. The approach permits the upload of both clinical data and mass spectrometry steroidomics profiles, automated analysis and results interpretation with a user-friendly presentation for clinicians.

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FT18:

Prevalence of primary aldosteronism in pafients with acute stroke: a prospective study

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Background: Primary aldosteronism (PA) is likely the most common secondary cause of hypertension and is associated with increased risk of stroke. We aimed to investigate the prevalence of PA in patients with recent stroke.

Methods: 300 patients admitted to a single-centre acute stroke unit with diagnosis of cerebrovascular accident (haemorrhagic/ischemic) or transient ischemic attack were recruited. Patients underwent screening blood test with the Aldosterone-to-Renin Ratio (ARR) at 2 to 4 months post-stroke. Patients with a positive screening test subsequently underwent confirmatory seated saline-loading test (SLT).

Results: 192 of 300 patients completed ARR screening with mean age of 56.7#10.6 years and 55 (28.6%) were females. 26 of 192 (13.5%) patients were ARR positive. 3 of 14 patients who underwent SLT confirmatory test had post-saline aldosterone >5ng/dl. Another three were diagnosed with PA using other confirmatory criteria. In total, six were diagnosed with PA, yielding a prevalence of 3.1% in the entire cohort and 4.6% amongst those with hypertension. Prevalence rates were higher amongst young stroke patients aged \leq 50 years (3 of 49, 6.1%), patients with cardioembolic strokes (2 of 21, 9.5%), patients with hypertension and hypokalemia (2 of 12, 16.7%), hypertension and AF (3 of 10, 30.0%). Using hypokalemia or young stroke as pre-requisites for screening would have missed half of the patients.

Conclusion: PA may be a common underlying treatable cause of stroke. In hypertensive patients who suffer a stroke and are at particularly high risk for recurrent strokes, screening for PA may be important to reduce the risk of recurrent events.



FT19:

Sublethal hyperthermia in combination with Heat Shock Protein Inhibitors as an adrenal sparing, targeted disruption of hyper functional nodules in APA's

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Introduction: Primary Aldosteronism (PA) is the most common secondary cause of hypertension. First-line treatment; adrenalectomy resects adrenal nodules and adjacent normal tissue thereby limiting suitability to those who present with unilateral disease (30-40% cases) to avoid development of the significant adrenal insufficiency associated with bilateral adrenalectomy. Use of heat shock protein inhibitors (HSPi) in combination with sub-lethal hyperthermia (45°C) presents as a possible minimally invasive therapy for unilateral and bilateral disease, to target and disrupt hypersecreting aldosterone producing adenomas (APA), while preserving adjacent normal adrenal cortex.

Methodology: Steroidogenic adrenocortical cell lines (H295R and HAC15) were co-treated with HSPi (Novobiocin) and thermal therapy at temperatures between 37-50°C using a water bath, with subsequent stimulation using forskolin and angiotensin II respectively. Cell death and steroid secretion were analysed immediately and 7-days post- treatment using Annexin V / Propidium Iodide (PI) (flow cytometry) and HPLC Mass Spectrometry quantification of cortisol, aldosterone and androstenedione in cell supernatants.

Results: Sub-lethal hyperthermia (45°C) alone significantly reduced Aldosterone secretion (Mean Difference -11533pmol/L vs ctrl p<0.001), which was enhanced with novobiocin co-treatment. Cell death did not occur with use of sub-lethal hyperthermia (45° C) either as a monotherapy (P>0.05 vs ctrl) or in combination with HSPi co-treatment (P>0.05 vs ctrl). Ablative hyperthermia (48-50°C) induced necrotic cell death without apoptosis and markedly reduced both cortisol and aldosterone secretion which was sustained up to 7-days post treatment (P<0.001 vs ctrl).

Conclusion: Sublethal hyperthermia alone and with HSPi Co- treatment significantly decreases steroidogenesis. Given the small size of the adrenal gland, co-treatment of sublethal hyperthermia with HSPi may present as a targeted safer alternative to ablative therapy by disrupting the hypersecreting functional nodule while (i) mitigating excessive damage to surrounding healthy tissue (ii) maintaining normal adrenocortical function (iii) reducing the risk of peri-procedural hypertensive catecholamine crisis.



FT20:

Japan Endocrine Society Clinical Practice Guideline for the Diagnosis and Management of Primary Aldosteronism 2021

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As primary aldosteronism (PA) is associated with higher cardiovascular morbidity and mortality rates than those of essential hypertension, appropriate diagnosis and treatment are essential for clinical practice of hypertension. The Japan Endocrine Society has developed a 2021 guideline for the management of PA, based on evidence especially from Japan. Hypertensive patients with a high prevalence of PA should be preferentially screened with an aldosterone to renin ratio (ARR) \geq 200 and plasma aldosterone concentration \geq 60 pg/mL as a cut-off of positive results. While excess production of aldosterone should be confirmed by one confirmatory test, it could be bypassed in patients with typical clinical and biochemical findings of PA. Considering a significant change of the plasma aldosterone values due to changes in assay methods from RIA to CLEIA, borderline ranges were set both for screening and confirmatory test and provisionally designated as positive. For the patients placed in the borderline range, individualized medicine for the next step of diagnosis and treatment is recommended considering the patient's needs and clinical findings. A dexamethasone suppression test is recommended to evaluate cortisol co-secretion in patients with adrenal macroadenomas on CT. Although adrenal venous sampling is recommended to distinguish unilateral lesions from bilateral lesions before adrenal surgery, it should be carefully indicated for select patients, rather than all patients; it could be bypassed in young patients with typical PA findings. Successful catheterization and diagnosis of the unilateral subtype are defined by a selectivity index \geq 5 and lateralization index > 4 after adrenocorticotropic hormone stimulation, respectively. Contralateral suppression of aldosterone secretion is considered for strict lateralization. Recommended treatment is adrenalectomy for unilateral PA and mineralocorticoid receptor antagonists for bilateral PA. Systematic as well as individualized clinical practice is always warranted. This Japan Endocrine Society guideline 2021 provides updated rational evidence and recommendations for clinical practice of PA within the framework of medical insurance system in Japan, leading to improved quality of clinical practice of hypertension and promotion of national health in Japan.



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