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ABSTRACT BOOK

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Development of two new ACC patient-derived cell lines to improve the landscape of available preclinical models

Andrea Abate[1], Mariangela Tamburello[1], Claudia Bonera[1], Sara Baldelli[2], Mattia Carini[2], Duilio Brugnoni[2], Marta Leporati[3], Domenico Cosseddu[3], Maria Scatolini[4], Alessia Inglesi[4], Marta Laganà[5], Deborah Cosentini[5], Guido Alberto Massimo Tiberio[6], Alfredo Berruti[5] and Sandra Sigala[1].

[1] Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.
[2] Central Clinical Laboratory, ASST Spedali Civili di Brescia, Brescia, Italy.
[3] Laboratory Medicine, AO Ordine Mauriziano, Turin, Italy.
[4] Molecular Oncology Laboratory, Fondazione Edo ed Elvo Tempia, Ponderano, BI, Italy.
[5] Medical Oncology Unit, ASST Spedali Civili, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, Brescia, Italy.
[6] Surgical Clinic, Department of Clinical and Experimental Sciences, University of Brescia at ASST Spedali Civili di Brescia, Brescia, Italy.

Background
AdrenoCortical Carcinoma (ACC) is an aggressive rare malignancy. Mitotane is the reference drug alone or combined with etoposide, doxorubicine and cisplatin (EDP-M). Due to the high heterogeneity of ACC, the preclinical investigation requires different experimental models. For this reason, the development of new cellular tools is of high interest. Here we reported the development and characterization of two new human ACC cell lines, namely SMAC-2 and SMAC-3.

Materials and Methods
Cell cultures were established from patient diagnosed with ACC and operated on. Mutational status was investigated by NGS and Sanger methods. Gene expression was analysed by qRT-PCR, while protein expression was evaluated by immunofluorescence. Hormonal secretion was investigated by LC-MS/MS. Cell proliferation was evaluated by BrdU-incorporation assay.

Results
SMAC-2 derived from a metastatic ACC EDP-M-treated female patient diagnosed with Cushing and hyperandrogenism, while SMAC-3 derived from a male patient with a mitotane-treated local recurrence. Genetic characterisation revealed for both cell lines a *TP53* mutant status. SMAC-2 harbours also pathogenic alterations on *CTTNB1* and *CDKN2A* genes, while SMAC-3 on *MSH2* gene. Analysis of basal hormonal secretion showed a peculiar finger-print for each of the two models. Also, gene and protein expression of steroid hormone receptors presents a model-specific pattern. In accordance with the clinical manifestation of the original patient, SMAC-2 secretes high levels of cortisol. SMAC-3 is also able to secrete cortisol at baseline, although in a low amount. Mitotane displayed in both cell lines a low potency. Experiments in a wide range of passages were carried out to study the stability of the two cell lines

Conclusions
SMAC-2 and SMAC-3 cell lines show a range of peculiar characteristics that establish their role as two new experimental cell models useful in ACC preclinical research in addition to the other today-available well-known models.

Validation of LC-MS–Based Post-Saline Infusion Test Aldosterone Thresholds for the Diagnosis of Primary Aldosteronism

Sara Ali[1], Zin Htut[1], Sophie Barnes[2], Florian Wernig[2]

[1]Imperial College London, London, UK,
[2]Imperial College Healthcare NHS Trust, London, UK

Background

Primary aldosteronism (PA) is the most common cause of secondary hypertension but often remains undiagnosed due to limitations in screening and confirmatory test. As laboratories shift from immunoassays to liquid chromatography–mass spectrometry (LC-MS) for aldosterone measurement, improved accuracy have prompted a re-evaluation of diagnostic thresholds—particularly for the saline infusion test (SIT). However, validated LC-MS–specific cut-offs remain scarce. This study aimed to define and validate SIT thresholds using LC-MS to support more reliable PA diagnosis.

Methods

We conducted a retrospective cohort study of patients evaluated for PA between January 2019 and March 2025. Patients underwent SIT, captopril challenge test (CCT), and/or adrenal vein sampling (AVS). Receiver operating characteristic curve analysis was used to determine the optimal SIT cut-off, with PA diagnosis confirmed by AVS and/or CCT

Results

Of the 46 patients initially screened, 41 completed the SIT and were included in the final analysis. PA was confirmed in 36 patients, while 5 were excluded based on negative confirmatory testing with CCT or AVS. Mean age was 49.6 ± 9.6 years, with 58.5% male and 41.5% female. Average BMI was 31.6 ± 7.7 kg/m². Clinical histories included cardiac disease (7.3%), stroke (2.4%), diabetes (14.6%), and chronic kidney disease (CKD; 12.2%). Post-SIT aldosterone showed excellent diagnostic performance for PA confirmation, with an area under the curve of 0.978. The optimal diagnostic threshold was 165 pmol/L (Youden Index), yielding 91.2% sensitivity and 100% sensitivity (PPV=100%; NPV=57%). All patients with CKD (n=5) had PA.

Conclusion

A post-SIT plasma aldosterone cut-off of 165 pmol/L using LC-MS demonstrates high diagnostic accuracy for PA and supports its use in real-world clinical practice.

Sex- and age-related differences, hormonal profiling and long-term outcome in adrenocortical carcinoma: evidence from the largest single-center cohort since 2000

Altieri Barbara[1,2], Megerle Felix[1], Kimpel Otilia[1], Detomas Mario[1], Max Kurlbaum[1,3], Küppers Sarah[1], Dischinger Ulrich[1], Kircher Stefan[4], Kroiss Matthias[1], Hahner Stefanie[1], Fassnacht Martin[1,3]

[1] Division of Endocrinology and Diabetes, Department of Internal Medicine I, University Hospital, University of Würzburg, Würzburg, Germany
[2] Bavarian Cancer Research Center (BZKF), University Hospital, University of Würzburg, Würzburg, Germany
[3] Central Laboratory, Core Unit Clinical Mass Spectrometry, University Hospital Würzburg, Würzburg, Germany.
[4] Institute of Pathology, University of Würzburg, Würzburg, Germany.

Background: Adrenocortical carcinoma (ACC) is a rare cancer with limited large cohort studies. We analyzed the largest single-center ACC cohort to date, spanning 20 years, focusing on sex- and age-related differences, hormonal profile, and impact of metastasis type on outcome.

Methods: Retrospective cohort study of patients with ACC treated at our center (01/2000-05/2024). Clinicopathological characteristics were analyzed by sex and age. Hormone profiling via LC-MS/MS for 15 steroids was performed in a subgroup of 79 patients and compared with standard immunoassays. Primary outcome was overall survival (OS).

Results: 1,516 patients with ACC (median age 51 years; 62% female) were included. Median OS was 59 months (95%CI=50.1-87.9). No significant sex-related differences were observed in age, tumor stage, metastatic patterns, or OS. However, men had more inactive (36% vs 20%, p<0.001) and larger tumors (median 12 vs 10 cm). Hormone secretion varied by age, with androgens (44% vs 12%) and estrogens (5% vs 1%) more frequent in young (≤17 years-old) (p<0.001). Young patients had longer OS (median not-reached) than young/adult (18-40 years-old) (91 months, 95%CI=69.3-122.7), adult (63 months, 95%CI=48.6-77.8) and elderly (≥61 years-old) (35 months, 95%CI=28.7-41.3) at multivariate regression (HR range 0.3-0.8, p<0.001). Endocrine-inactive (HR=0.56) and androgens-secreting tumors (HR=0.63) were associated with longer OS than other hormone-secretions at Cox regression (p<0.001). LC-MS/MS detected a median of 4 (IQR=2-6) elevated steroids *per* patient. Only 3 out 10 tumors labeled inactive by standard assays had no detectable steroid excess. 11-deoxycortisol and 11-deoxycorticosterone were frequently elevated (81% and 39%), even in endocrine-inactive cases. In advanced stages at diagnosis (n=412), liver, lymph nodes, and bone metastases were associated with shorter OS (p<0.01).

Conclusion: While ACC is more frequent in women, sex-dimorphism does not impact outcome. Younger age and androgen/inactive hormonal profiles predict better outcome. LC-MS/MS improves detection of steroid excess, revealing most ACCs are hormonally active.

Plasma Circulating Tumor DNA (ctDNA) as a Prognostic and Monitoring Biomarker in Metastatic Pheochromocytoma and Paraganglioma: Updated Results from an International Liquid Biopsy Study

Arenillas Carlota[1], Talvacchio Sara[2] Prodanov Tamara[2], Lim Eugenie S.[3], Remde Hanna[4], Bioletto Fabio[5], Casteràs Anna[6], Laganà Marta[7], Moreno-Cárdenas Ana Belén[1], Datta Debayan[1], Louhamane Aya[1], Calahorro Alma María[1], Palma Helena[8], Huynh Thanh[2], Charles Kailah[2], García-Alvarez Alejandro[8], Hernando Jorge[8], Liquid Biopsy International Consortium, Del Olmo-García María Isabel[9], Drake William[3], Parasiliti-Caprino Mirko[5], Berruti Alfredo[7], Dahia Patricia L.M.[10], Capdevila Jaume[8], Akker Scott[3], Fassnacht Martin[4], Pacak Karel[2], Toledo Rodrigo A[1]

1. Biomarkers and Clonal Dynamics Group, Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain.
2. Section on Medical Neuroendocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA.
3. Department of Endocrinology, William Harvey Research Institute, Queen Mary University of London, London, United Kingdom; Department of Endocrinology, St. Bartholomew's Hospital, Barts Health NHS Trust, London, United Kingdom.
4. Department of Internal Medicine I, Division of Endocrinology and Diabetes, University Hospital, University of Würzburg, Würzburg, Germany; Comprehensive Cancer Center Mainfranken, University of Würzburg, Würzburg, Germany.
5. Department of Medical Sciences, University of Turin, Turin, Italy.
6. Endocrinology Department, Diabetes and Metabolism Research Unit, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain.
7. Medical Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia. ASST Spedali Civili, Brescia, Italy.
8. Medical Oncology Department, Gastrointestinal and Endocrine Tumor Unit, Vall d´Hebron Barcelona Hospital Campus, Vall Hebron Institute of Oncology (VHIO), Barcelona, Spain.
9. Endocrinology and Nutrition Department, University and Polytechnic Hospital La Fe, Valencia, Spain.
10. Department of Medicine, Division of Hematology and Medical Oncology, Mays Cancer Center, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Background: Pheochromocytomas and paragangliomas (PPGLs) are often effectively managed with surgery. While most patients remain stable for years, a subset eventually progresses to metastatic disease (mPPGL) with a sudden and aggressive clinical deterioration and death. Although some biomarkers have been associated with tumor aggressiveness, there is currently no prognostic biomarker to predict patient outcomes. This study evaluates liquid biopsy as a novel prognostic tool to address this unmet clinical need.
Aim: To assess plasma circulating tumor DNA (ctDNA) of mPPGLs using shallow whole-genome sequencing (shWGS) as a potential prognostic marker.
Results: A total of 298 plasma cell-free DNA samples were collected from 197 PPGL patients through ENS@T and A5 networks, each with curated clinical data. Among them, 156 patients presented metastatic disease. Genomics sequencing was performed on all samples.
At baseline, ctDNA was detectable in 32 patients. Notably, these patients exhibited lower overall survival (34%) compared to those with non-detectable ctDNA (76%, n=157) over 24 months (hazard ratio: 17.3, p<0.001), supporting its role as a prognostic marker. Among ctDNA-positive patients with treatment data available (n=30), those receiving therapies with clinical efficacy had improved survival (68% n=15) versus patients receiving other systemic therapies (28%, n=15) over 12 months, (hazard ratio: 3.65, p=0.02).
Longitudinal analysis showed that ctDNA levels correlated with disease progression, highlighting its utility for real-time monitoring and patient stratification. Case reports illustrate how ctDNA levels anticipated and matched radiological progression. Finally, 24 ctDNA-positive samples paired with germline DNA were analyzed by whole-exome sequencing, identifying candidate driver mutations of tumor progression.
Conclusion: Baseline ctDNA detection in mPPGL patients may serve as a prognostic biomarker, enable disease monitoring, and uncover actionable genomic alterations to inform treatment strategies.

Shared signaling pathways uncover the contribution of Cancer-Associated Fibroblasts (CAFs) to PPGL progression

Ascione Francesco[1], Rouaud Ernest[1], Amore Francesca[2], Rapizzi Elena[3,4,5], Pellegata Natalia Simona[1,6]

1. Department of Biology and Biotechnology, University of Pavia, Pavia, Italy.
2. Department Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy.
3. Department Experimental and Clinical Medicine, University of Florence, Florence, Italy.
4. Centro di Ricerca e Innovazione sulle Patologie Surrenaliche, AOU Careggi, Florence, Italy.
5. ENS@T Center of Excellence, Florence, Italy.
6. Institute for Diabetes and Cancer, Helmholtz Zentrum München, Neuherberg, Germany.

Background
Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors (NETs) arising from chromaffin cells in the adrenal medulla or paraganglia. While the tumor microenvironment (TME)—particularly cancer-associated fibroblasts (CAFs)—has been implicated in PPGL progression, the molecular mechanisms underlying CAF contributions remain largely unknown. In this study, we examined the crosstalk between CAFs and tumor cells by analyzing the transcriptional profiles of patient-derived fibroblasts cultured alone or in co-culture with PPGL cells, aiming to uncover mechanisms of CAF involvement and identify novel therapeutic targets.

Methods
Primary fibroblasts were isolated from three PPGL patients—two with germline SDHB mutations and one without. These fibroblasts were cultured alone or co-cultured with either hPheo1 WT or hPheo1 SDHB-KO human PPGL cell lines. RNAseq was performed, and differential gene expression was assessed using the DESeq2 R package. Enrichment analyses (GO, GSEA, and EnrichR) were conducted to identify dysregulated pathways. Comparisons across fibroblast batches were used to detect consistent gene expression patterns and shared biological responses to tumor co-culture.

Results
Transcriptomic profiling revealed more upregulated than downregulated genes ($|\log_2 FC| > 2$) across all co-culture conditions, suggesting strong transcriptional activation. Notably, fibroblasts co-cultured with hPheo1 SDHB-KO cells exhibited a higher number of differentially expressed genes (DEGs) than those co-cultured with hPheo1 WT. While each fibroblast culture showed unique transcriptional responses, 14 enriched pathways—particularly involving IL2-STAT5 and IL6-JAK-STAT3 cytokine signaling, as well as cell cycle regulation—were consistently shared across all groups.

Conclusions
Our findings reveal that co-culture with PPGL cells, especially the SDHB-KO variant, induces significant and consistent transcriptional changes in patient-derived fibroblasts, which could mediate the pro-tumorigenic effects of the CAFs. The identification of common DEGs and enriched pathways supports the presence of shared mechanisms of CAF–tumor communication, offering valuable insights into potential targets within the TME for PPGL therapy.

Sphingosine Kinase 2 (SPHK2) inhibition as a therapeutic strategy for Adrenocortical Carcinoma (ACC)

Asif Kanwal[1], Altieri Barbara[2], Pittaway James[1], Kwong Ruth[1], Smith Chris[1], Hantel Constanze[3], Sigala Sandra[4], Berruti Alfredo[4], Sbiera Silviu[2], Fassnacht Martin[2], Guasti Leonardo[1], Metherell Lou[1], Prasad Rathi[1]

1. Centre for Endocrinology, William Harvey Research Institute, Queen Mary University of London, London, UK
2. Division of Endocrinology and Diabetes, Dept. of Medicine, University Hospital, University of Würzburg, Würzburg, Germany
3. Department of Endocrinology, Diabetology and Clinical Nutrition, University Hospital Zurich (USZ) and University of Zurich (UZH), Zurich, Switzerland
4. Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

Background:
Sphingolipid metabolism plays a role in adrenal homeostasis, as evidenced by the association of sphingolipid enzyme deficiency with adrenal insufficiency. In contrast, high transcriptomic expression of several sphingolipid enzymes is associated with poor prognosis in adrenocortical carcinoma (ACC). This study is focused on SPHK2, high transcriptomic expression of which is associated with poor overall survival in ACC (analysis of the TCGA-ACC data).
Methods:
Immunohistochemical staining of SPHK2 was undertaken in 125 ACC (where clinical data were available for correlation), 21 adrenocortical adenomas (ACA), and 5 normal adrenal tissues from University Hospital Würzburg. Lentiviral driven stable overexpression of SPHK2 was established in 3 ACC lines (H295R, TVBF-7, MUC-1) with cell proliferation, migration, response to mitotane treatment and selective SPHK2 inhibition assayed.
Results:
SPHK2 expression was significantly higher in ACC compared to ACA but showed no correlation with established prognostic factors, clinical scores, or steroid production. Although there was a trend toward reduced overall survival with high SPHK2 expression, it did not reach statistical significance, and no impact was seen on progression or recurrence free survival.
All three ACC cell lines (H295R, TVBF-7, MUC-1) express SPHK2. Overexpressing SPHK2 did not affect cell proliferation assayed at 72 hours in the 3 cell lines. SPHK2 overexpression did, however, promote cell migration in the H295R cell line. Opaganib (ABC294640), a selective SPHK2 inhibitor, reduced cell viability to a similar extent as mitotane in all three cell lines. Interestingly, SPHK2 overexpression in TVBF-7 cells induced mitotane resistance; this did not affect the susceptibility of the cells to SPHK2 inhibition.
Conclusion:
SPHK2 inhibition, in phase II clinical trials for other cancers, may present an alternative/adjunctive therapy in ACC. Further work is ongoing to identify the biological pathways disrupted by SPHK2 inhibition and determine how they differ from those targeted by mitotane.

The incidence of adrenal incidentalomas in oncological patients: a monocentric study in a tertiary Greek centre

Asimakopoulou Stavroula[1], Papalexis Petros[2], Siampanopoulou Vanessa[2], Lafioniatis Alexandros[2], Chalmantzi Varvara[2], Tsirou Georgia[2], Anastasopoulou Amalia[1], Ziogas Dimitrios[1], Gogas Helen[1], Angelousi Anna[2]

1 First Department of Medicine, Laiko General Hospital, School of Medicine, National and Kapodistrian University of Athens, Greece2 First Department of Internal Medicine, Unit of Endocrinology, Center of Excellence for Rare Adrenal Diseases (ENDO-ERN), Laikon General Hospital, National and Kapodistrian University of Athens, Greece

Introduction: While overt Cushing syndrome (CS) has been linked to malignancy, data on mild autonomous cortisol secretion (MACS) and nonfunctional adrenal incidentalomas (NFAIs) in oncological patients remain limited.

Objective: To assess the prevalence and characteristics of benign adrenal incidentalomas (AIs) in patients with malignancy (Group I) and compare comorbidities (hypertension [HTN], diabetes mellitus [DM], osteoporosis) with patients with AI but no malignancy (Group II) and healthy controls (Group III).

Methods: Medical records of 370 cancer patients (Group I), 18 non-oncological AI patients (Group II), and 51 healthy controls (Group III) were reviewed. Adrenal metastases were excluded. Hormonal evaluations were performed prior to oncologic treatment.

Results: Benign AIs were detected in 48/370 (12.9%) oncology patients (median age 66). Most (56.3%) had unilateral adenomas (median size: 16 mm in Group I vs. 10 mm in Group II). Post-1 mg dexamethasone suppression test cortisol levels were higher in Group I (1.4 μg/dL) vs. Group II (1.06 μg/dL). In Group I, 69.8% had NFAIs and 30.2% MACS, compared to 80% and 20% respectively in Group II. No patient had overt CS. DM and HTN rates did not differ significantly between groups. Regarding timing, 23 patients had concurrent AI and malignancy diagnoses, 16 had malignancy preceding AI (median: 5.5 years), and 5 had AI prior to malignancy (median: 1 year). Osteoporosis prevalence was significantly higher in Group I (61%) than Group II (17.6%) and Group III (7.8%) ($p < 0.001$).

Conclusion: Benign AIs were present in 13% of oncology patients. These individuals more frequently had MACS, larger adrenal lesions, and a significantly higher prevalence of osteoporosis compared to controls.

Systemic alterations in B-vitamin status, the kynurenine pathway and microbiota-dervied metabolites in patients with mild autonomous cortisol secretion

Ueland Grethe Åstrøm[1], Christensen Monika[1], Løvås Kristian[1], Breivik Lars[1,2], Sulen Andre[1,2], Methlie Paal[1,2], Husebye Eystein[1,2]

1 Department of Medicine, Haukeland University Hospital, Bergen, Norway
2 Department of Clinical Science, University of Bergen, Bergen, Norway

Context:
Mild autonomous cortisol secretion (MACS) is a common condition associated with the metabolic syndrome and cardiovascular disease. Recent studies have revealed an immune response in these patients involving multiple inflammation-related proteins.
Objective:
This work aimed to characterize the kynurenine pathway, activated during interferon-γ- and Interleukin 6- mediated inflammation, in patients with MACS.
Methods:
We analyzed 26 biomarkers by mass spectrometry in serum samples from 99 patients with MACS and 99 age and gender matched healthy controls. Postoperative samples were available for 17 MACS patients, median 24 months (range 6-40) after surgery and biochemical curation. The analytes included 10 metabolites and 3 indices from the kynurenine pathway, 6 microbiota-derived metabolites (indoles), and 10 B-vitamines.
Results:
MACS patients showed decreased levels of 6, and increased level of 2 kynerenine metabolites compared with healthy controls (P <0 .01). This gave two elevated indices, Par -index and HK-ratio, both markers of inflammation and oxidative stress. Also, the levels of vitamin B6 and B1 were lowered in MACS patients, while vitamin B2 status was significantly elevated. Finally, two indoles (microbiota-derived metabolites) were significantly higher in MACS patients (imidazole propionate and 3-indoxyl sulfate) compared with controls. The operated patients showed a trend towards normalization for all biomarkers after operation and cure, but not fully restored metabolite levels.
Conclusion:
This study confirms metabolic alterations on a cellular level in patients with MACS, based on findings of IFN-γ/ IL-6 induced inflammation, altered levels of B-vitamins, and changes in indoles that are associated with insulin resistance and kidney failure.

Urinary Steroid Profiling Reveals Distinct Clusters in Adrenocortical Carcinoma

Bain JT (1), Chortis V (1), Papathomas T (1), Shaheen F (1), Veen R (2), Bancos I (1), Sitch AJ (3), Jenkinson C (4),Davidge-Pitts CJ (5), Lang K (1), Tsagarakis S (6), Macech M (7), Riester A (8), Deutschbein T (9), Pupovac ID (10), Kienitz T (11), Prete A (1), Papathomas TG (1), Gilligan LC (1), Bancos C (5), Reimondo G (12), Haissaguerre M (13), Marina L (14), Grytaas MA (15), Sajwani A (16), Langton K (17), Ivison HE (1), Shackleton CHL (1), Erickson D (5), Asia M (18), Palimeri S (6), Kondracka A (7), Spyroglou A (8), Ronchi CL (1), Simunov B (10), Delivanis DA (5), Sutcliffe RP (19), Tsirou I (6), Bednarczuk T (7), Reincke M (8), Burger-Stritt S (9), Feelders RA (20), Canu L (21), Haak HR (22), Eisenhofer G (17), Dennedy MC (16), Ueland GA, Ivovic M (15), Tabarin A (13), Terzolo M (12), Quinkler M (11), Kastelan D (10), Fassnacht M (9), Beuschlein F (23), Ambroziak U (7), Vassiliadi DA (6), O'Reilly MW (1), Young WF Jr (5), Biehl M, Deeks JJ (24), Arlt W (4), Taylor AE (1),

ENSAT EURINE-ACT Investigators

(1) Metabolism and Systems Science, University of Birmingham, Birmingham, UK; Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham, UK.
(2) University of Groningen, Bernoulli Institute for Mathematics, Computer Science and Artificial Intelligence
(3) Institute of Applied Health Research, University of Birmingham, Birmingham, UK; NIHR Birmingham Biomedical Research Centre, University Hospitals Birmingham NHS Foundation Trust and University of Birmingham, Birmingham, UK.
(4) MRC Laboratory of Medical Sciences (LMS), London, United Kingdom
(5) Division of Endocrinology, Diabetes, Metabolism and Nutrition, Mayo Clinic, Rochester, MN, USA.
(6) Department of Endocrinology, Diabetes and Metabolism, Evangelismos Hospital, Athens, Greece.
(7) Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Warsaw, Poland.
(8) Medizinische Klinik and Poliklinik IV, Klinikum der Universität, Ludwig-Maximilians-Universität München, Munich, Germany.
(9) Division of Endocrinology and Diabetes, Department of Internal Medicine I, University Hospital Würzburg, University of Würzburg, Würzburg, Germany.
(10) Department of Endocrinology, University Hospital Centre Zagreb, Zagreb, Croatia.
(11) Endocrinology in Charlottenburg, Berlin, Germany.
(12) Department of Clinical and Biological Sciences, San Luigi Hospital, University of Turin, Turin, Italy.
(13) Department of Endocrinology, Hôpital Haut Lévêque, CHU de Bordeaux, Pessac, France.
(14) Department for Obesity, Reproductive and Metabolic Disorders, Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Centre of Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia.
(15) Department of Clinical Science, University of Bergen, Bergen, Norway; Department of Medicine, Haukeland University Hospital, Bergen, Norway.
(16) School of Medicine, National University of Ireland Galway, Galway, Ireland.
(17) Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Carl Gustav Carus, Technical University, Dresden, Germany.
(18) Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham, UK; Department of Endocrinology, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK
(19) Department of Hepato-Pancreato-Biliary and Liver Transplant Surgery, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK.
(20) Department of Internal Medicine, Division of Endocrinology, Erasmus University Medical Centre, Rotterdam, Netherlands.
(21) Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy.
(22) Department of Internal Medicine, Maxima Medisch Centrum, Eindhoven, Netherlands; Department of Health Services Research and CAPHRI School for Public Health and Primary Care, Maastricht University, Maastricht, Netherlands.
(23) Medizinische Klinik and Poliklinik IV, Klinikum der Universität, Ludwig-Maximilians-Universität München, Munich, Germany; Klinik für Endokrinologie, Diabetologie und Klinische Ernährung, Universitätsspital Zürich, Zurich, Switzerland.
(24) Bernoulli Institute for Mathematics, Computer Science and Artificial Intelligence, University of Groningen, Groningen, Netherlands.

Adrenocortical carcinoma (ACC) is a rare and aggressive cancer with poor prognosis. Our group has shown the Urinary Steroid Profiling (USP) can identify distinct steroid signatures that define malignancy, allowing differentiation between benign adrenocortical adenomas (ACA) and ACC with high sensitivity and specificity (1).

Studies into steroid metabolism in patients with ACC are difficult due to the rarity of ACC, with large multi-centre studies required to gather sufficient samples for metabolic investigations. Therefore, in ACC research a key question remains: Can USP predict prognosis, recurrence risk, and treatment outcomes in patients with ACC?

Through the ENS@T/EURINE-ACT study, we recruited 89 patients to explore ACC subtypes based on their USP. 24-hour urine samples were collected from each patient while the tumour was in-situ. Experimentally steroids were deconjugated from their conjugate esters, derivatised concentrated and quantified using gas chromatography-mass spectrometry (GC-MS) at the Steroid Metabolome Analysis Core (SMAC). Thirty-one steroid metabolites were quantified as micrograms per 24 hours.

The data were assessed using unsupervised clustering by K-means. This computational analysis identified three distinct clusters, these can be broadly classed based on their unique metabolic characteristics: cluster one-androgen excess, cluster two-glucocorticoid excess and cluster three-mixed steroid excess. Cluster two contained patients with the lowest proportion of localised disease, cluster three had the highest proportion of localised disease. Cluster two also had the highest Ki67 proliferation index (none below <10%). There was a non-significant trend towards worse overall survival for cluster two. Further investigation is underway to probe associations between specific clusters and response to chemotherapy or mitotane.

USP combined with cluster analysis enables the identification of distinct ACC subtypes, offering potential insights into prognosis and personalized treatment strategies.

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Activation of calcium signaling, using chemogenetic tools, leads to the development of hyperaldosteronism and adrenal remodeling in mice

Fedlaoui Bakhta[1], Hadri Rania[1], Faedda Nicolo[1], Fayad May[1], Giscos-Douriez Isabelle[1], Magnus Chris[2], Sternson Scott M[2], Baron Stephanie[3], Fernandes-Rosa Fabio L[1], Zennaro Maria-Christina[1,4] and Boulkroun Sheerazed[1]

[1] Université Paris Cité, Inserm, PARCC, Paris France
[2] Howard Hughes Medical Institute & Department of Neurosciences, University of California, San Diego, San Diego, United States.
[3] Service de Physiologie, Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Européen Georges Pompidou, Paris, France
[4] Service de Génétique, Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Européen Georges Pompidou, Paris, France

Primary aldosteronism (PA) is the most frequent form of secondary hypertension and is due to autonomous aldosterone production by the adrenal gland. The most frequent genetic cause of aldosterone producing adenoma (APA) are somatic mutations in the potassium channel KCNJ5.

To investigate how *KCNJ5* mutations lead to the development of APA, we developped a mouse model expressing specifically in the adrenal cortex a chimeric ion channel receptor named ⟨7-5HT3-R. (Cyp11b2-Cre::⟨7-5HT3-R). Activation of the ⟨7-5HT3-R by the selective agonist uPSEM-817 induces sodium influx into the cells, mimicking the effects of *KCNJ5* mutations.

In an adrenocortical cell model (H295R-S2 cells), we previously demonstrated that the expression of ⟨7-5HT3-R leads to an increase of sodium entry into the cells, resulting in cell membrane depolarization, the opening of voltage-gated calcium channel, an increase in intracellular calcium concentration, and an upregulation of *CYP11B2* expression and aldosterone biosynthesis. Additionally, we found that this sodium influx reduces cell proliferation and promote apoptosis. Moreover, RNA sequencing and steroidome analyses revealed unique profiles associated with sodium entry, with only partial overlap with changes induced by angiotensin II and potassium. These findings suggest that additional events may be required for the development of an APA with *KCNJ5* mutation.

In Cyp11b2-Cre::⟨7-5HT3-R mice, four weeks of treatment with uPSEM-817 induces, in both male and female, an increase in plasma aldosterone and 18-hydroxycorticosterone concentrations associated with an increase in *Cyp11b2* expression and to a lesser extent, a disorganization of the adrenal cortex. After four weeks of treatment, we did not observe an increase in blood pressure or cardiac remodeling. Further investigations and longer treatments are ongoing to thoroughly characterize this mouse model.

This mouse model, in which we can modulate calcium entry, provides a valuable tool for dissecting the mechanisms underlying APA development and assessing new therapeutic strategies.

Development and preliminary psychometric validation of a Quality-of-Life questionnaire for Adrenocortical Carcinoma patients in Italy

Bettini Davide Lorenzo[1], Laganà Marta[1], Cosentini Deborah[1], Francesca Consoli[1], Trevisan Benedetta[1], Cremaschi Valentina[1], Rodella Sara[1], Ripamonti Carla Ida[1], Berruti Alfredo[1], Chiesi Francesca[2]

1. Medical Oncology Unit, University of Brescia, Brescia, Italy.
2. Department of Neuroscience, Psychology, Drug, and Child's Health (NEUROFARBA), University of Florence, Florence, Italy.

Adrenocortical carcinoma (ACC) patients' quality of life may be impacted by symptoms related to cancer or treatments, as well as emotional, spiritual, existential, social, economic, and sexual aspects. This project's aim was the development and psychometric validation of a new instrument (QoLACC) for measuring Patient Reported Outcome in patients with ACC.

Comprehensibility, difficulty and clinical relevance of the items included in the QoLACC questionnaire were assessed by selected ACC patients and clinicians of the Oncology Unit of Brescia's ASST Spedali Civili. Validated scales were co-administered with the developed QoLACC questionnaire to assess physical and psychological symptoms, the test-retest reliability and construct validity psychometric validation were performed.

Fifteen patients and 15 physicians graded the comprehensibility/difficulty and relevance of the QoLACC items whereas 37 consecutive patients assessed psychometric validity. The QoLACC items showed excellent content validity [0.93-1.00] and acceptable to excellent comprehensibility [0.80-1.00] and difficulty [0.80-1.00], with minor exceptions. Item analysis showed good discriminant capability and response variability. The total questionnaire has good reliability (the intraclass correlation coefficient was 0.870 [95% CI: 0.734-0.937]) and construct validity proved by the moderate to strong correlations [0.39-0.62] between the QoLACC and the EORTC item 29 and item 30, the QoLACC financial section and the COST (0.47), and the QoLACC spiritual section and the JSWBS (0.60).

We defined and validated this Italian questionnaire aiming to explore ACC patients' quality of life, in order to help Health Care Professionals manage and support these patients.

Prevalence of risk features for primary aldosteronism in the general hypertensive population

Bioletto Fabio[1], Bollati Martina[1], Lopez Chiara[1], Procopio Matteo[1], Arata Stefano[1], Ghigo Ezio[1], Maccario Mauro[1], Parasiliti-Caprino Mirko[1]

[1] Division of Endocrinology, Diabetes and Metabolism; Department of Medical Sciences; University of Turin; Turin, Italy.

Background. Current ES Guidelines suggest screening for primary aldosteronism in hypertensive patients presenting specific risk features (i.e., sustained blood pressure ≥150/100 mmHg, resistant hypertension, hypokalemia, young-onset hypertension [<40 years], obstructive sleep apnea, or adrenal incidentaloma). Extension of screening to all hypertensives is a subject of debate. The potential impact of this change on healthcare systems is unclear, due to the lack of reliable estimates of the proportion of hypertensives that should be screened according to current guidelines, as compared to universal screening. The aim of this study was to assess the prevalence of risk features for primary aldosteronism in the general U.S. hypertensive population.

Methods. We analyzed individual data of 10618 hypertensive patients included in the biennial cycles of the National Health and Nutrition Examination Survey between 2009 and 2018, representative of the general U.S. hypertensive population following appropriate weighting as recommended by the NCHS. Relevant registries were linked through a unique patient identifier, and pertinent data for the evaluation of risk features for primary aldosteronism were extracted.

Results. The percentage of hypertensive patients presenting at least one risk feature for primary aldosteronism was 41.3% in the 2009-2010 cohort and 47.0% in the 2017-2018 cohort, with a significant increase over time (p-for-trend=0.02). In the 2017-2018 cohort, the most common risk feature for primary aldosteronism was young-onset hypertension (reported by 23.8% of hypertensive patients), followed by sustained blood pressure ≥150/100 mmHg (21.8%), resistant hypertension (9.0%), and hypokalemia (4.5%).

Conclusions. This analysis provides reliable estimates of the proportion of U.S. hypertensive patients who present risk features for primary aldosteronism according to current ES Guidelines. This proportion increased over time, equaling 47.0% in its most recent estimate. These data are instrumental for estimating the healthcare costs that may result from expanding primary aldosteronism screening from a targeted approach to universal screening.

Cancer-associated fibroblasts modulate PPGL cell behavior through direct crosstalk

Bistika Margarita[1], Amore Francesca[2,5], Catania Marta[1], Rapizzi Elena[3,4,5], Pellegata Natalia Simona[1,6]

1. Department of Biology and Biotechnology, University of Pavia, Pavia, Italy.
2. Department Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy.
3. Department Experimental and Clinical Medicine, University of Florence, Florence, Italy.
4. Centro di Ricerca e Innovazione sulle Patologie Surrenaliche, AOU Careggi, Florence, Italy.
5. ENS@T Center of Excellence, Florence, Italy.
6. Institute for Diabetes and Cancer, Helmholtz Zentrum München, Neuherberg, Germany.

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors notable for their high heritability and metastatic potential. Emerging data suggest that the tumor microenvironment (TME), particularly cancer-associated fibroblasts (CAFs), plays a critical role in PPGL progression, though the mechanisms remain unclear. This study investigates the interactions between PPGL cells and CAFs to uncover new therapeutic targets.

The study utilizes the hPheo1 human pheochromocytoma cell line and its *SDHB*-deficient variant, hPheo1 SDHB KO, along with CAFs isolated from four patients—two with *SDHB*-mutant paragangliomas and two with *SDHB*-wildtype pheochromocytomas. Functional assays were performed to evaluate the effects of CAFs on tumour cell behaviour. In 2D migration assays, tumour cell motility was assessed upon exposure to CAF-derived conditioned media (CM). For 3D invasion assays in Matrigel, the invasive capacity of tumour spheroids was compared among three conditions: serum-free media, CAF-CM, and direct tumor cells-CAF co-cultures (1:1 ratio). Alongside functional assays, candidate cytokine profiling is in progress. A TurboID-based proximity labelling strategy is being implemented via lentiviral transduction to generate the appropriate tumor cell models for secretome analysis using mass spectrometry.

In 2D migration assays, CAF-CM enhanced the motility of SDHB KO cells, while parental cells showed a distinct response, suggesting *SDHB* status alters the sensitivity to CAF-derived signals. In 3D invasion assays, direct co-culture with CAFs consistently led to the highest tumor cell invasion across all conditions, indicating that direct cell–cell interaction and crosstalk may modulate CAF secretory profiles and enhance tumor invasiveness.

These findings highlight a pivotal role for CAFs in enhancing the invasive behavior of PPGL cells, particularly through direct interaction and in an *SDHB*-dependent manner. This supports the hypothesis that the TME actively contributes to tumor progression and suggests that targeting CAF-tumor cell interactions may present a novel therapeutic strategy for PPGLs.

Elevated ZWILCH Expression Is Associated with Poor Prognosis in Adrenocortical Carcinoma PatientsElevated ZWILCH Expression Is Associated with Poor Prognosis in Adrenocortical Carcinoma Patients

Małgorzata Blatkiewicz, Marta Szyszka, Anna Olechnowicz, Hanna Komarowska, Marcin Ruciński

Zwilch kinetochore protein (ZWILCH) plays a key role in proper cell proliferation. The upregulation of the *ZWILCH* gene was observed in many types of cancers, but the association of ZWILCH with adrenocortical carcinoma (ACC) was not investigated so far. The main aim of the presented study was to verify if the enhanced level of the *ZWILCH* gene can be used as a diagnostic marker for ACC development and progression, as well as a predictor of survival time for ACC patients. The performed analyses included investigation of the *ZWILCH* expression profile in tumors with publicly available TCGA (The Cancer Genome Atlas) datasets and transcriptomic data from the Gene Expression Omnibus (GEO) database, as well as, in human biological samples of normal adrenal, adrenocortical carcinoma and in commercially available tissue microarrays. The findings demonstrate statistically significant higher *ZWILCH* gene expression in ACC tissue in comparison with normal adrenal glands. Furthermore, there is a strong correlation between *ZWILCH*upregulation and tumor mitotic rate and the probability of patient survival. The enhanced *ZWILCH*level is also connected with the activation of genes involved in cell proliferation and the inhibition of genes related to the immune system. This work contributes to a better understanding of the role of ZWILCH as an ACC biomarker and diagnostic tool.

Analysis of serum metanephrine levels in adrenocortical carcinoma, adrenal adenoma and pheochromocytoma – a retrospective, tertiary center study

Malgorzata Bobrowicz*, Zuzanna Roszkowska*, Adrianna Gladka, Katarzyna Slominska, Urszula Ambroziak

*contributed equally

Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Poland Banacha 1a, 02-097, Warsaw, Poland

Background:
Measurement of plasma free metanephrines or urinary fractionated metanephrines to exclude pheochromocytoma (PCC) is recommended in all patients with adrenal lesions that lack typical features of a benign adenoma. While markedly elevated metanephrine levels are considered specific for PCC, it has been proposed that mildly elevated levels (<2-fold above the upper reference limit) may occur in patients with adrenocortical carcinoma (ACC), particularly in those with large tumors, and may be non-specific. Additionally, a few published case reports have described elevated plasma metanephrine levels in patients with ACC. These observations prompted further investigation of metanephrine levels in a cohort of patients with ACC, PCC, and adrenocortical adenoma (ACA).

Methods:
We retrospectively analyzed plasma concentrations of metanephrine, normetanephrine, and methoxytyramine in patients with histopathologically confirmed adrenal tumors. The cohort included individuals with PCC (n=30), ACA (n=30), and ACC (n=20). Clinical parameters such as tumor volume, age, and cortisol secretion status were recorded. Group comparisons were performed using the Kruskal–Wallis test, and receiver operating characteristic (ROC) curve analysis was conducted to evaluate the discriminatory performance of each plasma marker.

Results:
Plasma metanephrine and normetanephrine levels were significantly elevated in PCC compared to ACA and ACC ($p<0.001$). Median normetanephrine concentrations exceeded 6000 pg/mL in PCC, whereas levels remained consistently below 1000 pg/mL in both ACA and ACC. Methoxytyramine levels were also elevated in PCC, although with some intergroup overlap. While ACCs were associated with larger tumor volumes, these did not correlate with increased plasma metanephrine levels. Cortisol secretion was observed in subsets of ACA and ACC but had no apparent effect on metanephrine profiles. ROC analysis demonstrated excellent discriminatory performance of normetanephrine for identifying PCC (AUC = 0.96).

Conclusion:
Our findings confirm that plasma normetanephrine is a highly specific and sensitive biomarker for PCC, with minimal overlap in ACA and ACC. ACC, even when large or cortisol-secreting, generally does not lead to elevated plasma metanephrine levels. These results support the continued use of metanephrine testing in the preoperative evaluation of adrenal masses and suggest that elevated metanephrines in ACC represent an exception rather than a rule. However, the primary limitation of this study is the relatively small sample size. Larger, multicenter studies are warranted to validate these findings.

Ectopic Cushing of unknown origin

Barbara Bromińska MD, PhD[1#], Prof. Nadia Sawicka-Gutaj MD, PhD [1#], Prof. Ewelina Szczepanek – Parulska MD, PhD [1], Tomasz Wierzbicki MD, PhD[2], Małgorzata Janicka – Jedyńska MD, PhD[3], Prof. Marek Ruchała MD, PhD [1]

# Those authors contributed equally

1 Department of Endocrinology, Metabolism and Internal Medicine, Poznan University of Medical Sciences, 60-355 Poznan, Poland
2 Department of General, Gastroenterological and Endocrine Surgery, Poznan University of Medical Sciences, 60-355 Poznan, Poland
3 Department of Clinical Pathomorphology, Poznan University of Medical Sciences, 60-355 Poznan, Poland

Introduction
Ectopic ACTH Syndrome (EAS) represents a rare endocrine disorder marked by chronic hypercortisolaemia. EAS accounts for approximately 9-18% of ACTH-dependent Cushing's syndrome cases. The ectopic ACTH/CTR-producing neoplasms can originate in various tissues, each displaying unique histopathological traits and varying degrees of malignancy.

Presentation
A 73-year-old women was admitted to the cardiology unit with suspected NSTEMI, which was ruled out. The patient was then referred to endocrinology due to hypercortisolaemia. Ectopic Cushing syndrome was conformed. We performed extensive imaging including CT scans of the abdomen, pelvis, and chest, revealing adrenal enlargement without a distinguishable mass. Additional diagnostics included a whole-body scan, SPECT imaging (Figure 1B), Gallium-68 labelled somatostatin receptor PET/CT, MRI of the pituitary gland and bronchoscopy. However, no lesions indicating ectopic ACTH secretion were identified.

Management
Metyrapone therapy was initiated but required progressively increasing doses, reaching 2500 mg daily before adrenalectomy qualification. Due to the therapy's limited effectiveness and the life-threatening condition, the patient underwent bilateral adrenalectomy. Histological analysis revealed diffuse adrenal cortex hyperplasia with immunohistochemical staining showing ACTH negativity and CRH positivity. Hydrocortisone replacement therapy was implemented. After adrenalectomy ACTH levels normalized and remained stable during a five-year observation period.

Conclusions
Management of EAS is demanding. In severe cases, rapid diagnostic intervention is critical, with the primary objective being the regulation of hypercortisolism. Identifying the tumour responsible for ectopic Cushing syndrome remains a complex challenge.

Correlation Between Depressive Dimension And Sleep Disturbance/Disorder In Overt Hypercortisolism/Cushing's Syndrome And Mild Autonomous Cortisol Secretion (MACS)

Bronzatti Liliana[1,2], Arena Jan [2], Aloini Maria Elena [1], Maggio Roberta[1], Lionetto Luana[3], Del Casale Antonio [2], Stigliano Antonio [1]

[1]Endocrinology, Department of Clinical and Molecular Medicine, Sant'Andrea University Hospital, Faculty of Medicine and Psychology, "Sapienza" University of Rome, 00189 Rome, Italy, [2]Psychiatry, Department of Neuroscience, Mental Health and Sensory Organs, Faculty of Medicine and Psychology, "Sapienza" University of Rome, 00189 Rome, Italy.

Background. Sleep disturbances, cognitive dysfunctions, and depression are commonly observed in Cushing's syndrome (CS) and mild autonomous cortisol secretion (MACS). However, the distinct neuropsychological correlates of these diseases remain poorly explored.

Methods. We conducted a retrospective cross-sectional observational study on a clinical sample of patients diagnosed with hypercortisolism who had been receiving pharmacological treatment for at least six months. To examine the relationship between sleep disturbances and depressive symptoms, we applied ANOVA and binomial logistic regression analyses. Sleep quality and depressive symptoms were assessed using validated self-report psychometric tools, specifically the Pittsburgh Sleep Quality Index (PSQI) and the Beck Depression Inventory-II (BDI-II).

Results. We included 52 patients, 28 in the Mild Autonomous Cortisol Secretion subsample and 24 in the Cushing subsample. Our findings indicate that depressive symptoms significantly contribute to the variance in insomnia, particularly affecting sleep disturbances and the use of hypnotic medications. Binomial logistic regression analysis revealed that depressive symptoms had a stronger predictive value than diagnosis alone in determining membership in the subgroup of patients with sleep disturbances.

Conclusion. Insomnia is highly prevalent in patients with hypercortisolism, with undiagnosed depressive symptoms contributing to sleep disturbances and the need for medication. Screening for depressive symptoms and sleep disturbances is essential for tailored treatment in both populations. The psychopathological burden is similar in both conditions, highlighting the need for further research on MACS Mild Autonomous Cortisol Secretion to refine its characterization and therapeutic targets.

A new inactivating mechanism of *SDHx* genes undetectable by conventional sequencing technologies

Moyret Timothée[1], Lussey-Lepoutre Charlotte[2,3], Auribault Karine[1], Favier Judith[3], Burnichon Nelly[1,3], Gimenez-Roqueplo Anne-Paule[1,3], Buffet Alexandre[1,3]

[1] Assistance Publique-Hôpitaux de Paris (AP-HP) Centre, Hôpital Européen Georges Pompidou, Département de Médecine Génomique des Tumeurs et des Cancers, Fédération de Génétique et de Médecine Génomique F-75015 Paris, France.
[2] Sorbonne Université, AP-HP, Hôpital Pitié-Salpêtrière, Service de Médecine Nucléaire, 75013 Paris, France.
[3] Inserm, Centre de recherche des Cordeliers, Université Paris-Cité, Sorbonne Université, Equipe Labellisée Ligue Contre le Cancer, Paris, France.

Paragangliomas (PGLs) are rare neuroendocrine neoplasms, frequently associated with alterations in the *SDHx* genes. However, some familial cases remain unexplained after conventional genetic investigations by NGS, highlighting the need for broader diagnostic approaches.

We report herein a family with multiple cervical paragangliomas in a father and son. Immunohistochemical analysis of the tumours showed a loss of SDHB protein expression, strongly suggestive of an altered *SDHx* gene. However, conventional molecular analyses, including trio whole genome sequencing, were negative. In this context, long read sequencing (Oxford Nanopore technology on a PromethION sequencer) was carried out in this family. This analysis identified the insertion of an SVA-type retrotransposon (SINE-VNTR-Alu) of around 2,700 bp within intron 3 of the *SDHD* gene, with concordant familial segregation. Genomic and transcriptomic analysis of tumour tissues revealed a predominance of the mutated allele in favour of a loss of heterozygosity and the presence of abnormal *SDHD* gene mRNAs, confirming the splicing anomaly predicted by *in silico* tools.

This is the first time that the insertion of a large transposable element has been demonstrated in a PGL susceptibility gene. This event, undetectable by the usual diagnostic tools, highlights the value of long read sequencing for the identification of complex structural variations, particularly in patients with a strong suspicion of a genetically determined form without a molecular diagnosis.

Adrenal steroidal response to prolonged stimulation with ACTH 1-24 in healthy subjects and patients with Cushing's disease in remission

Florian Busch, Sonja Kunz, Sharmilee Vetrivel, Martin Bidlingmaier, Martin Reincke, Silviu Sbiera, Andrea Oßwald-Kopp, Anna Riester

Patients with Cushing's disease develop after successful transsphenoidal surgery a prolonged phase of adrenal insufficiency. Recovery takes usually one to two years, and patients may complain of clinical symptoms even after biochemical normalization.
Furthermore, there are hints for sexual dimorphism in the adrenal stress response.
This study aims to investigate the sensitivity and capacity of the adrenal stress response upon stimulation with increasing doses of Synacthen® (ACTH 1-24) during a prolonged ACTH stimulation test.

Our interventional clinical study will include 12 male healthy probands, 12 female healthy probands and 12 female patients with Cushing's disease after remission.
After suppression of the endogenous ACTH production with dexamethasone the study starts with a continuously infusion of very low dose Synacthen® (25ng/m^2 BSA/h, e.g. 43ng/h in BSA 1,73m^2). The dose is doubled hourly to the highest dose of 800 ng/ m^2 BSA/h (e.g. 1384ng/h in BSA 1,73m^2). The steroidome, including 15 different steroids and salivary 11-oxy-androgens, is measured every hour with LC MS/MS to investigate the sensitivity to ACTH. After that a final bolus injection of 200µg Synacthen® follows to test the maximum capacity of the adrenal stress response.

Our preliminary data in healthy probands show relevant differences in the threshold to ACTH 1-24 stimulation: Whereas cortisol increases already in response to the lowest dose of 25ng/m^2 BSA/h, responses of 11-deoxycortisol, 21-deoxycortisol and DHEA were observed after the infusion of 200ng/m^2 BSA/h.
In salivary samples, we observed a 5-fold increase of over baseline 11-ketotestosteron and 11ß-hydroxyandrostendion had following ACTH stimulation.

The sensitivity to ACTH stimulation is different between steroid hormones. The steroid profile of additional healthy male and female probands and Cushing's disease patients will allow statistically robust analysis in the following months.

SDHB Deficiency in Human Pheochromocytoma Cells Enhances TSPO Expression and Mitochondrial-Nucleus Contacts (NAM)

Francesca Amore[1,2,5*], Eva Sidlauskaite[2*], Lorenzo Zanatta[3,9], Letizia Canu[1,3,4,5], Mario Maggi[1,3,4,5], Jiri Neuzil[6,7,8], Elena Rapizzi[3,4,5,9*] and Michelangelo Campanella[2,9,10*]

[1] Department Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy;
[2] William Harvey Research Institute, Queen Mary University of London, London, United Kingdom;
[3] Endocrinology Unit, Careggi University Hospital, Florence, Italy;
[4] Centro di Ricerca e Innovazione sulle Patologie Surrenaliche, AOU Careggi, Florence, Italy;
[5] ENS@T Center of Excellence, Florence, Italy;
[6] Institute of Biotechnology, Czech Academy of Sciences, Prague-West, Czech Republic;
[7] School of Pharmacy and Medical Science, Griffith University, Southport, Queensland, Australia;
[8] Faculty of Science and 1st Medical Faculty, Charles University, Prague, Czech Republic;
[9] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy;
[10] Institute Gustave Roussy, Villejuif, France;
[11] Department of Biomedical Sciences, University of Padua, Padova, Italy.
*Equally contributed

Introduction: Succinate dehydrogenase (SDH) is a key player in the tricarboxylic acid cycle and the electron transport chain, acting as a crucial tumour suppressor gene with its four subunits (A-D) associated with pheochromocytomas and paragangliomas (PPGL). Notably, it is mutations in the SDHB subunit that significantly heighten the risk of metastasis. Effective communication among intracellular organelles is vital for preserving cellular balance, coordinating metabolic processes, and responding to environmental shifts. This intricate communication often occurs through specialised contact sites. Recent studies have illuminated the role of the mitochondrial translocator protein (TSPO) in enhancing pro-survival responses by promoting connections between mitochondria and the nucleus, known as Nucleus Associated Mitochondria (NAM)[1,2]. Our study aimed to probe the effects of NAM in an SDHB-mutated human pheochromocytoma cell line[3,4].

Results: Remarkably, our electron microscopy findings indicated that SDHB knockout (KO) cells displayed disorganized mitochondrial cristae and reduced distances between mitochondria and the nucleus. Western blot analysis highlighted a significantly increase of TSPO levels in SDHB KO cells. Immunofluorescence and cell fractionation techniques revealed significantly increased TSPO localization in the perinuclear region of mutated cells compared to their wild-type (WT) counterparts. The Proximity Ligation Assay (PLA) underscored TSPO's critical role in NAM formation, showing a dramatic decrease in contact numbers upon TSPO knockdown. Additionally, the knockdown of TSPO not only inhibited cancer cell migration but also curtailed proliferation and viability, strongly suggesting that this protein plays a pivotal role in fostering an invasive cancer phenotype.

Conclusion: Collectively, these findings provide compelling evidence that higher TSPO levels are sufficient to drive various malignant traits. Targeting TSPO with specific ligands, alongside other agents aimed at mitochondria, could emerge as a groundbreaking therapeutic strategy.

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Adrenal insufficiency & adrenal neoplasms: when things get messy

Borges-Canha, Marta[1,2] & Gonçalves, Juliana[1,2]; Festas-Silva, Diana[1,2]; Meira, Inês [1,2]; Ferreira, Patrícia[1,2]; Costa, Mafalda[1]; Queirós, Joana[1]

1 Serviço de Endocrinologia, Diabetes e Metabolismo, ULS São João, Porto, Portugal
2 Faculdade de Medicina da Universidade do Porto, Porto, Portugal

Introduction: Diagnosing primary adrenal insufficiency (PAI) can be challenging, particularly when adrenal neoplasms are the underlying cause. Adrenal neoplasms often require thorough evaluation to rule out malignancy and hormonal excess. While most are benign, they can represent metastatic disease or, less commonly, primary adrenal malignancy. In patients with hematologic malignancies, the possibility of adrenal infiltration becomes a critical differential diagnosis.

Clinical case: This is the case of a 62 year-old man who's past medical history includes acute myeloid leukemia (AML) diagnosed in December 2024 and is otherwise unremarkable. In January 2025, he was admitted in the Hematology Department for AML treatment, and after a few days an endocrinology consultation was requested for *de novo* hyponatremia (Na^+ 125mg/dL). At the clinical examination, he reported fatigue and anorexia. The patient was hypotensive, tachycardic and was slightly dehydrated. Baseline laboratory results for hyponatremia revealed normal glucose, thyroid function, total bilirubin, and estimated glomerular filtration rate. However, significant decreases in albumin (28.2 g/L) and total protein (49.3 g/L) were observed. Serum and urine osmolalities were deemed unreliable due to fluid therapy administration. Low morning cortisol (2.4 µg/dL) and elevated adrenocorticotropic hormone (ACTH 136 pg/mL) were recorded, strongly suggesting of PAI. A thoracic CT scan identified bilateral adrenal nodules (24 mm right, 21 mm left), raising suspicion for adrenal infiltration by the underlying AML. After starting hydrocortisone and fludrocortisone, the patient progressively started feeling better, tensional profile became normal and hyponatremia was resolved.

Conclusion: This case report explores the unique presentation of PAI, likely due to infiltration by leukemia. We emphasize the need for a comprehensive approach, combining imaging and biochemical assessments together with the clinical setting.

Presurgical Succinate MetAstatic Risk Tool (P-SMART) in paragangliomas

Elena Rapizzi[1,2,3]*, Lorenzo Zanatta[1,2,3]*, Alice Santi[4], Fabio Staderini[1], Niccolò Galeotti[5], Tonino Ercolino[5], Francesca Amore[1,2,3], Clotilde Sparano[4], Mario Maggi[2,3,4], Letizia Canu[2,3,4]

[1] University of Florence, Dept. Experimental and Clinical Medicine, Florence, Italy;
[2] Centro di Ricerca e Innovazione sulle Patologie Surrenaliche, AOU Careggi, Florence, Italy;
[3] ENS@T Centre of Excellence, Florence, Italy;
[4] University of Florence, Dept. Experimental and Clinical Biomedical Sciences, Florence, Italy;
[5] Careggi University Hospital, Florence, Italy;
*These authors equally contributed to this work

Paragangliomas (PGLs) are rare neuroendocrine tumours characterized by a strong genetic determinism and heterogeneity across more than 20 susceptibility genes. Identifying PGLs with metastatic potential remains one of the greatest challenges, as there are not reliable pathological features to predict their clinical course.

This retrospective cross-sectional study investigates serum succinate as a novel biomarker for PGL genetic clustering, and metastatic risk assessment.

We enrolled 70 PGL patients evaluated at the University Hospital of Florence between 2006 and 2023. Clinical, biological, and imaging data were collected. Germline genetic variants were analysed via Sanger sequencing or NGS through a targeted panel of susceptibility genes. Serum succinate levels were quantified using GC-MS.

Succinate levels were significantly elevated in patients with Cluster 1 genetic variants ($p<0.001$), extra-adrenal tumours ($p=0.006$), and metastatic disease ($p=0.024$). A succinate threshold of ≥8.85μM predicted Cluster 1 genetic variants with 92.9% sensitivity and 55.4% specificity, while a level ≥8.95μM identified metastatic disease with 92.3% sensitivity and 56.1% specificity.

We developed a novel preoperative risk assessment tool, the P-SMART (Preoperative Succinate MetAstatic Risk Tool), combining serum succinate levels, tumour size, and location. In our cohort P-SMART demonstrated superior performance in predicting metastatic disease compared to the ASES score (AUC 0.891 vs. 0.752, $p=0.005$), with a threshold score >5.5 achieving 72.7% sensitivity and 83% specificity.

Though limited by sample size and retrospective design, our findings suggest that succinate could be a minimally invasive biomarker with dual utility: differentiating Cluster 1-associated lesions from other genetic Clusters and enhancing preoperative risk stratification when integrated into a multiparametric score, such as P-SMART.

Despite larger prospective studies are needed to validate succinate's role in the management of these challenging tumours, P-SMART could optimize clinical decision, particularly for biochemically silent tumours and could refine patient selection for whole-body imaging, reducing unnecessary radiation exposure, and informing surveillance strategies.

Exploring the impact of cortisol secretion and pathogenic variants on tumor-associated macrophage (TAM) polarization in adrenocortical adenomas

Catalano Rosa[1,2], Cristofolini Giacomo[1,3], Secener Ali Kerim[4], Detomas Mario[1], Schauer Marc P.[1], Kimpel Otilia[1], Herterich Sabine[5], Kircher Stefan[6], Ronchi Cristina L.[7], Sbiera Silviu[1], Peverelli Erika[2,8], Mantovani Giovanna[2,8], Fassnacht Martin[1], Altieri Barbara[1,9]

[1]Division of Endocrinology and Diabetes, Department of Internal Medicine I, University Hospital, University of Würzburg, Würzburg, Germany
[2]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy
[3]Division Department of Biomedical Sciences, Humanitas University, Milan, Italy
[4]Max Delbrück Center for Molecular Medicine, Berlin, Germany
[5]Central Laboratory University Hospital Würzburg, Würzburg, Germany.
[6]Institute of Pathology, University of Würzburg, Würzburg, Germany
[7]Department of Metabolism and System Science, University of Birmingham, Birmingham, UK
[8]Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[9]Bavarian Cancer Research Center (BZKF), University Hospital, University of Würzburg, Würzburg, Germany

Background: While prevalence of M2-macrophages has been described in normal adrenal gland (NAG) and adrenocortical carcinoma, their role in adrenocortical adenomas (ACA) remains poorly characterized.
Aim: To characterize tumor-associated macrophages (TAM) in ACA and investigate associations with clinicopathological features.
Methods: Immunohistochemistry for CD163 and CD206 (M2-specific markers), CD68 (pan-macrophage marker), and CD16 (immune-infiltrate marker) was performed on 102 ACA and 16 adjacent-NAG. Immunostaining was quantified using QuPath-0.5.1. Pathogenic variants in *CTNNB1, PRKACA,* and *GNAS* were assessed by Sanger sequencing. TAM associations with clinical, hormonal parameters and circulating monocytes were analyzed. Myeloid profile was further explored in single-nucleus RNA sequencing (snRNA-Seq, DOI:10.1002/ctm2.1798) using Seurat.
Results: In NAG, median CD16 (8.23%) and CD206 (6.58%) expression were higher than CD68 (5.59%; p=0.006 and p=0.02), while CD163 expression was similar. In ACA, CD16 (6.80%) and CD163 (6.86%) were more expressed than CD206 and CD68 (1.60% and 1.69%, all p<0.001), with CD68 positively correlating with CD163 (ρ=0.41) and CD16 (ρ=0.34) (p<0.001).
Compared to NAG, ACA showed reduced CD206 expression, confirmed by snRNA-Seq (p<0.001). Consistently, CD163/CD206 ratio was higher in ACA (3.11 vs 0.84, p<0.001) indicating a shift toward CD163 polarization.
In ACA, CD16 expression correlated with circulating monocytes (ρ=0.29, p=0.02). Cortisol excess was associated with lower CD68 and CD16, and higher CD163/CD68 and CD163/CD206 ratios (all p<0.05). In women, CD163/CD206 positively correlated with cortisol after 1 mg-DST (ρ=0.27, p=0.04), and CD206/CD16 was lower in cortisol-producing ACA (CPA) (p=0.01), suggesting sex-specific immune modulation.
ACA with *PRKACA* showed lower CD206 and CD16 (p<0.01), and higher CD163/CD206 ratio (17.42 vs 3.21, p=0.002) compared to those without. These associations remained significant within CPA and were confirmed by multivariate logistic regression, supporting a PRKACA-driven effect.
Conclusion: TAM in ACA display distinct polarization, with elevated CD163/CD206 ratio. This is influenced by cortisol levels, sex, and *PRKACA*, highlighting a complex immune microenvironment.

Rationally designed cholesterol-coated peptide Nanosponge as a novel therapeutic strategy in the management of Adrenocortical Carcinoma

Ritihaas Surya Challapalli [1], Mariam Hassany [1, 2], Obdulia Covarrubias-Zambrano [3], Sarah Feely [1], Solene Fiachetti [1], Michaela Luconi [2], Martin O'Halloran [6], Constanze Hantel [4, 5], Punit Prakash [8, 9], Stefan H. Bossmann [3], Michael Conall Dennedy [1]

[1] Discipline of Pharmacology and Therapeutics, School of Medicine, University of Galway, Galway, H91 V4AY, Ireland
[2] Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy
[3] Department of Cancer Biology, The University of Kansas Medical Center, Kansas City, KS. USA
[4] Department of Endocrinology, Diabetes, and Clinical Nutrition, University Hospital Zurich, Zurich, Switzerland
[5] Medizinische Klinik und Poliklinik III, University Hospital Carl Gustav Carus Dresden, Dresden, Germany
[6] Centre for Microscopy & Imaging, University of Galway, Ireland
[7] Translational Medical Device Lab, University of Galway, Galway, Ireland
[8] Department of Electrical and Computer Engineering, Kansas State University, Manhattan, KS. USA
[9] Department of Biomedical Engineering, The George Washington University, Washington, DC, USA

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Introduction: Management of adrenocortical carcinoma (ACC) is difficult, often diagnosed at an advanced stage. Surgical resection is potentially curative for localized ACC, but recurrence rates remain high (75–85%). Mitotane, the only approved drug for advanced ACC, has limited effectiveness (<30% of cases), a narrow therapeutic window, and poor tolerability. Nanotheranostics offers a novel approach, using nanomaterials for combined diagnosis, drug delivery, and treatment. Most FDA-approved nanomedicines rely on the enhanced permeability and retention (EPR) effect, which enables passive nanoparticle accumulation in tumours. While this enhances drug delivery and reduces side effects, EPR-based delivery varies by patient and tumour type. Targeted nanoparticles could address this variability. Since ACC cells depend heavily on cholesterol—for steroid synthesis, membrane integrity, and energy—we developed tri-maleimide peptide-based nanosponges (NS), functionalized with two cholesterol molecules and a Rhodamine B fluorescent tag.
Methods: NS were tested for uptake and cytotoxicity in three ACC cell lines: H295R (chemo-sensitive), HAC-15 and MUC-1 (chemo-resistant). Sytox Blue staining assessed cell death, while SR-B1 involvement in NS uptake was tested using the inhibitor BLT-1. Caspase dependence was examined using z-VAD (a pan-caspase inhibitor), and markers of apoptosis, ER stress, and autophagy (Caspases 3/8, CHOP, LC3) were evaluated. LC-MS/MS measured steroidogenesis.
Results: NS showed rapid uptake and significant cytotoxicity: at 20 µg/mL in H295R ($p<0.01$), 50 µg/mL in HAC-15 ($p<0.01$), and 100 µg/mL in MUC-1 ($p<0.05$). BLT-1 did not block NS uptake, suggesting alternative entry mechanisms. Confocal imaging showed mitochondrial accumulation. Cell death occurred via a caspase-independent pathway—caspase-3 was not upregulated, z-VAD had no protective effect, and autophagy markers increased. NS also reduced forskolin-stimulated cortisol production in H295R cells.
Conclusion: These cholesterol-functionalized NS offer a promising theranostic strategy for ACC, inducing potent, targeted cytotoxicity with reduced toxicity. Future studies will focus on loading NS with lipid-soluble drugs like mitotane.

Screening for Pheochromocytoma in Neurofibromatosis type 1: Defining the Most Effective Approach

Contarino Andrea[1,2], Rodari Giulia[2], Cesaretti Claudia[3], Lopez Gianluca[4,5], Cassinotti Elisa[6], Renda Riccardo[2,7], Boni Luigi[6,7], Natacci Federica[3], Mantovani Giovanna[2,7], Palmieri Serena[2]

[1]Department of Health Sciences, University of Milan, Milan, Italy
[2]Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[3]Medical Genetics Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[4]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy
[5]Division of Pathology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[6]General and Minimally-Invasive Surgery Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[7]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

Background: Neurofibromatosis type 1 (NF1) is a complex genetic syndrome associated with an increased risk of malignancies. Among endocrine tumours, pheochromocytoma (PHEO) is the most common, with a prevalence ranging from 0.8% to 14.6% depending on the screening strategy. Currently, NF1 guidelines do not recommend routine screening for PHEO in asymptomatic individuals.

Methods: We conducted a retrospective study involving adult NF1 patients referred to our Institution since January 2014. Clinical records were reviewed to assess screening practices based on symptoms, urinary fractionated metanephrines (uFM) and/or adrenal imaging. The diagnosis of PHEO was confirmed histologically after surgery.

Results: A total of 312 NF1 patients (60.6% female) underwent PHEO screening. PHEO was diagnosed in 20 patients (6.4%), with median age at diagnosis of 41.4 years. Only 2 patients underwent PHEO screening because of suggestive symptoms, both affected by PHEO (100%). Among the 195 patients (62.5%) screened primarily with uFM alone, metanephrines levels were within normal limits in 190 (97.4%), while elevated levels (>1× upper limit of normal, ULN) were observed in 5 patients (2.6%), all of whom were diagnosed with PHEO. Adrenal imaging was the initial investigation in 115 patients (36.9%), leading to the detection of PHEO in 13 out of 17 patients with incidentally discovered adrenal lesions (11.3%). Only 7 out of 20 PHEO patients (35%) reported symptoms of catecholamine excess. Nonetheless, all cases had adrenergic hypersecretion (median metanephrines level 1.886×ULN, IQR 1.539–2.340), with concomitant normetanephrines elevation in 5 patients. Synchronous bilateral PHEOs were found in 2/20 patients (10%), multifocal unilateral PHEO in 1/20, and metastatic disease in 1/20 (5%).

Conclusions: We reported a high prevalence of PHEO in asymptomatic NF1 patients. These tumours are often mildly secreting, which may contribute to underdiagnosis. Thus, a combined screening approach – including both uFM testing and imaging – may represent the most effective solution.

Systemic inflammation in a large cohort of non-secreting and cortisol-secreting adrenal incidentalomas: association with cortisol excess burden

Coscia Kimberly[1,2], Bovo Giulia[3,4], Donnarumma Francesca[1,2], Ramundo Giada[1,2], Tizianel Irene[3,4], Vicennati Valentina[1,2], Pagotto Uberto[1,2], Ceccato Filippo[3,4], Di Dalmazi Guido[1,2]

1 Division of Endocrinology and Diabetes Prevention and Care, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
2 Department of Medical and Surgical Sciences (DIMEC), Alma Mater Studiorum University of Bologna, Bologna, Italy
3 Department of Medicine DIMED, University of Padova, Padova, Italy
4 Endocrine Disease Unit, University-Hospital of Padova, Padova, Italy

Background. Altered serum inflammation-based scores (SIS) were linked to hypercortisolism, but their relationship with varying cortisol levels in mild autonomous cortisol secretion (MACS) remains unclear.

Aim. To assess the association between SIS and varying degrees of cortisol excess in patients with MACS and non-secreting (NS) benign adrenal incidentalomas.

Methods. We included 899 patients with benign adrenal tumors, classified as NS (n=558) or MACS (n=341) based on cortisol levels after the overnight 1-mg dexamethasone suppression test (DST). At diagnosis, the following SIS were calculated: neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), and systemic immune-inflammation index (SII; platelet count×NLR). Group comparisons were performed using Student's T-test. Associations with cortisol levels were evaluated via Pearson's correlation and generalized linear models adjusted for sex, age, body mass index, glucose metabolism, cardiovascular comorbidities, and smoking status.

Results. Mean age of the study population was 62±12 years, with MACS patients significantly older than NS (p=0.01). NLR, PLR, and SII were significantly higher in MACS compared to NS (p=0.02 for NLR; p=0.024 for PLR; p=0.001 for SII), while LMR showed no significant difference between groups. A positive correlation was observed between cortisol levels after DST and NLR (p<0.01, r=0.124), PLR (p=0.02, r=0.104), and SII (p<0.01, r=0.140). Cortisol levels after DST positively correlated with NLR (r=0.124, p<0.01), PLR (r=0.104, p=0.02), and SII (r=0.140, p<0.01). Higher post-DST cortisol levels were independently associated with increased NLR (B=0.009; 95%CI: 0.001–0.017; p=0.027) and PLR (B=0.007; 95%CI: 0.533–0.670; p=0.003). Female sex was protective against increasing NLR values (B=-0.472; 95%CI: -0.722 to 0.222, p<0.001). Non-smokers showed significantly higher PLR values than smokers (B=0.311; 95%CI: 0.156–0.465; p<0.001).

Conclusion. The significant association of NLR and PLR with cortisol excess, along with greater alterations of SIS in MACS compared to NS, might suggest a potential contribution of cortisol to systemic inflammation.

Serums from patients with autonomous cortisol secretion drive pro-resolving macrophage polarisation

Ana Crastin[1,6,7], Ezra Leander David Onesimu [1], Lorenzo Tucci [1,,8], Alessandra Mangone[1,5] Vittoria Favero[1,4], Chiara Parazzoli[1,5],Oskar Podstawka[1], Mengjie Xu[1,3], Alessandro Prete[1,2,7], Rowan S. Hardy[1,6,7], Cristina L. Ronchi[1,2]

[1]Department of Metabolism and Systems Science, University of Birmingham, Birmingham, UK; [2]Department of Endocrinology, Queen Elizabeth Hospital Birmingham NHS Trust, Birmingham, UK; [3]Taihe Hospital, Hubei University of Medicine, Shiyan, P.R. China, [4]Department of Biotechnology and Translational Medicine, University of Milan, Milan 20010, Italy [5]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy [6]Department of Biomedical Sciences, University of Birmingham, Birmingham[7] National Institute for Health and Care Research (NIHR) Birmingham Biomedical Research Centre, Birmingham, UK; [8]Medical and Surgical Sciences Department, Alma Mater Studiorum University of Bologna, Bologna, Italy

Background: Endogenous Cushing's syndrome (CS) is characterized by chronic cortisol excess, disrupting innate and adaptive immunity. While systemic immunomodulatory effects of CS are known, the direct impact on tissue macrophages is less defined. Moreover, the effects of mild autonomous cortisol secretion (MACS) in incidentally discovered cortisol-producing adrenocortical adenomas (CPA) without clinical stigmata of CS remain poorly understood. We hypothesize that macrophage polarization and activation are altered in both CPA-CS and CPA-MACS.

Methods: Human macrophages were polarized to an M1-like inflammatory state using TNFα (10 ng/ml) and IFNγ (20 ng/ml), followed by co-treatment with 10% patient serum. Samples were collected from 18 patients (12 CPA-MACS [8 women], 6 CPA-CS [4 women]) and 10 age- and sex-matched controls with endocrine-inactive adenomas (EIA). Cytokines and gene expression were assessed via ELISA and RT-qPCR. MTT assay measured mitochondrial viability, and phagocytosis was assessed after 48 hours. Data were correlated with cortisol secretion and clinical characteristics.

Results: Pro-inflammatory marker IL6 was decreased in M1-like polarised macrophages when treated with CPA-MACS(p=0.0705,0.72-fold change) and CPA-CS patients serums (p=0.0039,0.5-fold change) when compared to EIA, while gene expression was non-significant decreased in CPA-MACS(p=0.4549,0.815-fold change) and CPA-CS(p=0.2474,0.694-fold change) groups.

CD163, an M2-like marker, increased slightly in CPA-CS (p=0.2578,1.324-fold change) but not in CPA-MACS (p=0.9709,1.037-fold change) compared with EIA. CD163 expression shown a trend toward a correlation with cortisol post-dexamethasone suppression (r=0.2626,p=0.1857) and tumour size(r=0.2482,p=0.2029). TGFβ was elevated in CPA-CS(p=0.0121) at protein levels. The IL6/CD163 ratio was increased in CPA-CS(p= 0.0027,2.41-fold change). Also interestingly, mitochondrial viability increased in CPA-MACS(p=0.0585) and CPA-CS(p=0.0047) compared to EIA. Phagocytosis was impaired in CPA-MACS(p=0.0138) and CPA-CS(p=0.0762) compared to EIA.

Conclusions: Both CPA-MACS and CPA-CS groups showed suppressed M1-like activity and a shift toward M2-like polarization, with reduced phagocytosis and altered mitochondrial function. These findings suggest that even MACS can modulate macrophage behaviour, contributing to immune suppression.

Serum inflammation-based scores predict clinical outcome and response to adjuvant mitotane treatment in patients with adrenocortical carcinoma: an ENSAT multicenter study

Cristofolini Giacomo[1,2], Detomas Mario[1], Puglisi Soraya[3], Bahrani Fard Bahar [4], Nekić Anja Barac[5], Elhassan Yasir S.[6], Bobrowicz Malgorzata[7], Angelousi Anna[8], Kimpel Otilia[1], Novizio Roberto[9], Canu Letizia[10], Lasolle Helene[11], Ceccato Filippo[12], Pittaway James[13], Mangone Alessandra[14], Araujo-Castro Marta[15], Basile Vittoria[3], Powell Cayse[4], Ziolkowska Barbara[16], Quinkler Marcus[17], Stigliano Antonio[18], Lania Andrea G.[2], Luconi Michaela[10], Kaltsas Gregory[19], Ambroziak Urszula[7], Kastelan Darko[5], Bancos Irina[4], Terzolo Massimo[3], Ronchi Cristina L.[6], Fassnacht Martin[1,20], Altieri Barbara[1,20]

[1] Department of Medicine, Division of Endocrinology and Diabetes, University Hospital, University of Würzburg, Würzburg, Germany.
[2] Endocrinology, Diabetology and Medical Andrology Unit, IRCCS Humanitas Research Hospital, Rozzano-Milan, Italy.
[3] Department of Clinical and Biological Sciences, Internal Medicine, San Luigi Hospital, University of Turin, Turin, Italy.
[4] Division of Endocrinology and Metabolism, Department of Medicine, Mayo Clinic, Rochester, MN, USA. [5] Department of Endocrinology, University Hospital Center Zagreb, Zagreb, Croatia.
[6] Department of Metabolism and Systems Science, University of Birmingham, Birmingham, United Kingdom.
[7] Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Warsaw, Poland.
[8] First Department of Internal Medicine, Unit of Endocrinology, Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece.
[9] Unit of Endocrinology, Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore-Fondazione Policlinico "Gemelli" IRCCS, Rome, Italy.
[10] Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy.
[11] Hospices Civils de Lyon, Groupement Hospitalier Est, Endocrinology Federation, Lyon, France.
[12] Endocrinology Unit, Department of Medicine, University of Padua, Italy
[13] Centre for Endocrinology, William Harvey Research Institute, Faculty of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom.
[14] Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.
[15] Endocrinology & Nutrition Department. Hospital Universitario Ramón y Cajal. Instituto de Investigación Biomédica Ramón y Cajal (IRYCIS), Madrid, Spain.
[16] Maria Skłodowska-Curie National Institute of Oncology, Gliwice Branch, Poland.
[17] Endocrinology in Charlottenburg, Berlin, Germany.
[18] Azienda Ospedaliera Sant'Andrea, Endocrinology, Department of Clinical and Molecular Medicine, Sant'Andrea University Hospital, Sapienza University of Rome, Rome, Italy.
[19] First Department of Propaedeutic and Internal Medicine, Laikon Hospital, National and Kapodistrian University of Athens, Athens, Greece.
[20] Bavarian Cancer Research Center (BZKF), University Hospital, University of Würzburg, Würzburg, Germany

Background: Inflammation plays a key role in cancer progression, and inflammation-based scores have demonstrated prognostic value in various malignancies. In adrenocortical carcinoma (ACC), these scores are predictive in palliative settings, but their relevance during adjuvant mitotane therapy remains unclear.

Aim: To assess the prognostic value of inflammation-based scores in ACC patients receiving adjuvant mitotane.

Methods: Multicenter ENSAT retrospective study including patients with ACC treated with adjuvant mitotane as first-line treatment. Inflammation scores - neutrophil-to-lymphocyte ratio (NLR), derived NLR (dNLR), systemic immune-inflammation index (SII), and pan-immune-inflammation value (PIV) - were calculated at diagnosis (pre-surgery) and at mitotane start. Median values at diagnosis defined low and high levels. Primary endpoints were overall survival (OS) and time to progression (TTP).

Results: A total of 395 patients were included (66% women; median age: 51 years). Median time to mitotane start was 54 days (IQR:32–90 days). At diagnosis, all scores were higher in patients with glucocorticoid excess (n=84) compared to those without (n=104) (median NLR: 3.61 vs 2.53; dNLR:2.31 vs 1.72; SII: 864.1 vs 679.3; PIV: 531.6 vs 343.1; all $p \leq 0.005$). All scores significantly decreased after surgery ($p<0.05$).

At diagnosis, high levels of all inflammation-scores were associated with shorter OS (NLR: HR=3.85, 95%CI=1.95–7.59, $p<0.001$; dNLR: HR=3.19, 95%CI=1.67-6-08, $p<0.001$; SII: HR=1.97, 95%CI = 1.07–3.61, $p=0.03$; PIV: HR=2.11, 95%CI=1.13-3.95, $p=0.02$). However, only NLR and dNLR remained significant at multivariate analysis including ENSAT tumor stage, Ki67 index, and resection status ($p<0.05$).

At mitotane start (n=372), high SII and PIV were associated with shorter TTP (SII: HR=1.36, 95%CI=1.02-1.82, $p=0.03$; PIV: HR=1.40, 95%CI=1.04-1-87, $p=0.02$), with only SII remaining significant in multivariate regression ($p=0.04$).

Conclusion: Inflammation-based scores, including NLR and dNLR at diagnosis and SII at mitotane start, are associated with clinical outcome in patients with ACC undergoing adjuvant therapy. These markers may help improve prognostic stratification in clinical practice.

First-line Platinum-based Chemotherapy with or without Doxorubicine in Advanced Adrenocortical Carcinoma: An ENS@T Multi-center Cohort Study

Crona Joakim[1], Baudin Eric*[2], Kimpel Otilia*[3], Habra Mouhammed Amir*[4], Balderrama-Brondani Vania[4], Kroiss Matthias[5], Laganà Marta[6], Dischinger Ulrich[3], Quinkler Marcus[7], Skogseid Britt[1], Stell Anthony[8], Eriksson Niclas[26], Boudina Maria[9], Wadsley Jonathan[10], Canu Letizia[11], Trofimiuk-Müldner Malgorzata[12], Lamas Cristina[13], Arvat Emanuela[14], De Martino Maria Cristina[15], Sajous Christophe[16], Altieri Barbara[3], Hadoux Julien[2], Haissaguerre Magalie[17], Kastelan Darko[18], Puglisi Soraya[19], Ronchi Cristina L[20], Pittaway James[21], Libe Rossella[22], Fuss Carmina T[3], Lamartina Livia[2], Terzolo Massimo[19], Cosentini Deborah[6], Moog Sophie[2], Berruti Alfredo[6], Fassnacht Martin[3, 23], Beuschlein Felix[5, 24, 25]
*Shared authorship

1) Department of Medical Sciences, Uppsala University, Uppsala, Sweden
2) Gustave Roussy, Université Paris-Saclay, Département d'imagerie, Service d'oncologie endocrinienne, Villejuif, France
3) Department of Medicine, Division of Endocrinology and Diabetes, University Hospital, University of Würzburg, Würzburg, Germany
4) Department of Endocrine Neoplasia and Hormonal Disorders, The University of Texas MD Anderson Cancer Center, Houston, TX, United States
5) Department of Internal Medicine IV, University Hospital, Ludwig-Maximilians-Universität München, München, Germany
6) Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, Medical Oncology, University of Brescia at ASST Spedali Civili, Brescia, Italy
7) Endocrinology in Charlottenburg, Berlin, Germany
8) School of Computing and Information Systems, University of Melbourne, Melbourne, Victoria, Australia
9) Department of Endocrinology, Theagenio Cancer Hospital, Thessaloniki, Greece
10) Weston Park Hospital, Sheffield, UK
11) Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy
12) Department of Endocrinology, Jagiellonian University Medical College, Kraków, Poland
13) Endocrinology Department, Complejo Hospitalario Universitario de Albacete, Albacete, Spain
14) Oncological Endocrinology; City of Health and Science University Hospital; Department of Medical Sciences; University of Turin, Turin, Italy
15) Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II di Napoli, Naples, Italy
16) Hospices Civils de Lyon, Fédération d'Endocrinologie, Université Claude Bernard Lyon 1, Lyon, France
17) Department of Endocrinology and Endocrine Oncology, Haut Leveque Hospital, University Hospital of Bordeaux, Bordeaux, France
18) Department of Endocrinology, University Hospital Centre Zagreb, Zagreb, Croatia
19) Department of Clinical and Biological Sciences, University of Turin at San Luigi Hospital, Orbassano, Italy
20) Department of Metabolism and Systems Science, College of Medicine and Health, University of Birmingham, Birmingham, UK
21) Department of Endocrinology, St Bartholomews Hospital, London, UK
22) Service Endocrinologie, AP-HP, Hôpital Cochin, Paris, France
23) Comprehensive Cancer Center Mainfranken, University of Würzburg, Germany, and National Center for Tumor Diseases (NCT) WERA, Würzburg, Germany
24) Department of Endocrinology, Diabetology and Clinical Nutrition, University of Zürich (USZ) and University of Zürich (UZH), Zürich, Switzerland
25) The LOOP Zurich Medical Research Center, Zurich, Switzerland
26) Uppsala Clinical Research Center, Uppsala, Sweden

Background: Etoposide, doxorubicin, cisplatin and mitotane (EDP-M) is considered as the first-line chemotherapy for metastatic adrenocortical carcinoma (ACC). As many patients experience serious adverse events it has been hypothesized that omitting doxorubicin would increase tolerability, without relevant loss of efficacy.

Patients and methods: A multi-centric cohort study aiming to compare the efficacy of EDP-M with cisplatin, etoposide, and mitotane (EP-M) in a real-world setting. Patients (>18 years) had advanced ACC and started first-line chemotherapy treatment with EDP-M or EP-M between 2010 and 2020. Difference in overall survival was examined using log-rank and Cox regression analyses. Radiological response was evaluated by using local criteria.

Result: 517 ACC patients from 21 centers were included, 392 receiving EDP-M and 125 EP-M. A number of prognostic markers were unbalanced between the groups: Median age was 47 (EDP-M) and 51 years (EP-M, $P = 0.012$) and oligometastatic ACC (Stage IVa and ≤5 metastases) was present in 26% (EDP-M) and 12% (EP-M, $P < 0.001$) of patients. Overall survival for the EDP-M group was 634 days compared to 379 days in the EP-M group (hazard ratio [HR] 0.56 [95% confidence interval 0.45-0.7], $P < 0.0001$). After multiple imputation and adjustment for eight prognostic markers, the overall survival comparison remained favorable for EDP-M with an HR of 0.68 (0.53-0.87), $P = 0.002$. Similarly, overall survival in the EDP-M group was better in both subgroups of oligometastatic (HR 0.49 [0.27-0.88]) and non-oligometastatic patients (HR 0.6 [0.47-0.76]). Response by local radiological criteria was seen in 28% of the EDP-M and 16% in the EP-M group ($P = 0.017$).

Discussion/Conclusion: This study validates the activity of both EDP-M and EP-M in a first-line, real-world setting. While the less favorable characteristics of the EP-M group may lead to bias, the clear survival difference suggests superior antitumor activity of EDP-M.

Complete Responses in Metastatic Adrenocortical Carcinoma: Systematic Review and Individual Patient Meta-analysis

Crona Joakim[1], Zhang Liang[1], Kimpel Otilia[2], Fassnacht Martin[2], Burger-Stritt Stephanie[1]

1) Department of Medical Sciences, Uppsala University, Uppsala, Sweden
2) Department of Medicine, Division of Endocrinology and Diabetes, University Hospital, University of Würzburg, Würzburg, Germany

Background: The aim of systemic therapy in metastatic Adrenocortical Carcinoma (ACC) is to achieve disease control in order to improve quality of life and prolong survival. It has been recognized that rare cases may respond exceptionally with complete disappearance of viable tumour lesions: whether such patients are cured or will eventually relapse is unknown.

Hypotheses: We hypothesize that some patients with metastatic ACC are cured by systemic therapy. A systematic review and meta-analysis could provide information to guide patients and caregivers in this unique situation.

Aims: (1) To perform a literature search to identify cases with metastatic ACC that achieved complete response on systemic therapy. (2) To characterize ACC patients with complete response to describe patient and disease characteristics as well as outcomes in terms of disease cure (patients free of disease at 5 years) or time of recurrence.

Methods: Systematic literature review and individual patient meta-analysis using Pubmed search terms to identify publications describing ACC with complete response. Information relevant to address study aims will be extracted from the identified articles.

Results: The literature search generated 2820 hits, 313 abstracts were screened and 24 publications were included. A total of 40 patients with metastatic ACC having complete response were identified: 12 were treated with mitotane monotherapy, 24 had mitotane plus chemotherapy, 2 had mitotane plus anti PD1 antibodies and 2 had chemotherapy only. Median follow-up time was 29 months (data available in 20 patients) and among 5 patients with a follow-up time ≥5 years, 4 were disease free and thus considered as cured.

Conclusions: Metastatic ACC have demonstrated exceptional responses to various combinations of systemic therapy. There is limited data available in the literature to guide the care of patients that experience complete response.

Role of circulating cell-free DNA concentrations in adrenal pheochromocytoma: a pilot study

[1]Ezra Leander Santhosh David, [1,2]Lorenzo Tucci, [1]Ana Crastin, [3]Miriam Asia, [1]Oskar Podstawka, [1,4]Mengjie Xu, [5]John Ayuk, [1,3,5]Vasileios Chortis, [1,3.5]Cristina L Ronchi

1 Department of Metabolism and Systems Science, University of Birmingham, Birmingham, UK.
2 Medical and Surgical Sciences Department, Alma Mater Studiorum University of Bologna, Bologna, Italy.
3 Department of Endocrinology, Queen Elizabeth Hospital Birmingham NHS Trust, Birmingham, UK.
4 Department of Endocrinology, Taihe Hospital, Hubei University of Medicine, Shiyan, P. R. China.
5 Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham, UK.

Background: Our group previously demonstrated that circulating cell-free DNA (ccfDNA) concentrations are low in patients with endocrine-inactive adenomas (EIA) and elevated in those with adrenocortical carcinoma (ACC). However, the ccfDNA levels in patients with pheochromocytoma (Pheo) remains unclear.

Objectives: Our main aim was to investigate total ccfDNA concentrations in patients with Pheo compared to other adrenal tumour types and healthy controls. Secondary aim was to assess whether they correlate with plasma metanephrine (MN) and normetanephrine (NMN) levels.

Methods: A cohort of 37 patients with adrenal tumors was analyzed, including Pheo (n=6, 1 female, median age 64.5yr), EIA (n=13, 8 females, 56yr) and ACC (n=18, 13 females, 50yr). Fifteen healthy subjects (HS) were used as controls (8 females, 34yr). Blood samples were collected at baseline evaluation. ccfDNA was extracted using a commercial kit and quantified by fluorimeter. Quality control was performed by Tapestation. Plasma MN and NMN levels were measured using liquid chromatography-tandem mass spectrometry according to our adrenal tumour diagnostic pathway. Statistical analyses were performed to compare ccfDNA levels across tumor types and assess correlations with biochemical markers.

Results: ccfDNA concentrations in patients with Pheo (median 0.069 ng/µl, range: 0.001-0.320) were similar to those observed in EIA (0.091 ng/µl, 0.042-0.253) and HS (0.025 ng/µl, 0-0.186) and lower than those recorded in ACC (0.263 ng/µl, 0.049-1.680, *P=0.047* by Kruskall-Wallis test). As expected, ccfDNA concentrations were significantly higher in ACC than in both EIA (*P=0.024*) and HS (*P=0.001*). Considering all patients with different tumour types and available MN/NMN data (n=31), we there was no significant correlation between ccfDNA concentrations and MN/NMN levels.

Conclusions: Total ccfDNA concentrations in Pheo are similar to EIA and HS, but lower than in ACC. We will investigate a larger cohort of patients with Pheo to validate these findings and perform additional correlations with clinical/hormonal parameters.

Multiple genetic analysis of unilateral coexisting adrenal cortical adenoma and carcinoma in MEN1 syndrome to investigate tumor evolution

Francesca Cioppi[1,2], Tommaso Orioli[3], Giulia Cantini[2,4,5], Tonino Ercolino[5,6], Federica Cioppi[6], Guillaume Assié[7], Anne Jouinot[7], Anna Aurora Dedonno[2,4], Raffaella Santi[8], Ronald De Krijger[9,10], Mario Maggi[2,4,5,6] Matteo Benelli[3], Letizia Canu[2,4,5,6], Gabriella Nesi[2,5,8], Michaela Luconi[2,4,5]

[1] Department of Experimental and Clinical Medicine, University of Florence, 50139 Florence, Italy
[2] European Network for the Study of Adrenal Tumours (ENSAT) Centre of Excellence, University of Florence, 50139 Florence, Italy
[3] Department of Experimental and Clinical Biomedical Sciences, CAOS, University of Florence, 50139 Florence, Italy
[4] Department of Experimental and Clinical Biomedical Sciences, Endocrinology Section, University of Florence, 50139 Florence, Italy
[5] Centro di Ricerca & Innovazione Sulle Patologie Surrenaliche, AOU Careggi, 50134 Florence, Italy
[6] Azienda Ospedaliero-Universitaria Careggi, (AOUC), 50139 Florence, Italy
[7] Université Paris Cité, CNRS, INSERM, Institut Cochin, F-75014 Paris, France
[8] Department of Health Sciences, University of Florence, 50139 Florence, Italy
[9] Princess Maxima Center for Paediatric Oncology, Utrecht, Netherlands
[10] Department of Pathology, University Medical Center Utrecht, Utrecht 3584 CX, The Netherlands

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Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal dominant inherited genetic syndrome that predisposes to endocrine tumors including adrenal lesions. The possible evolution of a malignant (ACC) from a benign (ACA) adrenocortical tumor is particularly relevant to be clarified for patient management.

We report a unique case of a MEN1 patient diagnosed with a large central ACC coexistent with a small peripheral ACA. Interestingly, the patient relapsed after 2 years. Comparative immunohistochemistry confirmed the malignant features shared by the recurrence and ACC (marked positivity for SF1, β-catenin and p53, high Ki67, with disruption of the reticulum), while ACA was almost negative for Ki67 and p53 with intact reticulum. Expression profile signatures analyzed with a PCA transcriptome-based model coherently classified ACA and ACC separately. Whole Exome Sequencing (WES) analysis of the three masses revealed a total of 6 ACA-exclusive benign SNVs, 36 ACA/ACC-common variants, two of which, classified as Tier III, affected KANK1 and REN genes, and 69 variants common to ACC and recurrence, of whom ten were Tier III and two were considered as pathogenic (Tier I): a missense mutation in the exon 5 of *TP53* (NM_000546.6:c.376T>G, p.Tyr126Asp) along with an *NF1* donor splice site mutation (NM_001042492.3:c.4333-1G>C). *TP53* and *NF1* are well-known driver genes in ACC, and their exclusive presence in the malignancy and recurrence suggests that they are mainly involved in the clonal evolution of the ACC. Interestingly, MEN1 locus showed Loss of Heterozygosity (LOH) in both ACC and recurrence but not in ACA. Clonal evolution analysis of variants showed that no clones present in ACA that disappeared in ACC.

These findings support the hypothesis of a malignant clonal evolution from a benign adrenocortical tumor in an MEN1 context and underscore the importance of tumor profiling by WES towards a better clinical personalized management of these patients.

Manipulation of insulin receptor (IR) alternative splicing as a novel therapeutic strategy to block IGF2 autocrine proliferative loop in adrenocortical carcinoma (ACC)

Di Bari Sonia[1,2], Catalano Rosa[1], Nozza Emma[1,2], Esposito Emanuela[1], Sigala Sandra[3], Di Dalmazi Guido[4,5], Palmieri Serena[6], Ferrante Emanuele[6], Mantovani Giovanna[1,6], Peverelli Erika[1,6]

[1]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy;
[2]PhD Program in Experimental Medicine, University of Milan, Milan, Italy;
[3]Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.
[4]Division of Endocrinology and Diabetes Prevention and Care, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy;
[5]Department of Medical and Surgical Sciences, Alma Mater University of Bologna, Bologna, Italy.
[6]SC Endocrinology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy;

The majority of ACC overexpress insulin-like growth factor 2 (IGF2), which binds IGF1R, insulin receptor and IGF2R and the consequent activation of IGF signaling system promotes cancer cells growth in an autocrine loop. Among these receptors, an emerging role has been recently shown for IR whose alternative splicing of exon 11 generates two isoforms: IRA, mediating mitogenic effects, and IRB, involved in metabolic regulation. A higher IRA than IRB expression was found in ACC samples and in ACC cell lines H295R and TVBF-7.

The aim of this study is to manipulate IR splicing to imbalance the ratio between the two IR isoforms favoring the metabolic isoform at expense of the mitogenic one and to evaluate the downstream effects of the IR splice-switching in ACC cell lines and primary cultured cells.
To intervene in IR splicing we used 3 strategies: 1) targeting the core spliceosome with SF3B1 inhibitor pladienolide-B: 2) inhibiting the splicing regulatory protein SRPK1 using SRPIN340; 3) interfering with splicing factors binding sites using splice switching oligonucleotides (SSOs).

We found that the first two strategies were not effective in switching the IR splicing from isoform A to B in H295R and TVBF-7. In contrast, SSOs have shown to be effective in reducing IRA levels, resulting in a halving of the IRA/IRB ratio in H295R and TVBF7 cell lines and in one ACC primary culture.
Furthermore, consistent with changes in IRA/IRB ratio, cell proliferation decreased in H295R (-27.56(13.32)% $p<0.05$), in TVBF7 (-43.27(13.13)% $p<0.05$) and in primary cultured cells (-57.73(18.31)%) and apoptosis augmented (+24.93(10.7)%, +105.7(44.2)% and +41.29% respectively), after 250 nM SSOs transfection.

In conclusion, our results suggest new potential therapeutic approaches targeting IRA/IRB ratio in ACC.

Long-Term Cardiovascular outcomes after adrenalectomy in mild autonomous cortisol secretion: results from the multicentric ENSAT NAPACA Outcome study

Di Dalmazi Guido[1,2], Terzolo Massimo[3], Deutschbein Timo[4,5], Bancos Irina[6,7], Letizia Claudio[8], Tucci Lorenzo[1,2], Puglisi Soraya[3], Giordano Roberta[9], Falhammar Henrik[10,11], Prete Alessandro[12], Zacharieva Sabina[13], Alexandraki Krystallenia[14], Reincke Martin[15], Loli Paola[16], Chiodini Iacopo[17], Stigliano Antonio[18], Ceccato Filippo[19], Altieri Barbara[4], Salama Bahaa[6,7], Petramala Luigi[8], Parasiliti-Caprino Mirko[9], Juhlin C. Christofer[20], Ferreira Elio[6,7], Circosta Francesco[8], Paccapelo Alexandro[21], Reimondo Giuseppe[3], Fassnacht Martin[4]

[1]Division of Endocrinology and Diabetes Prevention and Care, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy. [2]Department of Medical and Surgical Sciences (DIMEC), Alma Mater Studiorum University of Bologna, Bologna, Italy. [3]Internal Medicine, Department of Clinical and Biological Sciences, San Luigi Hospital, University of Turin, Orbassano, Italy. [4]Department of Internal Medicine, Division of Endocrinology and Diabetes, University Hospital Würzburg, Würzburg, Germany. [5]Medicover Oldenburg MVZ, Oldenburg, Germany. [6]Division of Endocrinology, Mayo Clinic, Rochester, MN, USA. [7]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, US. [8]Department of Clinical, Internal, Anesthesiological and Cadiological Sciences, University Sapienza, Rome, Italy. [9]Division of Endocrinology, Diabetes and Metabolism, University of Turin, Turin, Italy. [10]Department of Molecular Medicine and Surgery, Karolinska Institutet, 171 76 Stockholm, Sweden. [11]Department of Endocrinology, Karolinska University Hospital, 171 76 Stockholm, Sweden. [12]Department of Metabolism and Systems Science, College of Medicine and Health, University of Birmingham, Birmingham B152TT, United Kingdom. [13]Department of Endocrinology, Faculty of Medicine, Medical University-Sofia, USHATE "Acad. Iv. Penchev", 2, Zdrave Str., 1431 Sofia, Bulgaria. [14]2nd Department of Surgery, Aretaieio Hospital, National and Kapodistrian University of Athens, Athens, Greece. [15]Department of Medicine IV, LMU University Hospital, LMU Munich, Munich, Germany. [16]Endocrinology, Clinica Polispecialistica San Carlo, Paderno, Milano. [17]Endocrinology Department of Endocrine and Metabolic Diseases, IRCCS Istituto Auxologico Italiano, 20100 Milan, Italy. [18]Azienda Ospedaliera Sant'Andrea, Endocrinology, Department of Clinical and Molecular Medicine, Sant'Andrea University Hospital, Sapienza University of Rome, Rome, Italy. [19]Endocrinology Unit, Department of Medicine-DIMED, University of Padova, Via Ospedale Civile, Padova, 105 - 35128, Italy. [20]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden and Department of Pathology and Cancer Diagnostics, Karolinska University Hospital, Stockholm, Sweden. [21]Research and Innovation Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy.

Background. The efficacy of adrenalectomy in adenomas associated with Mild Autonomous Cortisol Secretion (MACS) is poorly investigated. A few small randomized trials and retrospective studies showed beneficial effects of adrenalectomy on hypertension. Long-term data on cardiovascular outcomes are missing.

Aim. To investigate cardiovascular outcomes in patients with unilateral adrenal adenomas and MACS after adrenalectomy.

Methods. Patients with MACS due to unilateral adrenal adenomas (serum cortisol after dexamethasone suppression test [DST] >1.8 mcg/dL) from 15 ENSAT centers were included. Clinical data were retrieved at the time of initial evaluation (before adrenal surgery) and at last follow-up. From each center, MACS patients with and without surgery were matched 1:1. The control group belonged to a previously published study on long-term outcomes of MACS (1). Matching was performed by propensity score using age and sex. We considered new cardiovascular events (CVE), CVE and death from cardiovascular causes (composite-CVE), and death from all causes.

Results. We included 610 patients: 305 were treated by adrenalectomy and 305 underwent follow-up. Median age was 58.2 (IQR 50.1-65.0) and 58.7 (51.9-66.5) years for follow-up and adrenalectomy groups, respectively (P=0.296). Prevalence of female sex was 66.9% (n=204) vs 68.2% (n=208), respectively (P=0.795). The prevalence of hypertension and diabetes at baseline

was not different between groups (P=0.526 and P=0.700, respectively). Values of post-DST cortisol were higher in operated patients than in non-operated ones (3.4 [2.5-5.9] vs 2.7 [2.2-4.0] mcg/dL; P<0.001), as well as tumor size (35 [26-45] vs 25 [18-30] cm; P<0.00).

After a mean follow-up of 6.9±4.5 years, survival analysis showed a significant increased rate of events in non-operated patients for new-CVE (log-rank X^2 6.45; P=0.011) and composite CVE (log-rank X^2 10.79; P=0.001). No significant difference was found for all-cause mortality (log-rank X^2 3.35; P=0.067). The multivariable Cox-regression analysis confirmed the significant beneficial effect of surgery on new CVE (HR: 0.477, 95%CI: 0.284-0.801, P=0.005), composite-CVE (HR: 0.494, 95%CI: 0.340-0.717, P<0.001), and all-cause mortality (HR: 0.638, 95%CI: 0.412-0.988, P=0.044), after adjustment for age, sex, hypertension, diabetes, and cortisol after DST .

Conclusion. Treatment of unilateral adenomas and MACS with adrenalectomy might improve long-term cardiovascular outcomes.

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Optimising Glucocorticoid Replacement in ACC: The Role of ACTH Monitoring

Paniz Dogaheh[1], Zin Htut[1], Einas Mohammed[2], Michael Seckl[2], Sheba Jervis[1], Florian Wernig[2]

[1]Imperial College London, London, United Kingdom
[2]Imperial College Healthcare NHS Trust, London, United Kingdom

Background
Mitotane is an adrenolytic drug used to treat adrenocortical carcinoma (ACC). It increases steroid metabolism and corticosteroid binding globulin (CBG), necessitating high-dose glucocorticoid replacement. Prednisolone is preferred over hydrocortisone at Imperial College Healthcare NHS Trust due to its once-daily dosing and stable pharmacokinetics. However, biomarkers for adjusting glucocorticoid dosing in mitotane-treated ACC patients remain undefined.

Methods
Out of 25 ACC patients identified, 6 patients (median age 63.5 years, range 20–72) met inclusion criteria: mitotane therapy, once-daily prednisolone replacement, and available prednisolone and ACTH day curves. Clinical and treatment data were collected. Descriptive statistics were used to explore prednisolone and ACTH trends across day curves. The Friedman test and paired Wilcoxon tests were used to compare ACTH measurements at 2-, 4-, 6- and 8 hours post-dose. Spearman's correlation assessed the relationship between prednisolone and ACTH measurements. ACTH reference range was 10–20 ng/L, based on optimally replaced primary adrenal insufficiency patients.

Results
Median mitotane treatment duration was 13 months (range 8–20), with prednisolone doses ranging from 4–20 mg once daily. ACTH levels were stable and strongly correlated between 4–8 hours post-dose ($Rs > 0.85$, $p<0.0001$), while 2-hour levels differed significantly. No consistent correlation was observed between ACTH and prednisolone levels at any timepoint. ACTH trends better guided clinical dose changes than prednisolone levels. Median ACTH when doses were increased was 21.8 ng/L; when decreased, 2.5 ng/L. No adrenal crises occurred. CBG was elevated in 85% of cases, and thyroxine and mineralocorticoid supplementation were required in 67% and 50% of patients, respectively.

Conclusions
ACTH appears to be a reliable marker for guiding prednisolone dose adjustment in ACC patients on mitotane. Its stability between 4–8 hours post-dose supports a practical and individualised approach to glucocorticoid titration.

Exploration of the functional impact of overexpressed microRNAs in metastatic paraganglioma

Drossart Tom, Sanceau Julie, Jacquet Amandine, Westbrook Becky, Favier Judith

Inserm, Centre de recherche des Cordeliers, Université Paris-Cité, Paris, France

Approximately 15% of PGLs become metastatic and their malignancy is only defined by the appearance of metastases. Currently, the presence of a mutation in the *SDHB* gene is the main genetic risk factor for malignancy and poor prognosis in PGL. There are no robust biomarkers used in clinical practice that can predict malignancy.
MicroRNAs (miR) are small non-coding RNAs that are post-transcriptional regulators capable of turning off the expression of one or more target genes. Some of them have been shown to have oncogenic characteristics and can be secreted by cells.
Several studies have confirmed the overexpression of miR-96-5p, 182-5p, 183-5p and 483-5p in metastatic PGL compared with non-metastatic PGL. We hypothesised that overexpression of some miRs by tumour cells could modify their phenotype and the tumour microenvironment, promoting the development of metastases.

We used CRISPR-cas9 to generate 3 cell lines with a deletion of one miR of the 183-96-182 cluster enabling us to characterise the impact of these miR on the migration of *Sdhb -/-* imCCs and to identify Stmn1 as a potential new target of these miR. Several studies also highlighted the importance of miR-483-5p in PGL metastases, but this miR isn't expressed in the imCC cell line. We therefore overexpressed miR-483-5p in imCC WT and *Sdhb-/-* cells and demonstrated its role on the migration and proliferation of these cells. This result was confirmed by RNAseq, which also revealed the impact of miR-483-5p on the expression of genes involved in oxidative stress and glutathione synthesis pathways.

This work enables the study of the role of microRNAs in the metastatic progression of PGL and highlights their impact in this context.

18F-CETO-PET in the Diagnosis and Characterization of Adrenocortical Carcinoma

Edholm Adam*[1], Zhang Liang*[1], Åkerström Tobias[2], Welin Staffan[1], Hegedus Agnes[3], Lase Ieva [1], Stålberg Peter[2], Hellman Per[2], Löfgren Eir[1], Skogseid Britt[1], Samuel Backman[2], Sundin Anders[4], Crona Joakim[1]

*Shared authorship

Departments of 1) Medical Sciences, 2) Radiology & Molecular Imaging, 3) Immunology, Genetic and Pathology, and 4) Endocrine Surgery at Uppsala University Hospital, Uppsala, Sweden

Background: 11C-MTO, a PET-tracer binding to the CYP11B1/B2 enzymes, has been available to Adrenocortical Carcinoma (ACC) patients since the 1990s. While demonstrating excellent performance, its wide use was limited by challenging radiochemical properties. 18F-CETO, a novel PET tracer with similar enzymatic affinity, offers improved practicality and broader clinical applicability.
Aims: (1) To assess the diagnostic capacity of 18F-CETO-PET in ACC compared to 18F-FDG-PET. (2) To explore if 18F-CETO-PET or 11C-MTO-PET uptake correlates with ACC molecular characteristics.

Methods: This prospective study included newly diagnosed or progressive ACC patients for investigation with 18F-CETO-PET/CT and 18F-FDG-PET/CT. Patients undergoing 18F-CETO-PET/CT or 11C-MTO-PET/CT as part of their clinical evaluation at Uppsala University Hospital was used as validation. Paired tumor samples were analyzed via RNA sequencing to assess *CYP11B1/B2* expression, adrenal differentiation score and to perform unsupervised molecular clustering.

Results: Prospectively, 9 patients underwent 18F-CETO-PET/CT and 18F-FDG-PET/CT. All were 18F-FDG-PET positive. At initial diagnosis (n=3), all 18F-CETO and 18F-FDG scans were positive, whereas 18F-CETO was negative in 3/6 patients that were investigated for recurrence (during or after mitotane therapy). This pattern was confirmed in the validation cohort (18F-CETO: n=9; 11C-MTO: n=65) where all untreated ACCs showed uptake (18F-CETO n=3 and 11C-MTO n=30), while all negative scans (18F-CETO n=3, 11C-MTO n=4) were in patients treated with mitotane. In 27 unique tumor lesions from 20 patients with paired RNA and PET data, CETO and MTO-PET SUVmax correlated with CYP11B1 expression and negative tumors had a lower adrenal differentiation score. Among 12 untreated primary tumors, 8 were C1A-like and 4 were C1B-like. The mean SUVmax was 31.4 ±19.8 for C1A-like and 18.9 ±7.2 for C1B-like (P=0.145).

Discussion: 18F-CETO is a promising imaging tool for ACC, particularly in newly diagnosed, treatment-naïve tumors. Its uptake appears to reflect underlying tumor phenotype, offering a non-invasive method to investigate ACC biology in vivo.

Retrospective analysis of tumor evolution in ACC

Authors: Edholm, Adam[1,] Stenman, Adam[2 3], Zhang, Liang[1], Hegedus, Agnes[1], Juhlin, Christoffer[4]*, Crona, Joakim[1]*

1 Department of Medical Sciences, Uppsala University, 75185 Uppsala, Sweden
2 Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
3 Department of Breast, Endocrine Surgery and Sarcoma, Karolinska University Hospital, Stockholm, Sweden
4 Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden.

*Both authors contributed equally to this work.

Background: It is recognized that in many cancers, tumor genome and biology is dynamically changing and evolving during the course of the disease. Tumor evolution in ACC is poorly understood, with one limiting factor being the lack of longitudinal tissue samples. Clinical experience suggests that a subset of tumors increase their proliferation rate over time.

Aim: To characterize longitudinal changes in ACC biology and evaluate whether an increase in Ki-67 correlates to a poorer prognosis.

Method: In this bi-center, retrospective cohort study all ACC patients treated at Uppsala University Hospital and Karolinska University Hospital from 1984-2024 were screened. Those with multiple tumor tissue samples collected >6 months apart were included. Tumor material was reviewed by a senior pathologist to determine Ki-67 and evaluate morphology. Medical records were used to acquire patient data at baseline and at last follow-up; age, disease stage as well as clinical signs and/or biochemical confirmation of hormonal syndromes and the reason for acquiring the tissue.

Preliminary results: Of 147 patients screened, 42 patients with 2 or more longitudinal samples were identified. 21 were males and 21 had adrenal steroid hypersecretion. Of the tumor samples, 42 were primary tumors, 28 resections of recurrences and 21 were biopsies from metastases. 14 patients had documented Ki-67 values. Of them, 11 displayed an increased proliferation, with a mean increase of 20% in Ki-67 index from first to second PAD, while 3 patients had no or less than 5% increase. Patients with an increased proliferation >5% (N=11) had a mean survival of 72 months compared to a mean survival of 84 months for the 3 patients with <5% increase. Histopathological review of the tumor material is ongoing.

Conclusions: A significant proportion of ACC patients at our tertiary centers had longitudinal tumor samples, with most displaying an increase in Ki-67 over time.

Dual inhibition of PLK1 and multi-CDKs: a novel approach for the treatment of adrenocortical carcinomas

Emanuela Esposito[1,2], Lucy Beevors[3], Vasileios Chortis[3], Laura-Sophie Landwehr[4], Constanze Hantel[5,6], Sandra Sigala[7], Erika Peverelli[1,8], Giovanna Mantovani[1,8], Paul A. Foster[3,9], Cristina L Ronchi[3,9]

[1] *Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy*
[2] *PhD Programme in Experimental Medicine, University of Milan, Milan, Italy*
[3] *Metabolism and Systems Science, College of Medicine and Health, University of Birmingham, Birmingham, UK*
[4] *Department of Internal Medicine I, Division of Endocrinology and Diabetes, University Hospital Würzburg, University of Würzburg, Würzburg, Germany*
[5] *Department of Endocrinology, Diabetology and Clinical Nutrition, University Hospital Zurich (USZ) and University of Zurich (UZH), Switzerland*
[6] *Medizinische Klinik und Poliklinik III, University Hospital Carl Gustav Carus Dresden, Germany*
[7] *Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Italy*
[8] *Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan, Italy*
[9] *Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham, UK*

Adrenocortical carcinomas (ACC) are aggressive tumors with limited treatment options. Polo-like kinase 1 (*PLK1*) and cyclin-dependent kinases (*CDKs*) 1/2/4 are overexpressed in ACC human samples. Here, we tested the efficacy of 1) the polo-box domain (PBD)-targeting PLK1 inhibitor (PLK1i) Poloxin and the kinase domain (KD)-targeting PLK1i Plogosertib; 2) the CDK1/2 inhibitor (CDKi) Dinaciclib; 3) the combination of Plogosertib and Dinaciclib. Experiments were carried out in four ACC cell lines (H295R, MUC-1, TVBF-7, JIL-2266), using increasing drug concentrations for 72h. Cell proliferation and apoptosis were assessed by BrdU incorporation and caspase 3/7 activity. The “SynergyFinder” tool was used to analyse drug combinations.

PLK1i Poloxin reduced proliferation at high doses, reaching a maximum effect at 100μM ($p<0.01$ in MUC-1 and TVBF-7; $p<0.001$ in H295R and JIL-2266), and increased apoptosis at 10μM ($p<0.05$). At much lower doses, Plogosertib reduced cell proliferation ($p<0.05$ at 100nM in MUC-1 and JIL-2266; $p<0.01$ at 750nM in H295R and TVBF-7) and increased apoptosis ($p<0.05$ for H295R, TVBF-7, and JIL-2266 at 1μM). CDKi Dinaciclib drastically reduced cell proliferation at low nanomolar concentrations ($p<0.05$ at 20nM in MUC-1 and JIL-2266, and at 100nM in TVBF-7; $p<0.01$ at 100nM in H295R), and increased apoptosis ($p<0.05$ in MUC-1, TVBF-7, and JIL-2266 at 200nM).

In line with the observed differences in treatment sensitivity among cell lines, qRT-PCR analysis showed that CDK1/2 and PLK1 mRNA expression was particularly high in MUC-1 and JIL-2266.

Synergistic inhibition of cell proliferation by combined treatment with PLK1i Plogosertib and CDKi Dinaciclib was observed in H295R and TVBF-7 cells.

In conclusion, Plogosertib and Dinaciclib were the most effective inhibitors in all cell lines, representing interesting novel treatment options for ACC. Moreover, the combination of these drugs showed a synergistic effect, suggesting a potential benefit of using both PLK1i and multi CDKi that need to be further investigated *in vivo*.

Metastatic seeding clones: the driving force

Arroba Ester[1], Piñeiro-Yáñez Elena[2], Salazar-Hidalgo Milton E[1], Calsina Bruna[1], Leandro-García Luis Javier[1], Cascón Alberto[1,3],Díaz-Talavera Alberto [1,3], Al-Shahrour Fátima[3], Robledo Mercedes [1,3]

[1] Hereditary Endocrine Cancer Group, Human Genetics Programme, Spanish National Cancer Research Center (CNIO) Madrid, Spain
[2] Bioinformatics Unit, Structural Biology Programme, Spanish National Cancer Research Center (CNIO), Madrid, Spain
[3]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Institute of Health Carlos III (ISCIII) Madrid, Spain

Metastatic pheochromocytoma is an ultra rare tumor type with poor clinical outcome. Given its low incidence and the scarcity of metastatic samples, a cohort comprising 48 primary-metastatic paired samples from 15 unrelated patients represents a unique and valuable resource. This cohort includes 3 cases with multiple metastasis, 3 cases with primary-relapse-metastasis trios and 3 cases with multiregional sampling.

Leveraging this sample collection, we performed an in-depth analysis of clonal evolution. Our previous work has shown that high-impact mutations tend to be enriched in metastatic lesions [1]. In previous ENSAT meetings, we presented the evolutionary trajectories and dissemination patterns. However, identifying the alterations that specifically drive metastatic spread remains a major challenge.

In this study, we picked out the clone that escaped from the primary tumor, entered circulation and was able to metastasize: the metastatic seeding clone. By analyzing clonal composition and abundance across all samples, we were able to categorize clone types as either sample-specific or shared between primary and metastatic sites. We identified the metastatic seeding clone for each patient, characterized by a consistent enrichment in high-impact mutations. Notably, these mutations were present at higher cancer cell fractions (CCF), suggesting that a greater proportion of tumor cells within the clone carry these driver alterations.

Remarkably, in most cases, the seeding clone emerged early in the evolutionary timeline. By exploring the mutations present in the metastatic seeding clones, we were able to identify what we called “seeding clone mutational signature”, bringing us relevant information about the biological processes behind the metastatic spread.

These findings advance our understanding of metastatic pheochromocytoma by highlighting the early emergence and unique mutational landscape of the metastatic seeding clone, positioning it as a key driver of disease dissemination.

[1] Calsina *et al.* N*at Commun* 14, 1122 (2023).

Circadian fluctuation of lipid and amine metabolic pathways in states of cortisol excess and insufficiency

Fanelli Flaminia[2,3], Galante Greta[2,3], Carlo Mengucci[4], Tucci Lorenzo[1,2], Coscia Kimberly[1,2], Bissi Valentina[2,3], Colombin Giacomo[1,2], Perrone Alessandro[2,3], Vicennati Valentina[1,2], Pagotto Uberto[1,2], Capozzi Francesco[4], Di Dalmazi Guido[1,2]

1Division of Endocrinology and Diabetes Prevention and Care, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy.
2Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy.
3Center for Applied Biomedical Research, Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy.
4Department of Agricultural and Food Sciences DISTAL, Alma Mater Studiorum University of Bologna, Bologna, Italy

Introduction. Adrenal insufficiency (AI) under replacement therapy, adrenocortical tumours with mild autonomic cortisol secretion (MACS) and Cushing's syndrome (CS) display different alterations of cortisol rhythm. Circadian alterations of metabolic pathways in these diseases were not characterized yet.

Objective. To characterise circadian fluctuations of 187 metabolites in dried blood spots (DBS) from healthy subjects (HS) and from AI, MACS and CS patients.

Methods. We enrolled 10 HS, 8 AI taking dual-release hydrocortisone, 12 MACS and 9 newly diagnosed CS patients. All followed 7-days standardised isocaloric Mediterranean diet. On day seven, they collected DBS 30min before and 2h after breakfast, lunch and dinner, and at 11pm. We measured 42 amino acids (AA) and biogenic amines, 40 acylcarnitines, 15 sphingomyelins and 90 glycerophospholipids by LC-MS/MS. The BORUTA algorithm highlighted the informative features. Subsequently, factor analysis reduced data dimension. The linear combination between informative features and factor analysis coefficients calculated for the seven time-points returned two molecular patterns.

Results. In pattern A, HS had higher phosphatidylcholines (PC) PC-ae-C34:2, PC-ae-C34:3, PC-aa-C34:2, PC-aa-C36:2 and PC-aa-C36:0 compared to all patients, with largest difference found 2h after lunch ($p<0.05$). In pattern B, HS and AI showed lower PC-aa-C38:4, PC-aa-C36:4, lysoPC-a-C20:4, PC-aa-C36:5 and methionine compared to MACS and CS patients, with larger differences in the afternoon ($p<0.05$). For the same compounds, AI patients had lower levels than HS ($p<0.05$). By focusing on amines only, we obtained pattern A in which HS had higher serine, glycine, threonine, citrulline and histidine than all patients, with greater differences in the afternoon and at 11pm ($p<0.05$); and pattern B, in which AI had lower spermidine, taurine, glutamine and histidine than HS, MACS and CS, with larger differences at midday, and 30min before dinner ($p<0.05$).

Conclusions. Dysregulation of PC and AA in the afternoon appears a prominent feature in states of cortisol alteration.

Paragangliomas and Pheochromocytomas: correlation between genotype and phenotype, and relevant clinical implications in an Italian retrospective analysis

Martina Fano[1], Pasqualino Malandrino[2], Franz Sesti[3], Gianfranco Di Iasi[4], Roberta Lai[5], Rossella Mazzilli[1], Massimo Niola[4], Antonio Prinzi[2], Monica Verrico[3], Giuseppe Fanciulli[5], Elisa Giannetta[3], Roberta Modica[4], Antongiulio Faggiano[1]

1. Azienda Ospedaliero Universitaria Sant'Andrea, Rome, Italy
2. Azienda Ospedaliero Universitaria Policlinico “G. Rodolico - San Marco”, Catania, Italy
3. Clinica Ospedaliero Universitaria Policlinico Umberto I, Rome, Italy
4. Azienda Ospedaliera Universitaria Federico II - "Policlinico", Naples, Italy
5. Ospedale Civile Santissima Annunziata, Sassari, Italy

1. Introduction

Pheochromocytomas and paragangliomas (**PPGLs**) represent rare neuroendocrine neoplasms arising from the paraganglia system, with an estimated incidence of 0.1 to 0.6 cases per 100,000 population per year. PPGLs are characterized by a high degree of heritability, with about 30-35% of patients having germline mutations in susceptibility genes, each related to heterogeneous clinical phenotypes.

2. Aim of the study

The aim of the study is to compare the clinical phenotype of PPGLs with the genetic profile of the analysed patients.

3. Method applied

The multicentre retrospective study has involved 146 patients diagnosed with paraganglioma or pheochromocytoma from five Italian public hospitals. Clinical, demographic, and tumor characteristics as well as genetic data were collected. The study has included symptoms, tumor stage, genetic mutations and treatments received. Statistical analysis was conducted with R software, using association tests for categorical variables. A literature review was finally conducted to compare the results obtained with other epidemiological and genetic studies on PPGLs.

4. Results

Of 146 patients, 61.64% were females, showing a mean age at diagnosis of 46.42 years for paragangliomas/phaeochromocytomas, and an earlier age (40.7 years) in the presence of genetic mutations. Symptomatic diagnosis, the most common (55.17%), was associated with larger tumors (56.51 mm), while genetic screening (10.34%) enabled diagnosis 10-15 years earlier. A genetic analysis of 160 tumors detected germline mutations in 38.7% of cases, often associated to multifocal disease.

A strong correlation between familiarity and genetic mutations ($p=1.9\times10^{-10}$) was observed, although 19.7% of mutated patients had no family history, suggesting the importance of universal genetic screening.

5. Conclusion

The study conducted confirms the heterogeneity of these neoplasms, and the results underscore the crucial importance of genetic screening for early diagnosis, identification of smaller tumors, and personalization of therapeutic approach, even in the absence of documented familiarity.

Adrenalectomy reduces the risk of vertebral fractures in patients with mild autonomous cortisol secretion

Favero Vittoria [1,2], Morelli Valentina [3], Frigerio Sofia [4], Aresta Carmen[3], Pugliese Flavia [5], Salcuni Antonio [6], Risio Alessandro[2], Eller-Vainicher Cristina[4], Palmieri Serena [4], Cairoli Elisa [1], Corbetta Sabrina[1, 7], Scillitani Alfredo[5], Mantovani Giovanna [4, 8], Chiodini Iacopo[2, 3]

[1]Department of Medical Biotechnology and Translational Medicine, Milan, Italy
[2]ASST Grande Ospedale Metropolitano Niguarda, Unit of Endocrinology, Milan, Italy
[3]IRCCS Istituto Auxologico Italiano, Endocrinology Department of Endocrine and Metabolic Diseases, Milan,Italy
[4]Fondazione IRCCS Cà Granda-Ospedale Maggiore, Unit of Endocrinology, Milan, Italy
[5]IRCCS Ospedale Casa Sollievo della Sofferenza, Unit of Endocrinology and Diabetology, San Giovanni Rotondo (FG), Italy
[6]University-Hospital S. Maria della Misericordia, Unit of Endocrinology and Metabolism, Udine, Italy [7]University of Milan, Department of Biomedical, Surgical and Dental Sciences, Milan, Italy
[8]University of Milan, Department of Clinical Sciences and Community Health, Milan, Italy

Objective. Mild autonomous cortisol secretion (MACS) increase risk of vertebral fractures (VFx). However, the impact of recovery from MACS on bone health remains unclear.

Design. Retrospective Longitudinal Study (Study 1): 53 patients with MACS were followed for 35.2±18.6 months (mean±SD). Among these, 31 patients underwent surgery (Study 1-GroupA), while 22 patients were conservatively treated (Study 1-GroupB). Prospective Randomized Study (Study 2): 51 patients with MACS were randomly assigned to either adrenalectomy (Study 2-GroupA, 21 patients) or a conservative approach (Study 2-GroupB, 28 patients) and were followed for 24 months.

Methods. MACS was diagnosed in patients with adrenal incidentalomas>1 cm and cortisol after 1-mg dexamethasone suppression test (F-1mgDST)≥1.8 μg/dL. At baseline and at the end of the follow-up we assessed: mineral metabolism, bone mineral density (BMD) at the lumbar spine (LS), total hip (TH) and femoral neck (FN), and clinical and morphometric vertebral fractures (VFx).

Results. Study 1: Study 1-GroupB showed an increased incidence of VFx (n=11) at the end of the follow-up when compared to Study1-GroupA (n=3, $p<0.05$). In both groups, BMD at LS, FN and TH were similar at baseline and at the end of follow-up.

Study 2: Patients in both groups were comparable for demographic features (age, sex, BMI), adenoma's size, cortisol secretion parameters (F-1mgDST, urinary free cortisol, ACTH), prevalent VFx and BMD at baseline.

After 24 months, in Study 2-GroupA, we observed significant increases in calcium and phosphate levels compared to baseline ($p=0.03$ and $p=0.04$, respectively). Study 2-GroupB showed no difference from baseline to 24 months. At the end of follow up, BMD remained stable across both groups. Study 2-GroupB showed a significantly higher incidence (n=7, 25%) of VFx by the end of the follow-up period compared to Study 2-GroupA (n=1, 4.8%, $p=0.04$).

Conclusions: In patients with MACS, adrenalectomy significantly reduces the risk of vertebral fractures.

Role of the Mineralocorticoid Receptor in the Physiology of the Adrenal Cortex and the Development of Aldosterone Producing Adenomas

May Fayad[1,2], Rami M. El Zein[1], Nicolo Faedda[1], Rita Chamoun[1,3], Parisa Rezvanisanijouybari[1,2], Ourida Koual[1], Isabelle Giscos-Douriez[1], Oliver Domenig[4], Marko Poglitsch[4], Stephanie Baron[5], Fabio L. Fernandes-Rosa[1], Eleanor Davies[2], Sheerazed Boulkroun[1], Maria-Christina Zennaro[1,6]

[1]Université Paris Cité, Inserm, PARCC, Paris, France
[2]School of Cardiovascular and Metabolic Health, BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, United Kingdom
[3]Maastricht University Medical Center, Maastricht Limburg, The Netherlands
[4]Attoquant Diagnostics, Vienna, Austria
[5]Assistance publique–Hôpitaux de Paris (AP–HP), Hôpital Européen Georges Pompidou, Service de Physiologie, Paris, France
[6]Assistance Publique-Hôpitaux de Paris (AP–HP), Hôpital Européen Georges Pompidou, Service de génétique, Paris, France

Primary aldosteronism (PA) is the major cause of secondary arterial hypertension. The mineralocorticoid receptor (MR) binds aldosterone and is expressed in the adrenal cortex specifically in the zona glomerulosa (ZG) and in aldosterone-producing adenoma (APA). Given the role of MR in tissue remodeling and its expression in the ZG and APA, we hypothesized that aldosterone could be involved in the pathophysiology of APA through MR.

A new Cyp11b2-cre mouse model was generated and crossed with $Mr^{flx/flx}$ mice. Specific recombination in adrenals from $Cyp11b2^{+/Cre}$:$Mr^{flx/flx}$ ($MRKO^{ZG}$) mice was proven. MR expression was reduced as shown by RT-qPCR and RNAscope. The ZG size was increased in $MRKO^{ZG}$ female mice. *Cyp11b2* expression was decreased in $MRKO^{ZG}$ males and females, with an increased *Cyp11b1* expression in males only. These changes are associated with decreased cell proliferation in both sexes and activation of the PKA signaling pathway at 12 weeks of age. $MRKO^{ZG}$ mice were crossed with mTmG reporter mouse model ($MRKO^{ZG}$::mTmG) which allows following the cell lineage and transdifferentiation over time. Transdifferentiation from ZG to zona fasciculata appears to be faster in those mice compared to WT mice. $MRKO^{ZG}$::mTmG mice were challenged with low salt diet (LSD) and high salt diet (HSD). Following a 2-wks LSD, WT mice increase the size of the ZG and increase *Cyp11b2* expression; these changes were attenuated in $MRKO^{ZG}$ mice. In WT mice under LSD, proliferation is predominantly confined to the ZG, where it appears dense and region-specific. In contrast, cell proliferation is showing a widespread distribution in the cortex of $MRKO^{ZG}$::mTmG mice under LSD.

In conclusion, *Mr* inactivation in the ZG alters the adrenal phenotype and the response to physiological challenges. The identification of the underlying molecular mechanisms will allow to better understand cell lineage, differentiation and function of the adrenal cortex in relation to the development of PA.

Adrenal incidentaloma: when the diagnosis is challenging

Festas-Silva Diana1,2, Borges-Canha Marta1,2, Meira Inês1,2, Ferreira Patrícia1,2, Costa Mafalda1, Queirós Joana1

1. Serviço de Endocrinologia, Diabetes e Metabolismo, ULS São João, Porto, Portugal
2. Faculdade de Medicina da Universidade do Porto, Porto, Portugal

Introduction: adrenal incidentaloma (AI) is defined as an adrenal mass detected incidentally through imaging performed for reasons unrelated to adrenal pathology. The diagnosis of adrenal tumors, both in the preoperative and postoperative settings, can be particularly challenging.
Clinical Case: a 73-year-old female underwent imaging for the evaluation of abdominal pain, which showed a left AI of 59 mm in its largest dimension. The lesion was hypodense with cystic characteristics (15HU) on abdominal CT. Her medical history included type 2 diabetes mellitus managed with oral antidiabetic agents and well-controlled dyslipidemia. Physical examination was unremarkable for endocrinopathy. Endocrine evaluation included an overnight 1 mg dexamethasone suppression test, with a cortisol level of 1.3 µg/dL, and an ACTH level of 11 pg/mL. Fractionated metanephrines and 3-methoxytyramine in 24-hour urine collection were within normal limits. Due to diagnostic uncertainty, an adrenal-targeted MRI was performed, revealing a nonspecific solid mass, displaying heterogeneous hyperintensity on T2-weighted images and hypointensity on T1, with no signal loss on out-of-phase sequences. The lesion increased in size to 94mm (< than 6 months between imaging studies), raising concern for malignancy. Additional hormonal testing, with estradiol, total testosterone, DHEAS, androstenedione and 17- hydroxyprogesterone levels was normal. A thoracoabdominopelvic CT scan showed no evidence of secondary lesions, and Ga-68 DOTANOC PET/CT demonstrated no significant somatostatin receptor expression.
The patient underwent surgical resection. Histopathological analysis revealed a myxoid mesenchymal neoplasm, with a Ki-67<5%, and CD34 expression. A complementary molecular study using an extended NGS panel covering 464 genes was negative. The case was referred for external consultation with a specialized pathologist. The patient was subsequently referred to oncology for follow-up.
Conclusion: AI may present significant diagnostic challenges both pre- and postoperatively. This case highlights the importance of a multidisciplinary approach in the evaluation and management of indeterminate adrenal masses to ensure optimal patient care.

Unravelling Thermotolerance in Adrenocortical Carcinoma: Implications for Hyperthermia-Based Therapies

Solène Fiachetti[1], Nathan Mullen[1], Sarah Feely[1], Ritihaas Surya Challapalli[1], Padraig Donlon[1], Mariam Hassany[1], Martin O'Halloran[2], Constanze Hantel[3], Punit Prakash[4], Michael C Dennedy[1]

1 Discipline of Pharmacology and Therapeutics, School of Medicine, University of Galway, Galway, H91 V4AY, Ireland.
2 Translational Medical Device Laboratory, Discipline of Medicine, University of Galway, Galway, H91 V4AY, Ireland.
3 Department of Medicine IV, University Hospital, Ludwig Maximillan University of Munich, Munich, Germany
4 Department of Electrical and Computer Engineering, Kansas State University, Manhattan, KS, United States of America.

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Introduction: Adrenocortical carcinoma (ACC) is a rare, aggressive cancer with limited treatment options and frequent chemoresistance, highlighting the need for improved therapies. Hyperthermia is used to treat ACC metastases primarily through radiofrequency ablation to control disease burden. Incomplete tumour ablation risks exposure to sub-lethal hyperthermia in the transitional zone, potentially leading to thermotolerance development, where cells resist subsequent thermal stress after initial sublethal heat exposure.

We hypothesised that sublethal hyperthermia induces thermotolerance to subsequent exposure at 48°C or 50°C, mediated by the heat shock response and TMEM16F, a calcium-dependent scramblase involved in cellular repair.

Methods: ACC primary cell lines H295R and HAC15, and the metastatic line MUC-1 were pre-treated at 45°C, then rechallenged at 48°C or 50°C after 24 hours or 7 days. Rechallenge cells were compared to naïve (non-pretreated) cells. Cell death was assessed using Sytox Blue staining and flow cytometry. Protein expression of HSP70, HSP90, P-HSP27, and TMEM16F was analysed by Western Blot.

Results: Surviving ACC cells previously exposed to hyperthermia showed no evidence of thermotolerance. Viability at 48°C and 50°C was similar between pre-treated and naïve cells, though MUC-1 was more resistant at higher temperatures (≥48°C). While heat stress was evident following hyperthermia, no significant differences in HSP70 and HSP90 expression were observed between naïve and rechallenged cells. Additionally, P-HSP27 and TMEM16F expression was significantly decreased, in both naïve and rechallenge cells, at 48°C and 50°C, but reappeared 24 hours later at 48°C only.

Conclusion: Hyperthermia of 45°C did not confer thermotolerance in ACC cells. The transient but marked reduction in P-HSP27 and TMEM16F suggest a diminished and delayed heat shock response, insufficient to protect against subsequent thermal stress. These findings emphasise the complex ACC response to hyperthermia but suggest that thermotolerance may not develop in incompletely ablated cancers, whereby cells remain sensitive to further ablation challenges.

Preoperative and Surgical Management of Giant Pheochromocytomas

Raluca Maria Furnica, Stefan Matei Constantinescu, Damien Gruson, Michel Mourad, Antoine Buemi, Dominique Maiter

Introduction: Giant pheochromocytomas (PCs) are adrenal tumors exceeding 7 cm in size. The incidence and clinical presentation of large tumors are not well understood, with only a limited number of case reports available in the literature. Additionally, the preoperative and surgical management of giant PCs remains poorly defined.

Materials and Methods: This is a retrospective, monocentric study conducted at a tertiary center, involving 100 consecutive patients who underwent surgery for a pheochromocytoma between 1988 and 2024.

Results: Among the 100 patients, 25% had a giant PC, with a mean tumor diameter of 9.9 cm. There were no significant differences in the clinical manifestations at the time of diagnosis between these patients and those with smaller tumors. The diagnosis was primarily driven by PC-related symptoms in half of the cases, mass-related symptoms in 25%, during family genetic screening in 10%, and incidentally in 1 case. Giant PCs were associated with significantly higher levels of 24-hour urine metanephrines ($P = 0.028$) and normetanephrines ($P = 0.001$) compared to tumors smaller than 7 cm. Interestingly, noradrenergic PCs were the largest tumors ($P < 0.01$). The incidence of perioperative hemodynamic ($P = 0.008$) and surgical complications ($P = 0.032$) was positively correlated with tumor size. There was no significant difference in the cumulative dose of preoperative alpha-blockers when comparing tumor sizes. As anticipated, giant PCs were more likely to require open surgery (85% vs 23%). Moreover, giant PCs had a higher rate of recurrence ($P = 0.039$) and malignancy ($P < 0.001$).

Conclusion: Our study demonstrates that giant PCs are typically symptomatic and their clinical presentation is similar to that of smaller PCs. However, they are associated with a higher incidence of perioperative hemodynamic and surgical complications and tend to exhibit a more aggressive phenotype. These tumors require specialized, multidisciplinary management in expert centers.

Measurement of steroid hormones and metabolites in Dried Blood Spots (DBS) by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to explore circadian rhythms: a pilot analysis

Galante Greta[1,2], Galmiche Mathieu[2,3], Mao Federico [1,4], Bissi Valentina[1], Pizza Gianmarco [1,4], Gennai Alessia[1], Tribotti Laura [1,4], Donnarumma Francesca [1,4], Di Dalmazi Guido[1,4], Pagotto Uberto[1,4], Rudaz Serge[2,3], Fanelli Flaminia[1]

1. Center for Applied Biomedical Research and Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy.
2. School of Pharmaceutical Sciences, University of Geneva, CMU - Rue Michel-Servet 1, Geneva, Switzerland.
3. Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU - Rue Michel-Servet 1, Geneva, Switzerland
4. Division of Endocrinology and Diabetes Prevention and Care, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy.

Introduction. Perturbations of steroid hormones were reported in dysmetabolic conditions such as obesity and insulin resistance. However, the possible alteration of circadian steroid fluctuations in these states remains poorly investigated. Challenges consist in the need for frequent blood sampling and for highly sensitive and accurate measurements. DBS from finger-prick allows autonomous and non-invasive home sampling. Recent instrumentation in LC-MS/MS provides top sensitivity for measurements of small blood volumes, while quantitation based on internal calibration enhances throughput and ensures accuracy.

Aim. To evaluate the utility of DBS combined with LC-MS/MS and internal calibration quantitation as a tool to examine steroid circadian fluctuation.

Methods: DBS were collected from 6 healthy subjects (3 males, 3 females) at nine time points of an ordinary day (07:00, 08:30, 10:00, 12:30, 14:00, 16:00, 19:30, 21:00 and 23:00). After the addition of isotopically labelled internal calibrants, cortisol, cortisone, 11-deoxycortisol, 11-deoxycorticosterone, 17OH-progesterone, androstenedione and testosterone were analysed in extracts of two 6 mm DBS circles by LC-MS/MS. Data were reported in ng/mL and analysed by non-parametric tests.

Results: Overall daily cortisol levels were higher (median (IQR): 25.6 (18.7-43.9) vs 20.1 (8.13-32.7); p=0.025), and testosterone levels were lower (0.43 (0.14-0.49) vs 2.32 (2.01-2.77); p<0.001) in females compared to males. Levels of cortisone (8.87 (4.16-11.1)), 11-deoxycortisol (0.075 (0.054-0.121)), 11-deoxycorticosterone (0.022 (0.016-0.029)), 17OH-progesterone (0.309 (0.169-0.550)), progesterone (0.116 (0.089-0.301) and androstenedione (0.174 (0.115-0.338) showed no sex differences. Cortisol, cortisone, 11-deoxycortisol and androstenedione displayed circadian rhythm in both sexes (all p<0.003), whereas testosterone and 17OH-progesterone displayed significant rhythm in males only (all p<0.048). No circadian rhythm was detected for progesterone and 11-deoxycorticosterone.

Conclusions: These preliminary findings in a small control group demonstrate the feasibility of using DBS and internal calibration LC-MS/MS quantitation to explore circadian steroid patterns, paving the way for future studies in larger cohorts and diverse physiological states.

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Characterization of metastatic dissemination in Znrf3/Trp53 double knock-out mouse model

GARCIA-GARCIA Diana[1], Voisin Allison[1], Wilmouth Jr James[1], Guiton Rachel[1], Martinez Antoine[1], Val Pierre[1]

[1] GReD Institute - CNRS UMR6293, Clermont-Ferrand, France

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with very poor prognosis due to the lack of successful therapies for patients with metastatic or recurrent tumors. However, the urgent need for the development of new therapies clashes with the lack of preclinical models that accurately represent ACC. Using Cre-loxP technology to mimic inactivation of *ZNRF3* and *TP53*, the most commonly found alterations in ACC patients from the most aggressive subgroup, we developed a new clinically relevant mouse model allowing for the study of the molecular basis of metastatic ACC. Using a combination of Kaplan Meier analysis, bulk RNA sequencing and immunohistochemistry, we demonstrate that adrenal cortex specific ablation of *Trp53* and *Znrf3* results in development of metastatic and lethal ACC over a 6-month time course, associated with the molecular signature of C1A subgroup, immune poor, steroidogenic high, and proliferation high signatures or the overexpression of the EZH2 methyltransferase and a constitutive activation on WNT pathway.

Single nucleus RNA sequencing of primary tumors showed that acquisition of aggressive features is associated with amplification of a population of proliferative cells characterized by expression of *Dab2*, *Wnt4, Lef1, Mki67* and *Ezh2*. These molecular features are also found in lung metastases, as assessed by both immunohistochemistry and spatial transcriptomic analysis, suggesting that these originate from dissemination of this set of cells. In addition, metastatic dissemination is associated with upregulation of senescence-related signatures. Immunolabeling for P16 and SA-β-galactosidase revealed the presence of senescent macrophages in the most aggressive primary tumors and their corresponding metastases. Single cell transcriptomic analysis of the immune compartment showed that senescent macrophages also express immune-checkpoint and pro-tumoral signatures, suggesting a role for senescent cells in promoting metastatic progression by inhibiting the anti-tumor response. We are therefore currently evaluating the therapeutic potential of senolytics in our ACC mouse model. Altogether, these data shed light on the underpinnings of metastatic dissemination and establish these as a good *in vivo* model to investigate novel therapeutic options.

Genetic clusters and Somatostatin Receptor Positron Emission Tomography Imaging Characteristics in Pheochromocytomas and Paragangliomas

Maria Geuß, Meike Onkes, Paul Dahlmann, Katharina Wang, Isabel Stüfchen, Júnia R.O.L. Schweizer, Christian Lottspeich, Christine Schmid-Tannwald, Martin Reincke, Svenja Nölting, Rudolf Werner, Matthias Kroiss, Friederike Völter

Aim/Introduction:
Pheochromocytomas and paragangliomas (PPGLs) are highly heritable tumors. Based on underlying pathogenic variants (PVs), PPGLs are assigned to genetic clusters differing in clinical and biochemical tumor characteristics and metastatic risk. Current evidence suggests somatostatin receptor (SSTR) PET/CT as the recommended imaging modality for SDHx-associated and metastasized PPGLs. The uptake intensity on SSTR PET/CT is predictive for the tumor dose in peptide radioreceptor therapy (PRRT). Therefore, this study aimed to evaluate the radioligand uptake across different PPGL clusters on SSTR PET/CT.

Materials and Methods:
Patients with PPGLs who underwent Ga-68 DOTATOC PET/CT or F-18 SiTATE PET/CT and genetic testing at LMU Hospital were included. The metabolic tumor volume (MTV) was analyzed semi-manually using a threshold of SUVmax 5.0. Radioligand uptake and MTV were compared across different genetic clusters using the Mann-Whitney U test. Ki67 was compared to the radioligand uptake using Pearson's correlation analysis.

Results:
34 patients (14 male, mean age: 52.2, range:26-99) were analyzed, 15 with pheochromocytoma, 19 with paragangliomas. Patients were grouped into cluster 1A (n=15), 1B (n=2), 2 (n=1) and sporadic PPGLs (n=16). Radioligand uptake was significantly higher in cluster 1A (median SUVmax = 64.8 (35.3-112.5) compared to sporadic PPGLs (median SUVmax = 27.95, range 11.6-52.3, p = 0.01). The strongest radioligand uptake (SUVmax = 67.6 (52.4-121.2)) was noted in 11 SDHB-associated PPGLs. Cluster 1B and Cluster 2 PPGLs were not compared due to the small group size. MTV was not significantly different between the genetic subtypes. There was no significant correlation between SUVmax and Ki67.

Conclusion:
Cluster 1A including SDHB-associated PPGLs show significantly increased radioligand uptake on SSTR PET/CT compared to sporadic PPGLs as a prerequisite for an increased radiation dose in PRRT. Nevertheless, the therapeutic efficacy of PRRT is contingent on a multitude of factors and the relevance of genetic subtypes requires further clinical evaluation.

Granin family proteins and urinary steroid profiles in the diagnosis of patients with adrenal tumors

Glinicki Piotr[1,2], Szatko Alicja[1,2], Siejka Anna[3], Godlewska Marlena[4], Leszczyńska Dorota[2], Zielińska Magdalena[3],Kowalska-Ciszek Ewa[3], Klimiuk-Balas Joanna[3], Sawicka-Gutaj Nadia[5], Bętkowska Anna [4], Ambroziak Urszula[6], Bobrowicz Małgorzata[6], Ochocińska Agnieszka[3], Gaweł Damian[4], Ruchała Marek[5], Zgliczyński Wojciech[2]

[1]EndoLab Laboratory, Centre of Postgraduate Medical Education, Warsaw, Poland
[2]Department of Endocrinology, Centre of Postgraduate Medical Education, Warsaw, Poland
[3]Department of Clinical Biochemistry, Children's Memorial Health Institute, Warsaw, Poland;
[4]Department of Cell Biology and Immunology, Centre of Postgraduate Medical Education, Warsaw, Poland
[5]Department of Endocrinology, Metabolism and Internal Medicine, Poznan University of Medical Sciences, Poland
[6]Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Poland

Introduction

The application of metabolomics studies of determining the profile of steroid hormones or their metabolites in blood or urine opens new research directions in the diagnosis of adrenal diseases. In addition, studies of new biomarkers in the form of proteins, peptides, and neuropeptides can be useful at various stages of the diagnostic and therapeutic process.

Materials and methods

The study included 61 patients with various adrenal tumors (MACS, CS, PA). All patients with adrenal tumors were analyzed for 41 different steroid hormone metabolites in a 24-hour urine samples by GC/MS technique, and selected proteins / peptides: CgA, WE-14, Catestatin, Serpinin, proSAAS in blood by ELISA immunoassays.

Results

Comparison of CS vs. NFAA group revealed significant differences for: ET/AN ($p = 0.010$), THS ($p = 0.003$), THF ($p = 0.007$), Free cortisol ($p = 0.004$) and CgA ($p = 0.002$) with higher level in CS group in all cases. Significant difference for MACS vs. NFAA was confirmed for: AN ($p = 0.008$), An-3-ol ($p = 0.002$), with higher level in NFAA group and for THS ($p = 0.009$) with higher level in MACS group. For PA vs. NFAA there was a significant difference for THAldo ($p = 0.026$) with higher level in PA group.
The next stage of the project was the analysis of selected urinary steroid profile biomarkers (ET/AN, THS, THF, Free cortisol) and protein - chromogranin A in a group of patients with CS in order to assess the usefulness of this diagnostic tool. In the study of a panel of biomarkers (ET/AN + CgA, THS + CgA, THF + CgA, Free cortisol + CgA), the sensitivity and specificity rates were over 80-100%.

Conclusions

The study of urinary steroid profile and chromogranin A concentration has shown high usefulness in the diagnosis of Cushing's syndrome.

Lipotoxicity and Autophagy Induced by Cholesterol-Containing Peptide Nanosponges in Adrenocortical Carcinoma Cells

Mariam Hassany [1, 2], Ritihaas Surya Challapalli [1], Obdulia Covarrubias-Zambrano [3], Sarah Feely [1], Solene Fiachetti [1], Michaela Luconi [2], Martin O'Halloran [6], Constanze Hantel [4, 5], Punit Prakash [8, 9], Stefan H. Bossmann [3], Michael Conall Dennedy [1]

[1] Discipline of Pharmacology and Therapeutics, School of Medicine, University of Galway, Galway, H91 V4AY, Ireland
[2] Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy
[3] Department of Cancer Biology, The University of Kansas Medical Center, Kansas City, KS. USA
[4] Department of Endocrinology, Diabetes, and Clinical Nutrition, University Hospital Zurich, Zurich, Switzerland
[5] Medizinische Klinik und Poliklinik III, University Hospital Carl Gustav Carus Dresden, Dresden, Germany
[6] Centre for Microscopy & Imaging, University of Galway, Ireland
[7] Translational Medical Device Lab, University of Galway, Galway, Ireland
[8] Department of Electrical and Computer Engineering, Kansas State University, Manhattan, KS. USA
[9] Department of Biomedical Engineering, The George Washington University, Washington, DC, USA

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Abstract: Adrenocortical carcinoma (ACC) remains a difficult-to-treat malignancy, particularly in chemotherapy-resistant cases. Cholesterol-containing peptide nanosponges (NS) have demonstrated cytotoxic effects in ACC cells. Given the central role of cholesterol in steroidogenesis and cellular metabolism, we investigated how NS induce lipotoxicity and whether resistant ACC cells activate cholesterol efflux pathways as a survival mechanism.

We evaluated NS uptake and lipid-related toxicity in three ACC cell lines: H295R (chemotherapy-sensitive), HAC-15, and MUC-1 (both chemoresistant). Post-treatment, total lipid content, cholesterol esters, and triglyceride levels were measured. Free cholesterol (FC) levels were assessed using FILIPIN III staining, and gene expression of cholesterol efflux transporters LXRα, ABC A1, and ABC G1 was analyzed via qPCR in MUC-1 cells. Autophagy involvement was examined through western blot detection of LC3II/LC3I ratios.

NS treatment caused a marked reduction in total lipids across all cell lines, with altered cholesterol ester and triglyceride levels. FILIPIN III staining revealed increased FC specifically in the dead population of H295R cells, suggesting cholesterol overload may drive cytotoxicity. In MUC-1 cells, upregulation of LXRα, ABC A1, and ABC G1 suggested an adaptive response aimed at mitigating cholesterol-induced stress. Western blot analysis showed upregulation of autophagy markers, indicating that autophagy contributes to NS-induced cell death.

In conclusion, cholesterol-functionalized nanosponges trigger lipotoxicity in ACC cells, with chemoresistant cells responding via cholesterol efflux mechanisms. Free cholesterol accumulation and autophagy activation appear to mediate cytotoxicity. These findings highlight new therapeutic opportunities targeting cholesterol metabolism in ACC.

The role of FDG-PET/CT in managing indeterminate adrenal lesions - a retrospective, single-centre study

Hempel Justyna, Bobrowicz Małgorzata, Gladka Adrianna, Ambroziak Urszula

Medical University of Warsaw, Warsaw, Poland

Introduction:

The latest European Society of Endocrinology guidelines on the subject of the management of adrenal incidentalomas bring up the value of F-18-fluorodeoxyglucose (FDG) PET/CT which could be beneficial in patients with indistinguishable adrenal lesions.

FDG-PET is a nuclear imaging technique and a very valuable modality widely used in oncology diagnosis. Combining FDG-PET alongside computed tomography (CT) or magnetic resonance imaging (MRI) became a common method in terms of identifying and localising proliferative processes which were difficult to determine using standard instruments.

According to the guidelines, FDG-PET/CT could be considered in indefinite tumours (with unenhanced HU between 11 and 20) or lesions over 4 cm of size. The lack of uptake of the radiotracer (fluorodeoxyglucose) or the uptake lower than the liver indicate in favour of benignity. What is more, the technique is suggested to carry out staging before performing a surgery of a malignant adrenal mass. However, neither recommendation can be supported with evidence of sufficient quality.

Methods:

This study analysed the medical records of fifteen patients that were referred to undergo a PET-CT examination with fluorodeoxyglucose as a radiotracer in order to assess the possible malignancy of the adrenal lesions in the last three years. The initial diagnosis of adrenal lesion was made based on a CT scan or MRI. Our study was aimed at analysing whether performing a FDG-PET/CT altered the diagnostic process in terms of additional findings or decision making.

Results:

Six of the pathological masses were identified as benign. A further two were described as unclear or heterogenous, requiring a follow-up. One lesion was suggested to be differentiated with a renal pathology, one required evaluation using another examination (scintigraphy) and one combined morphology of adenoma and haematoma.

In the final four patients, features of malignancy or indeterminate character of the adrenal masses were present in the prefatory imaging. This resulted in three patients undergoing a unilateral adrenalectomy while one is being qualified for the surgery.

A histopathological examination followed the three adrenalectomies and stated two of them as benign adenomas and one as high-grade adrenocortical carcinoma.

Conclusions:

In conclusion, FDG-PET/CT appears to be an advantageous modality with regard to unclear adrenal lesions requiring additional examination or treatment, especially concerning malignant tumours. The major advantage of this tool is its low risk of false negative results, while the major drawback is the high cost and limited accessibility.

Influence of germline and somatic mutations on the expression of potential CAR-T cell targets in pheochromocytoma and paraganglioma

Huckert Paul[1], Remde Hanna[1], Weber Justus[5], Schauer Marc[1], Uchihara Masaki[1], Kircher Stefan[2], Sbiera Silviu[1], Pellegata Natalia[3], Mohr Hermine[3], Robledo Mercedes[4], Hudecek Michael[5], Fassnacht Martin[1] & Landwehr Laura-Sophie[1]

[1] Division of Endocrinology and Diabetes, Department of Internal Medicine I, University Hospital of Würzburg, Würzburg, Germany.
[2] Institute for Pathology, University of Würzburg, Würzburg, Germany.
[3] Institute for Diabetes and Cancer, Helmholtz Center Munich, Neuherberg, Germany.
[4] Hereditary Endocrine Cancer Group, Spanish National Cancer Research Center (CNIO), Madrid, Spain.
[5] Department of Internal Medicine II, University Hospital of Würzburg, Würzburg, Germany.

Background and aims: Pheochromocytomas and paragangliomas (PPGL) are rare tumors of the adrenal medulla that metastasize in 10-17% of cases and associated with a poor prognosis. Germline mutations have been observed in approximately 30-40% of PPGL patients, while somatic mutations are present in an additional 47%. To explore therapeutic options for advanced stages, we investigate the susceptibility of the receptor tyrosine kinase 1 (ROR1), -2 (ROR2) and L1 cell adhesion molecule (L1CAM) as potential targets for a chimeric antigen receptor (CAR)-T cell therapy considering the genetic background.

Methods: The study population comprised 159 patients with (non-)metastatic and non-metastatic PPGL. To ascertain the presence of frequent PPGL mutations within our cohort, DNA from blood, formalin-fixed paraffin-embedded (FFPE) and native tissue was analyzed via Sanger sequencing for germline or tumor mutations (n=144). The mRNA expression of ROR1, ROR2 and L1CAM were analyzed by RT-qPCR normalized to beta-actin (n=82). The protein expression levels of these antigens were quantified in FFPE tissue by immunohistochemistry (n=196) with subsequent image analysis using QuPath. Clinical correlations were performed using SPSS and Prism.

Results: RT-qPCR analysis revealed the mean mRNA expression levels of ROR2 (0.00228 fold change), L1CAM (0.12664 fold change) as well as ROR1 (0.00043 fold change). At protein level, the expression of the tumor specific antigens in PPGL (average H-scores) reflect its potential: L1CAM: 181, ROR2: 145, ROR1: 57. Analysis of the mutation data showed 27% germline and 31% somatic mutations in the cohort.

Conclusion: Our preliminary data indicates the potential of both L1CAM and ROR2 as targets for a CAR-T cell therapy in PPGL. However, ROR1 plays a minor role due to its low and inhomogeneous expression. The aims of this study is to establish a correlation between the genetic data and the expression of the tumor targets to identify patients with metastatic PPGL who could benefit from CAR-T cell therapy.

Study of the therapeutic potential of dimethylfumarate in SDH-deficient paragangliomas and pheochromocytomas: Induction of ferroptosis and clinical implications

Amandine Jacquet[1,2] and Judith Favier[1,2]

[1]CRC, INSERM UMR1138, Equipe Labellisée par la Ligue Contre le Cancer, Paris, France.
[2]Université de Paris, Paris, France

Paragangliomas and pheochromocytomas (PPGL) are rare neuroendocrine tumors whose metastatic forms are frequently associated with inherited deficiencies in succinate dehydrogenase (SDH), an enzyme at the crossroads of the Krebs cycle and the mitochondrial respiratory chain. Ferroptosis, an iron-regulated cell death mechanism characterized by the lethal accumulation of uncontrolled lipid peroxides, is currently emerging as a potentially exploitable cell death pathway in PPGL. Dimethylfumarate (DMF), used as a treatment for multiple sclerosis, is an activator of nuclear factor erythroid 2-related factor 2 (Nrf2) known for its role in modulating oxidative stress, and is attracting increasing interest as an inducer of ferroptosis.
We are exploring the mechanisms by which DMF induces ferroptosis in Sdh-deficient cells. Preliminary results showed an increase in mitochondrial ROS levels in Sdhb KO cells, as well as in lipid peroxidation. We also observed deregulation of NAD/NADH and NADP/NADPH levels.
DMF activates NRF2, increasing the expression of genes regulating glutathione metabolism/homeostasis and promoting a decrease in glutathione levels, sensitizing tumor cells to ferroptosis. In parallel, DMF could inhibit the activity of glutathione peroxidase 4 (GPX4), a key enzyme protecting cells against lipid peroxidation, via cysteine residues, thus contributing to the accumulation of lipid peroxides and the induction of cell death by ferroptosis.
The therapeutic implications of DMF induction of ferroptosis in SDH-deficient cancers are also discussed. By exploiting the sensitivity of tumor cells to ferroptosis, DMF could represent a new therapeutic approach for the treatment of SDH-deficient cancers, where the only effective treatment is surgical removal of the tumor.

Adrenal Tumours in Congenital Adrenal Hyperplasia (CAH) due to 21-Hydroxylase Deficiency: Therapeutic Pitfalls in a Severely Virilized Female Teenager

Januś Dominika, Iwańska Anna, Gawlik Zuzanna, Starzyk Jerzy

Department of Pediatric and Adolescent Endocrinology, University Children`s Hospital, Krakow, Poland
Department of Pediatric and Adolescent Endocrinology, Chair of Pediatrics, Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland

Abstract:
This case report presents the long-term clinical course of a female patient with classic congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD), highlighting the therapeutic challenges associated with poor compliance secondary to psychosocial stress. Diagnosed neonatally and initially well managed with glucocorticoid and mineralocorticoid therapy, the patient demonstrated excellent clinical and hormonal control in early childhood. Feminizing genital surgery was performed at 2.5 years of age. Despite transient stability, signs of hyperandrogenism recurred in mid-childhood, coinciding with precocious puberty and progressive virilization.

From age 11, her condition deteriorated markedly due to non-adherence to therapy, likely related to a difficult family situation—maternal abandonment and care by her father and ill grandmother. This led to sustained androgen excess, resulting in severe virilization including a masculine body habitus, 3 cm clitoromegaly, and hirsutism. Imaging revealed a 2 cm hypoechoic lesion in the left adrenal gland with loss of corticomedullary differentiation, suggestive of an ACTH-driven adrenal tumor.

At age 13, following initiation of psychotherapy and improved caregiver support, treatment compliance significantly improved. A combination of reduced hydrocortisone and added dexamethasone resulted in improved adrenal hormonal control and regression of the adrenal lesion. Despite partial reversal of virilization and normalization of ovarian and uterine morphology, primary amenorrhea persisted.

This case underscores the profound impact of psychosocial stress and non-compliance on long-term outcomes in CAH, with potentially reversible adrenal morphological changes. It highlights the critical importance of early psychological support, family engagement, and consistent interdisciplinary care in managing complex CAH cases.

Transcriptome signatures associated with response to treatment in advanced adrenocortical carcinoma: an ENSAT study

Jouinot Anne[1,2], Bouvard François[1], Lombard Margot[1], Violon Florian[1,3], Birtolo Francesca[1], Perlemoine Karine[1], Gaillard Martin[1,4], Bonnet-Serrano Fideline[1], Gravrand Victor[1], Bioletto Fabio[1], Benanteur Nesrine[1], Martin Yoann[1], Adoux Lucie[1], Angelousi Anna[5], Vaduva Patricia[1,6], Mandic Ante[7], Kastelan Darko[7], Dedonno Anna-Aurora[8], Luconi Michaela[8], Babinska Anna[9], Swiatkowska-Stodulska Renata[9], Hanzu Felicia[10], Maite Rodrigo[10], Kimpel Otilia[11], Fassnacht Martin[11], Libe Rossella[1,2], Bertherat Jérôme[1,2], Assié Guillaume[1,2]

1- Université Paris-Cité, Institut Cochin, INSERM U-1016, CNRS UMR-8104, F-75014, Paris, France
2- Endocrinology Department, AP-HP Hôpital Cochin, Paris, France
3- Pathology Department, AP-HP Hôpital Cochin, Paris, France
4- Department of Digestive and Endocrine Surgery, AP-HP Hôpital Cochin, Paris, France
5- First Department of Internal Medicine, Unit of Endocrinology, Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece
6- Department of Endocrinology, Diabetes and Nutrition, Rennes University Hospital, Rennes, France
7- Endocrinology Department, University Hospital Centre Zagreb, Kispaticeva 12, 10000 Zagreb, Croatia
8- Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy
9- Department of Endocrinology and Internal Medicine, Medical University of Gdansk, Gdańsk, Poland
10- Endocrinology Department, Hospital Clínic, Barcelona, Spain
11- Division of Endocrinology and Diabetes, Department of Medicine, University Hospital, University of Würzburg, 97070 Würzburg, Germany

Background:

Adrenocortical carcinoma (ACC) is an aggressive tumor with poor prognosis. Mitotane, platinum-based chemotherapy and immune checkpoints inhibitors (ICI) are rarely effective (< 20% of response), even though some extraordinary responses could be observed.
The aim of this study is to identify transcriptome signatures associated with response to treatment.

Methods:
One hundred and forty-one ACC patients treated with mitotane monotherapy, platinum-based chemotherapy or ICI in metastatic setting were sent from 8 ENSAT centers.
Transcriptome was determined on tumor RNA extracted from frozen or paraffin samples, using RNA-sequencing of 3' transcripts (QuantSeq, Lexogen and Illumina).
Primary endpoint was objective response rate, assessed by RECIST radiologic criteria.
Transcriptome analyses included unsupervised clustering and differential expression analyses (DESeq and GSEA) between responders and non-responders. Adjustment for multiple testing was performed with Benjamini-Hochberg correction.

Results:
Among the 83 patients with clinical data already available (69% female, median age 49 y), 14 patients were responders to mitotane (7 partial responses (PR) and 4 complete responses (CR)), 20 (14 PR and 6 CR) to platinum-based chemotherapy and 4 (4 PR) to ICI.
Unsupervised clustering did not identify specific clusters associated with response to treatment.
Comparison between responders and non-responders showed 45, 36 and 197 differentially expressed genes for mitotane, platinum and ICI respectively.
Response to mitotane was associated with expression of *PON3* – coding for paraoxonase 4 enzyme, involved in redox signaling – and *MRC2* – involved in extracellular matrix remodeling.

Response to platinum was associated with expression of *ACSM2A* and *ACSM2B*, encoding acyl-coA synthetase.
Response to ICI was associated with enrichment in pathways associated with immune response (antigen presentation, MHC class II, neutrophil chemotaxis and migration).

Conclusion:
Transcriptome identifies signatures associated with response to treatment in advanced ACC, which will need to be confirmed by future studies.

A Case Series of Patients with Primary Aldosteronism managed with Finerenone

Panagiotis Karageorgiou, Dimitra Sinni, Sofia Chatzi, Marinela Tzanela, Dimitra A Vassiliadi

Department of Endocrinology "D. Ikkos"- Diabetes Center- EndoERN-Evangelismos General Hospital, Athens, Greece

Background:
Finerenone, a selective non-steroidal mineralocorticoid receptor antagonist (MRA), has demonstrated efficacy in reducing cardiovascular and renal risk in patients with chronic kidney disease and hypertension. However, its role in the management of primary aldosteronism (PA) remains underexplored, particularly in individuals with inadequate response or intolerance to conventional steroidal MRAs such as spironolactone or eplerenone.

Methods:
We present a retrospective case series involving five patients with biochemically confirmed PA. Four patients were transitioned from spironolactone or eplerenone due to suboptimal blood pressure (BP) control and/or adverse effects. One patient received finerenone following partial clinical and biochemical improvement post-unilateral adrenalectomy. Finerenone therapy was initiated at a low dose and titrated to a maximum of 20 mg daily. Clinical parameters, including office BP, serum potassium, renal function, and antihypertensive medication burden, were monitored throughout the treatment period.

Results:
Finerenone therapy resulted in normalization of both systolic and diastolic BP in all five patients. This was accompanied by a marked reduction in the number and dosage of concomitant antihypertensive agents. Treatment was well tolerated, with no reported serious adverse events. Importantly, serum creatinine and potassium levels remained stable, and no cases of hyperkalemia or acute kidney injury were observed during follow-up.

Conclusion:
In this preliminary case series, finerenone was associated with effective blood pressure control and a favorable safety profile in patients with PA who were either resistant to or intolerant of conventional MRAs. These findings suggest that finerenone may represent a viable therapeutic alternative in select cases of PA, warranting further prospective investigation.

Adenosine receptor A2A as a potential target for diagnosis and therapy in pseudohypoxic Pheochromocytomas and Paragangliomas (PPGLs)

Karna Bhargavi[1], Mohr Hermine[1], Bechmann Nicole[2], Deuther-Conrad Winnie[3], Herzig Stephan[1], Pellegata Natalia S.[1]

[1]Institute for Diabetes and Cancer, Helmholtz Centre Munich, Neuherberg, Germany
[2]Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[3]Institute of Radiopharmaceutical Cancer Research, Helmholtz Zentrum Dresden-Rossendorf (HZDR), Forschungsstelle Leipzig, Leipzig, Germany

Background: In the tumor microenvironment, adenosine signaling via the A2A receptor (A2A/Adora2a) promotes immune evasion and is linked to tumor cell migration, branching, and proliferation. A2A expression can be induced by hypoxia-inducible factors (HIFs). We found A2A overexpressed in in vitro and in vivo models of pseudohypoxic PPGLs (p-PPGLs), aggressive tumors lacking effective therapies and associated with high morbidity and mortality. Given the need for novel therapeutic targets, we investigated A2A's role in p-PPGL progression.

Materials&Methods: mRNA and protein levels of A2A were analyzed in PC12 cells (with/without a pseudohypoxic signature) and p-PPGL tissues from MENX rats via qRT-PCR, immunostaining, and western blotting. Cell proliferation was assessed in the presence/absence of the A2A antagonist istradefylline using viability assays. Adenosine levels were measured in cells and tissues. MENX rats received istradefylline injections, and tumor size was monitored. Autoradiography of p-PPGLs was performed using an A2A-specific radiolabeled tracer.

Results: A2A and Hif2α (Epas1) expression was significantly higher in PC12 cells with a hypoxic signature than in those without. Adenosine levels were elevated in rat p-PPGLs compared to normal adrenal tissues. Advanced rat p-PPGLs showed increased expression of A2A-adenosine pathway genes (*Adora2a, Epas1, Entpd1*). A2A inhibition reduced viability in A2A-expressing PC12 cells. Mechanistically, blocking A2A signaling in p-PPGL cells decreased CREB phosphorylation and increased OXPHOS pathway.

Conclusions: Our findings indicate a role for A2A in p-PPGL growth, suggesting it as a potential therapeutic target. The availability of A2A-directed tracers and antagonists supports the translation of these findings into novel imaging and treatment options for p-PPGLs.

Rare but relevant: incidence, characteristics and clinical impact of rare metastases in adrenocortical carcinoma

Otilia Kimpel[1], Lara Friedel[1], Martin Fassnacht[1,2,3], Barbara Altieri[1,2]

1 Division of Endocrinology and Diabetes, Dept. of Medicine, University Hospital, University of Würzburg, Germany
2 Bavarian Cancer Research Center (BZKF), University Hospital, University of Würzburg, Germany
3 Comprehensive Cancer Center Mainfranken and NCT WERA, University of Würzburg, Germany

Background: Adrenocortical carcinoma (ACC) is a rare aggressive malignancy characterized by a high rate of metastatic spread.

Methods: Retrospective study including patients with ACC treated at our center. Clinical data including metastases, onset, associated symptoms, and specific treatment were collected from medical records. Rare metastases were defined as those not occurring in the liver, lung, bone, lymph nodes or peritoneal carcinomatosis.

Results: Until now, among 863 screened patients, 421 patients presented metastases. 237 patients had already metastases at primary diagnosis, but none at rare sites. During follow-up metastases in common regions such as liver (n=421), lung (n=497), bone (n=165), lymph nodes (n=363), and peritoneal carcinomatosis (n=158) occurred in 658 (76%) patients. Rare metastases were observed in 77 patients (9 %) during follow-up, affecting brain (n=13 (1.5%), pancreas (n=9, 1%), contralateral adrenal gland (n=8, 0.9%), skin (n=8, 0.9%), spleen (n=8, 0.9%), intestine (n=8, 0.9%), muscle (n=7, 0.8%), calvaria (n=6, 0.7%), diaphragm (n=6, 0.7%), uterus/adnexa/vagina (n=3, 0.3%), kidney (n=2, 0.2%), pericardium (n=2, 0.2%), stomach (n=1, 0.1%), urethra (n=1, 0.1%), jaw (n=1, 0.1%), breast (n=1, 0.1%) and sphenoorbital region (n=1, 0.1%).

Rare metastases appeared after a median of 25 months (range 0.6-293 months) since primary diagnosis.

Most of these metastases were asymptomatic and were detected during routine staging. Only 23 caused symptoms, including pain, swelling, perforation, ileus or neurological symptoms. Brain metastases were symptomatic in 8 of 13 cases, causing hemiplegia, seizure, headache, and in one case haemorrhage resulting in death. Half of cerebral metastases were multiple and treated with radiotherapy or surgery. Overall survival was reduced in patients with symptomatic cerebral metastases in comparison to asymptomatic brain metastases, but this difference was not significant (2.5 vs. 8.6 months, p=0.39).

Conclusion: Rare metastases developed in patients with widespread metastatic disease. Cerebral metastases were the most clinically relevant, often symptomatic and potentially affecting survival.

Patient reported outcome measurements (PROM) for the evaluation of the psychosomatic status in patients with adrenocortical carcinoma

Kimpel Otilia[1]*,Kiermeier Senta [2]*, Stahl Emely [1], Altieri Barbara [1,3], Fassnacht Martin [1,3,4]*, Maatouk Imad [2]*

1 Division of Endocrinology and Diabetes, Dept. of Medicine, University Hospital, University of Würzburg, Germany
2 Section of Psychosomatic Medicine, Psychotherapy and Psychooncology, Department of Internal Medicine II, Julius-Maximilian University Würzburg, Würzburg, Germany.
3 Bavarian Cancer Research Center (BZKF), University Hospital, University of Würzburg, Germany
4 Comprehensive Cancer Center Mainfranken, University of Würzburg, Germany

Background: Several studies have shown that distress, depression and fear of progression (FoP) are common in cancer patients and negatively impact quality of life (HRQoL). However, no studies have explored these aspects in patients with adrenocortical carcinoma (ACC).

Objectives: This observational study investigates FoP, distress and depression in patients with ACC and their impact on HRQoL.

Methods: Adults (≥18 years) with ACC were included during regular hospital visits. Patients filled out questionnaires addressing FoP (FoP-questionnaire, short-form), HRQoL (EORTC-QLQ-C30), distress (National Comprehensive Cancer Network distress thermometer) and depression (patient health questionnaire, PHQ-9). Patients were distributed according to different clinical parameters to assess possible influencing factors. Multiple linear regression on HRQoL subscales ('total symptoms', 'total functioning') was performed in order to identify associated psychosomatic variables.

Results: Over a 12-months period, 105 patients were included (56.2% female; median age = 49 years). Most patients demonstrated an elevated level of distress (62%) and a high level for FoP (59%). A major depression according to PHQ-9 was present in 41% of participants.The HRQoL questionnaire showed a higher level for functional problems (median = 62.2) in comparison to symptoms (median = 33.3). These results were neither influenced by tumour burden, hormone secretion nor by cancer treatment. In the multiple linear regression only depressive symptoms revealed a significant effect for HRQoL 'total symptoms' (B_{stand} = 0.620, p<.001) and for HRQoL 'total functioning' (B_{stand} = -0.673, p<.001). Most of our patients (80%) showed a need for psychosomatic counselling revealed by the questionnaires.

Conclusions: Patients with ACC show similar values for distress, FoP, depression and HRQoL independent of their clinical characteristics. The questionnaires revealed the need for psychooncological intervention that should be offered to all patients with ACC.

Is this a true recurrence of adrenocortical cancer?

Kluczyński Łukasz[1], Kotecka-Blicharz Agnieszka[1], Hasse-Lazar Kornelia[1], Handkiewicz-Junak Daria[1]

[1]National Research Institute of Oncology, Department of Nuclear Medicine and Endocrine Oncology, Gliwice, Poland

We present the case of a 53-year-old woman with coexistence of adrenocortical carcinoma and lung neuroendocrine tumour. Initially, diagnostics was conducted in Ukraine, where the patient underwent left adrenalectomy due to a large, hormonally inactive tumour measuring 110x100x80 mm (05/2023). Histopathological examination confirmed well-differentiated adrenocortical carcinoma, oxyphilic variant, Ki-67 5%. The assessment of the surgery radicality was not possible. During the follow-up, based on control computed tomography (CT) and positron emission tomography with fluorodeoxyglucose a suspicion of tumour recurrence (48x32 mm) in the left adrenal area was raised. In addition, a lesion in the right lung measuring 16x12 mm was described (the biopsy suggested a typical G1 lung carcinoid). Exploratory laparoscopy was performed – the mass in the adrenal area was considered unresectable; due to massive bleeding during the procedure, splenectomy was necessary. The unfavourable tumour location made the biopsy impossible. The patient was initially qualified by multidisciplinary team for mitotane treatment and radiotherapy of the lesion in left adrenal gland. Due to questionable nature of the tumour, control CT and magnetic resonance imaging were preformed - no progression was observed during one-year follow-up (radiological image suggested hematoma with postoperative changes and possible neoplasm infiltration). The decision about reoparation was made – abnormal mass was removed from the left adrenal region (03/2025). The examination revealed ingrown surgical swab with surrounding fibrosis, without suspicious neoplastic cells.

The patient is currently in good clinical condition, awaiting resection of the lung tumour. Due to coincidence of two neoplasms genetic tests was performed – no pathogenic mutations were detected in TP53 and MEN1 genes.

Suspicion of the adrenal cancer recurrence is often a challenge. Correct diagnosis requires thorough clinical, biochemical, radiological assessment and multidisciplinary approach.

Overnight Dexamethasone in Primary Aldosteronism Screening (ODEPRASC): Initial Results of a Diagnostic Accuracy Study

Piotr Kmieć[a, B], MD, PhD; Dominika Okroj[b*], MD; Małgorzata Zdrojewska[a,B*], MD, PhD; Jowita Fiszer[a,B #]; Sonia Zembrzuska[a,B #]; Renata ŚWIĄTKOWSKA-Stodulska[a,B], Profesor, MD, PhD.
* MZ and DO contributed equally, # JF and SZ contributed equally.

a) Department of Endocrinology and Internal Medicine, Medical University of Gdańsk, Gdańsk, Poland;
b) Department of Endocrinology and Internal Medicine, University Clinical Center, Gdańsk, Poland.

Background and Aim
The underdiagnosis of primary aldosteronism (PA) partially stems from the recommendation to withdraw medications that interfere with the renin–angiotensin–aldosterone system (RAAS) before testing. Previous studies showed that: (i) an aldosterone (Ald)-to-renin ratio (ARR) of 10 pg/uIU/ in patients receiving RAAS-interfering medications was shown to be equivalent to 20 pg/uIU off interfering drugs, (ii) 48-hour cosyntropin suppression was highly accurate in the PA work-up among HT patients off interfering drugs when combined with objective diagnostic thresholds.
This study aims to generate thresholds offering a 25% higher diagnostic accuracy in PA screening than baseline ARR determination despite interfering medications by applying overnight cosyntropin suppression.

Methods
A development and confirmation cohort have been planned. Patients with an adrenal incidentaloma are enrolled in a tertiary endocrine clinic. ARRs are determined by a radioimmunoassay before and after overnight intake of 1 mg dexamethasone (DXM) on, partially off, and off interfering medications. Exclusion criteria include hyperreninemia (above manufacturer's threshold).

Results
Until March 18, 2025, initial data have been gathered for 63 patients (63% female, aged 63+-8.1 years). All enrolled patients were normonatremic, all but three normokalaemic. Possible mild autonomous cortisol secretion was recorded in 13 patients (highest post-DXM cortisol: 78.7 nmol/l)
Baseline PA screening was negative in 25.4%, positive in 30.2%, and equivocal in 44.4% of patients. Post-DXM measurements allowed clearly classifing the ARR as positive (*i.e.* ARR of 2 ng/dl per mIU/l or above) in a total of 52.4% despite interfering medications; in one patient a positive initial ARR was equivocal post-DXM, which translates to a 20.6% improvement in the overall diagnostic accurracy (p=0.024, Fisher's exact test).
Modarate to strong correlations were recorded for pre- to post-DXM Ald, renin, and ARR.

Conclusions
The ODEPRASC study may provide a rationale for an optimized diagnostic approach in PA screening (ClinicalTrials.gov registration ID NCT06740838)

Role of the adrenal gland in postmenopausal cardiometabolic complications associated with yo-yo diet

Linghui Kong[1], Coralie Frimat[2], Nicolo Faedda[1], Bakhta Fedlaoui[1], Isabelle Giscos-Douriez[1], Fabio L Fernandes-Rosa[1], Bruno Feve[3,4,5], Antoine Ouvrard-Pascaud[2], Maria-Christina Zennaro[1,6] et Sheerazed Boulkroun[1]

[1] Université Paris Cité, Inserm, PARCC, Paris, France
[2] Inserm U1096, UFR santé, Rouen, France
[3] Sorbonne Université-Inserm, Centre de Recherche Saint-Antoine UMR_S938, Paris, France
[4] Institut hospitalo-universitaire de cardio-métabolisme et nutrition (ICAN), Paris, France
[5] Service d'endocrinologie-diabétologie, centre de référence des maladies rares de l'insulino-sécrétion et de l'insulino-sensibilité (PRISIS), Hôpital Saint-Antoine, Assistance publique-Hôpitaux de Paris, Paris, France
[6] Service de Génétique, Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Européen Georges Pompidou, F-75015 Paris, France

Introduction Overweight and obesity represent major public health problems and only 20% of overweight individuals achieve sustainable weight loss, leading to repeated cycles of weight loss and gain. Weight cycling is associated with an increased risk of developing metabolic syndrome. Furthermore, the menopausal transition is associated with a 60% increase in the incidence of metabolic syndrome, which is associated with increased cardiovascular mortality. Aldosterone, produced by the adrenal gland, plays a role in regulating metabolic processes and exhibits a pathophysiological link to visceral adiposity. Our objective is to investigate the role of the adrenal gland in postmenopausal cardiometabolic complications related to yo-yo dieting.

Materials and Methods Female mice, both ovariectomized and non-ovariectomized, were subjected to three successive cycles of a high-fat diet/standard diet (yoyo diet) or maintained exclusively on a standard diet for 33 weeks. Functional and molecular analyses were performed at the end of each phase of the high-fat diet or standard diet.

Results Ovariectomized mice on a yoyo diet exhibit an increase in adrenal gland weight. Histological and immunofluorescence staining demonstrated significant adrenal cortex dysplasia, characterized by loss of zona glomerulosa (ZG) and zona fasciculata (ZF) zonation. A decreased expression of both *Cyp11b2* and *Cyp11b1* was observed at the mRNA level in OVX mice, and Western blot revealed reduced CYP11B2 protein expression. Interestingly, plasma steroid profiling revealed that yoyo diet significantly increased aldosterone levels ($p=0.0025$), while simultaneously lowering corticosterone levels ($p=0.0461$). Furthermore, ovariectomized mice subjected to yoyo diet show higher fasting blood glucose and impaired glucose tolerance, and also demonstrated increased left ventricular cardiomyocyte cross-sectional area (CSA).

Conclusions
Ovariectomized mice on a yoyo diet developed multinodular adrenals, accompanied by increased aldosterone and decreased corticosterone levels, which may serve as the molecular basis for cardiovascular metabolic complications.

Integrative bioinformatics analysis of potential modulators affecting the expression of PINK1, DLGAP5, and BUB1B genes in ACC

Kožik B[1,*], Božović A. [1], Mandušić V. [1], Živaljević V. [2,3], Paunović I. [2,3], Stanojević B.[1], and Todorović L. [1]

[1] Laboratory for Radiobiology and Molecular Genetics, Vinča Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia
[2] Center for Endocrine Surgery, Clincal Center of Serbia, Belgrade, Serbia
[3] School of Medicine ,University of Belgrade, Belgrade, Serbia
* Correspondence: bojana86@vin.bg.ac.rs

Adrenocortical carcinoma (ACC) is an aggressive form of cancer that originates from the adrenal cortex and is associated with poor clinical outcomes. Current treatment options have not shown promising results, indicating a need for new prognostic and predictive factors in clinical practice.

Research has identified alterations in gene expression patterns in adrenocortical neoplasms compared to normal tissue. In addition to their role in tumor development, distinct gene expression signatures may help differentiate between various types of adrenocortical tumors. Our previous findings suggest that the expression levels of the PINK1, DLGAP5, and BUB1B genes may serve as valuable molecular predictors of malignancy and/or survival in patients with ACC.

In this study, we will conduct an integrative bioinformatics analysis to identify new molecular modulators of PINK1, DLGAP5, and BUB1B gene expression. We will also investigate their protein-protein interactions (PPI), perform clustering and signaling analysis using available ACC datasets from The Cancer Genome Atlas (TCGA). The results of this study will be evaluated for their potential prognostic or predictive roles and tested in silico for the identification of druggable targets.

Steroidomics in adrenal tumors

Lafioniatis Alexandros[1], Velesiotis Christos[2], Mytareli Chrysoula[1], Siampanopoulou Vasiliki[1], Papalexis Petros[1], Tsirou Georgia[1], Chalmantzi Varvara[1], Moutsatsou Paraskevi[2], Kaltsas Gregory[3], Angelousi Anna[1]

[1]First Department of Internal Medicine, European Reference Network on Rare Endocrine Conditions (ENDO-ERN), Laikon General Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece.
[2]Department of Clinical Biochemistry, National and Kapodistrian University of Athens, School of Medicine, "ATTIKON" University Hospital, Athens, Greece
[3]Department of Propaedeutic and Internal Medicine, European Reference Network on Rare Endocrine Conditions (ENDO-ERN), Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece

Background: Mild autonomous cortisol secretion (MACS) as well as non-functional adrenal incidentalomas (NFAI) are associated with increased metabolic and cardiovascular risk suggesting a subtle secretion of cortisol or of other precursors of steroidogenesis.

Aim: To investigate steroid metabolites levels in patients with MACS and NFAI benign adrenal tumours as well as in adrenocortical carcinomas (ACC). Controls were defined as patients with normal adrenals on imaging.

Methods: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for the steroidomics analysis of 18 steroid precursors in the blood of 35 patients. The categorisation of patients in functional or NFAI was based on functional hormonal blood tests [1 mg overnight dexamethasone suppression test (ODST)] based on current guidelines. All ACC cases were confirmed histopathologically.

Results: A total of 29 patients (8 males) with adrenal tumors [(n=17 with NFAI, n=8 with MACS and n=4 with ACC] and 6 controls were included. LC-MS/MS analysis showed that all the median blood steroid hormones levels were significantly higher ($p<0.05$) in ACC patients compared with NFAI, MACS and control patients except for aldosterone levels. Regarding patients with benign tumors, median levels of baseline morning cortisol, corticosterone, 11-Deoxycorticosterone and 21-Deoxycortisol were significantly higher in MACS compared with NFAI (p=0,04, p=0,03, p=0,047, p=0,043 respectively). Baseline median levels of cortisol, corticosterone, 11-Deoxycorticosterone and progesterone levels were significantly higher in MACS compared with controls (p=0,037, p=0,042, p=0,039, p=0,023 respectively). Baseline median levels of 11-Deoxycorticosterone were significantly higher in NFAI compared with controls (p=0,01) whereas progesterone and 17-OH progesterone were significantly lower in NFAI compared with controls (p=0,002, p=0,04).

Conclusions: All steroid hormones levels were significantly higher in ACC patients compared to patients with MACS and NFAI. Precursors such as 11- Deoxycorticosterone, and 21-deoxycortisol could play a supplementary role to the routine hormones profile for the distinction of NFAI from controls or MACS.

Radiomics Predictors of Outcome in Adrenocortical Carcinoma Treated with EDP-M

Laganà Marta[1], Bettini Davide Lorenzo[1], Rodella Sara[1], Casella Carlo[2], Leggeri Alice[2], Ambrosini Roberta[2], Cosentini Deborah[1], Gatelli Fabio[3], Signoroni Alberto[3], Ravanelli Marco[2], Grisanti Salvatore[1], Berruti Alfredo[1]

[1]Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia; ASST Spedali Civili di Brescia, Medical Oncology Unit, Brescia, Italy
[2]Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia; ASST Spedali Civili di Brescia, Radiology Unit, Brescia, Italy
[3]Department of Information Engineering, University of Brescia, Brescia, Italy

Background:
Adrenocortical carcinoma (ACC) is a rare malignancy with limited treatment options. Etoposide, Doxorubicin, and Cisplatin plus Mitotane (EDP-M) remains the standard first-line therapy for advanced ACC. Identifying imaging biomarkers that predict treatment outcomes could optimize patient management. This study investigates the potential of CT-based radiomics features to predict progression-free survival (PFS) and overall survival (OS) in metastatic ACC patients undergoing EDP-M.

Methods:
Twenty-eight patients with histologically confirmed metastatic ACC were retrospectively analyzed. All patients underwent contrast-enhanced CT before starting EDP-M. Radiomics features were extracted from the adrenal lesions and normalized using Z-score standardization. Feature selection was performed using the Mann–Whitney U test and Random Forest regression. Predictive modeling was conducted using linear regression with Leave-One-Out and 500-fold bootstrap cross-validation strategies. Kaplan-Meier analysis and log-rank tests were used to evaluate survival associations.

Results:
Significant class imbalance was observed in the dataset (PFS event rate: 89%; OS: 68%). The feature *log-sigma-6-0-mm-3Dfirstorder90Percentile* showed predictive ability for PFS, achieving an AUC of 0.623. Using Random Forest-selected features, *originalshapeSphericity* and *log-sigma-6-0-mm-3DglcmCorrelation* demonstrated improved performance (AUC: 0.826 and 0.737, respectively). For OS, *log-sigma-2-0-mm-3DglcmImc1* emerged as the top feature (AUC: 0.643). However, statistical significance was limited due to small sample size.

Conclusions:
This is the first study highlights the feasibility of CT-based radiomics in predicting outcomes in metastatic ACC patients treated with EDP-M. While several features showed promise, larger, prospective studies are warranted to validate these findings and refine predictive models. Radiomics is emerging as a non-invasive biomarker to aid clinical decision-making in ACC.

Steroidogenic Factor-1 regulates a core set of target genes to promote malignancy in adrenocortical carcinoma

João C. D. Muzzi[1,2], Carmen Ruggiero[3,4], Mabrouka Doghman-Bouguerra[3,4], Maísa E. Colodel[1], Jessica M. Magno[1,2], Jean S. S. Resende[1,2], Nelly Durand[3,4], Juliana F. de Moura[5], Larissa M. Alvarenga[5], Luciane R. Cavalli[1,6], Bonald C. Figueiredo[1,7], Enzo Lalli[3,4,8], Mauro A. A. Castro[2]

[1]Pelé Pequeno Príncipe Research Institute, Curitiba 80250-060, Brazil; [2]Bioinformatics and Systems Biology Laboratory, Federal University of Paraná (UFPR), Curitiba 81520-260, Brazil; [3]Institut de Pharmacologie Moleculaire et Cellulaire CNRS UMR 7275, 06560 Valbonne, France; [4]Université Côte d'Azur, 06560 Valbonne, France; [5]Department of Basic Pathology, Federal University of Paraná (UFPR), Curitiba 81530-990, Brazil; [6]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC 20057, USA; [7]Faculdades Pequeno Príncipe, Curitiba - PR, 80230-020, Brazil; [8]Inserm, 06560 Valbonne, France

Gene dosage is at the core of biological activity of the Steroidogenic Factor-1 (SF-1/NR5A1) transcription factor. Its overexpression in adrenocortical carcinoma (ACC) is associated with enhanced proliferation and invasive capacities, steroid modulation, immune suppression and poor prognosis. Surprisingly, three independent studies showed less than 10% agreement in identifying SF-1-regulated genes in the same H295R ACC cell line, raising concerns about technical reproducibility and methodological consistency.

Our study aimed to reconcile discrepancies in SF-1-regulated gene identification across independent studies using a systematic approach. We reanalysed datasets from those studies using an *in silico* SF-1 regulon obtained from ACC TCGA data as an external reference to evaluate transcriptional patterns. Additionally, we assessed how threshold selection impacts the overlap between experiments and optimized this process. Furthermore, we performed functional experiments to evaluate how variations in SF-1 dosage impact target gene expression. Our analysis revealed comparable transcriptional patterns across all studies, reconciling transcriptional signatures and phenotypes. Threshold optimization identified consensus sets of genes responsive to SF-1 perturbations. Functional experiments confirmed that variations in SF-1 dosage significantly impact gene expression, explaining discrepancies in previous studies, and evidenced negative autoregulation of the SF-1 transcript by its encoded protein both in ACC cells and in a mouse model of *Sf-1* overexpression in the adrenal cortex. Our findings deepen our understanding of SF-1 regulatory activity in ACC and demonstrate that dosage is critical for observed gene expression patterns. Our integrative approach improves reproducibility and biological interpretation, offering a framework to reconcile cross-study findings.

Exploring Tumor-Specific Neoantigens: Advancing T Cell Immunotherapy for Adrenocortical Carcinoma

Landwehr Laura-Sophie[1], Appenzeller Silke[2], Ölsner Laura[2], Weigand Isabel[4], Sbiera Silviu[1,2], Fassnacht Martin[1,2,3], Kroiss Matthias[1,2,4]

[1] Department of Internal Medicine I, Division of Endocrinology and Diabetes, University Hospital Würzburg, Würzburg, Germany
[2] Comprehensive Cancer Center Mainfranken, University of Würzburg, Würzburg, Germany
[3] Clinical Chemistry and Laboratory Medicine, University Hospital Würzburg, Würzburg, Germany
[4] Medizinische Klinik und Poliklinik IV, LMU Klinikum München, Munich, Germany

Adrenocortical carcinoma (ACC) is a highly aggressive endocrine cancer with a poor prognosis and limited treatment options. Immune checkpoint inhibitors have shown only modest efficacy, highlighting the need for novel immunotherapies such as tumor vaccines and T-cell-based treatments. The identification of suitable targets for these therapies is crucial. Tumor-specific mutant neoantigens, which can be recognized by T cells through the major histocompatibility complex (MHC) I, represent promising targets.

In this study, we performed whole-genome sequencing on 84 ACC samples and matched blood controls. Somatic mutations were detected using an in-house bioinformatics pipeline. We combined POLYSOLVER for HLA typing with netMHCpan to predict the binding affinity of tumor-specific neoantigens to MHC I based on both peptide and HLA sequence data. Strong binding was defined as a rank of <0.5%, weak binding as 0.5-1.9%, and no binding as >2.0%. Predicted neoantigens and reactive T cells were validated in *vitro* using flow cytometry with fluorochrome-labeled dextramer multimers, immunoprecipitation, and mass spectrometry.

We identified 1,067 unique somatic mutations in 989 different genes across all ACC patients. Binding affinity predictions were altered for 576 neoantigens. Mutant neoantigen load per patient ranged from 10 to 235 (mean 66.2) and was positively correlated with Ki67 proliferation index (R2 0.56, 95% CI 0.54-3.37; p = 0.013) and advanced ENSAT stage.

This is the first study to successfully perform *in silico* neoantigen profiling and validate the results *in vitro* for ACC. We found that mutant neoantigens are present in ACC tumors regardless of tumor mutational burden. These findings hold promise for the development of therapeutic cancer vaccines and T cell-based immunotherapies for ACC.

Successful Non-Surgical Treatment of Bilateral Macronodular Adrenal Hyperplasia with Osilodrostat

Laskowski Gustaw[1], Działach Łukasz[1], Witek Przemysław[1]

[1]Department of Internal Medicine, Endocrinology, and Diabetes, Medical University of Warsaw, Warsaw, Poland

Bilateral macronodular adrenal hyperplasia (BMAH) is a rare cause of endogenous Cushing's syndrome (CS). Untreated CS contributes to various comorbidities and is independently associated with increased mortality. The primary treatment for BMAH is bilateral or unilateral adrenalectomy. In patients who are not candidates for surgery, pharmacological therapy with steroidogenesis inhibitors remains the primary treatment option. Osilodrostat is a potent inhibitor of 11β-hydroxylase, a key enzyme involved in the final steps of cortisol biosynthesis. Data on osilodrostat use in patients with ACTH-independent CS are limited, even more so for BMAH.

We present the case of a 67-year-old woman diagnosed with BMAH and ACTH-independent CS, along with multiple comorbidities, including uncontrolled diabetes mellitus (HbA1c% 9,5%) requiring complex pharmacological management with metformin, dapagliflozin, semaglutide, and insulin therapy in basal/bolus regimen. Due to the comorbid conditions, the patient was deemed ineligible for surgical intervention.

Treatment with osilodrostat was initiated at a starting dose of 2 mg twice a day. During the initial phase of dosage titration (up to a maximum dose of 7 mg/day), the main complication was a pronounced effect of elevated concentrations of mineralocorticoid precursors (serum 11-deoxycorticosterone concentration: 377.0 ng/100 mL 15x ULN), which manifested as marked worsening of hypertension control and hypokalemia; however effectively managed with spironolactone and potassium supplementation. The maintenance dose of osilodrostat was 1 mg in the evening.

The therapy resulted in effective biochemical control of hypercortisolemia and led to marked clinical improvement in CS manifestations, including weight loss, resolution of proximal myopathy symptoms and enhanced physical capacity. Additionally, glycemic control improved substantially (HbA1c 5.8%), enabling insulin therapy discontinuation and continuation of diabetes management with oral antidiabetic agents.

This case highlights the efficacy and safety of osilodrostat in the management of BMAH and demonstrates its role as a viable therapeutic alternative when surgical treatment is not feasible.

Role of microRNA 139-5p in the regulation of aldosterone production and aldosterone-producing adenoma development

Xiaomin Li[1], Nicolo Faedda[1], Sheerazed Boulkroun[1], Nadia Cherradi[2], Maria-Christina Zennaro[1,3] and Fabio Fernandes-Rosa[1]

[1] Université Paris Cité, PARCC, Inserm, Paris, France
[2] University Grenoble Alpes, Inserm, CEA, U1292, Grenoble, France
[3] Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Génétique, Paris, France

The WNT/β-catenin signaling pathway is a key regulator of zona glomerulosa (zG) identity and plays a critical role in the pathogenesis of aldosterone-producing adenoma (APA). Mutations in β-catenin have been identified in 5% of APA cases, and β-catenin activation has been observed in two-thirds of tumors. In mice, β-catenin gain-of-function in the zG induces its expansion through upregulation of PDE2A, which in turn inhibits the cAMP/PKA-dependent establishment of the zona fasciculata. MiR-139-5p, a microRNA embedded within the PDE2A gene, is overexpressed in adrenocortical carcinoma and promotes cell migration; however, its role in APA and aldosterone production remains unclear. Its expression is tightly regulated by WNT/β-catenin signaling and correlates with that of its host gene, PDE2A. We hypothesize that miR-139-5p-mediated posttranscriptional regulation plays a role in adrenal cortex function and disease. The objectives of this study are: (i) to analyze the functional significance of miR-139-5p in aldosterone biosynthesis, and (ii) to evaluate its role in APA pathogenesis. In vitro knockdown experiments targeting miR-139-5p were performed in the human adrenocortical cell line H295R-S2. A 50% reduction in miR-139-5p expression was associated with decreased CYP11B2 levels following stimulation with potassium and angiotensin II. To further investigate the regulation of aldosterone production, we assessed miR-139-5p and PDE2A expression in mouse adrenal glands under high salt (HSD) and low salt (LSD) diet conditions, which modulate the renin-angiotensin-aldosterone system. In mice fed an HSD, both miR-139-5p and PDE2A expression were significantly downregulated. Additionally, miR-139-5p expression was reduced in female mice maintained on an LSD. Furthermore, using immunohistochemistry and RNAscope analyses on APA tissue sections, we observed that both PDE2A and miR-139-5p were specifically expressed in zG cells and in the majority of tumor cells. These results suggest a potential role for miR-139-5p in adrenal cortex function, particularly in aldosterone production, and in the pathogenesis of APA.

The combination of afternoon and midnight salivary cortisol improves the diagnosis of Cushing's syndrome

Lionetto Luana[1], Aloini Maria Elena[2], De Bernardini Donatella[1], Costanzi Giuseppe[1], Maggio Roberta[2], Lardo Pina [2], Salerno Gerardo [3], Simmaco Maurizio [1,3], Stigliano Antonio[2]

[1]Clinical Biochemistry, Mass Spectrometry Section, Sant'Andrea University Hospital, [2]Endocrinology, Department of Clinical and Molecular Medicine, Sant'Andrea University Hospital, Faculty of Medicine and Psychology, "Sapienza" University of Rome, 00189 Rome, Italy, [3]Department of Neuroscience, Mental Health and Sensory Organs, Faculty of Medicine and Psychology, "Sapienza" University of Rome, 00189 Rome, Italy.

Background. The diagnosis of Cushing's syndrome is challenging and often fraught with many pitfalls depending on several factors. We compare the diagnostic performance of AM serum cortisol (SC), 24-hour urinary free cortisol (UFC) and 0800 h, 1400 h and 2400 h salivary cortisol curve (SCC) in dexamethasone suppression test 1mg (1mg-DST) positive and negative patients.

Methods. 83 subjects performed measurements of SCC by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) method, exploiting the circadian rhythm of cortisol.

Results. The reproducibility and specificity of the test identify patients with hypercortisolism in 95% of cases at midnight. Interestingly, when considering two specific points (1400 h and 2400 h) on the SCC, the success rate rises to 100%.

Conclusion. The evaluation of the 1400 h and 2400 h assays lead to detection of the total number of patients with Cushing's syndrome. SCC is a non-invasive diagnostic strategy associated with elevated positive predictive value for hypercortisolism capable of enabling diagnosis. In addition, it can be considered for management of patient outcomes and monitoring of Cushing's syndrome pharmacological treatment.

Downregulation of tumoural antigen presentation is a hall mark of glucocorticoid excess in ACC

Maier Tanja[1], Landwehr Laura-Sophie[2], Triebig Alexandra[1], Schwarzlmueller Paul[1], Weigand Isabel[1], Kircher Stefan[3], Bernhardt Melissa[7], Schlosser Andreas[7], Knösel Thomas[4], Sbiera Silviu[2], Zimmermann Petra[5], Reincke Martin[1], Fassnacht Martin[1,6], Kroiss Matthias[1,2]

[1] Department of Medicine IV, LMU University Hospital, LMU Munich, Munich, Germany
[2] Department of Internal Medicine I, Division of Endocrinology and Diabetes, University Hospital, University Würzburg, Würzburg, Germany
[3] Institute of Pathology, University of Würzburg, Würzburg, Germany
[4] Institute of Pathology, LMU Munich, Munich, Germany
[5] Department of General, Visceral and Transplant Surgery, University Hospital, LMU Munich, Munich, Germany
[6] Comprehensive Cancer Center Mainfranken, University of Würzburg, Würzburg, Germany
[7] Rudolf Virchow Center for Experimental Biomedicine, University of Wuerzburg, Wuerzburg, Germany

Background: Advanced adrenocortical carcinoma (ACC) has a low response rate (~15%) to immune checkpoint inhibitors (ICIs). Glucocorticoid (GC) secretion and impaired antigen presentation may contribute to immune exclusion in the tumour microenvironment.

Objective: To investigate the role of GC in modulating antigen presentation pathways and its impact on the immunologically ´cold´ ACC tumour microenvironment.

Methods: Targeted RNA expression analysis of 486 immune-related genes was performed in 58 ACC specimens. qPCR, flow cytometry, and Western blot analyses assessed genes related to GC receptor activation and antigen presentation was performed in the ACC cell line JIL-2266. SILAC experiments examined HLA class I gene regulation through immunopeptidomic analysis in the JIL-2266 cell line.

Results: Targeted RNA analysis revealed significant downregulation of antigen presentation genes in ACC tissues, e.g., HLA-E (log2 fold change -1.74) or B2M (log2 fold change -1.58). Dexamethasone treatment of JIL-2266 cells significantly downregulated HLA class I-associated genes (B2M, HLA-B, HLA-E) at 0.1 µM by 2-fold at RNA level ($p<0.01$) after 6 hours and by 1.5-fold at protein level (pan-HLA class I marker) after 72 hours. Co-treatment with the selective GC receptor antagonist relacorilant reversed this effect to baseline. Global peptide presentation was decreased, HLA-peptides derived from NPTX2 showed enhanced presentation. Based on GEPIA, in ACC tumours NPTX2 was upregulated (log2(TPM+1) =450.88) by around 18-fold compared to healthy adrenal tissue (log2(TPM+1) =24.13).

Conclusion: GC signaling suppresses antigen presentation in ACC, potentially reducing tumour immunogenicity. GC receptor antagonists may offer a strategy to enhance immune responses in ACC.

A rare and challenging case of ectopic ACTH syndrome caused by a mediastinal paraganglioma

[1,2]Alessandra Mangone, [1,2]Andrea Contarino, [1,2]Riccardo Renda, [1,2]Giacomo Noschese, [1,2]Iulia Petria, [1,2]Giovanna Mantovani, [2]Emanuele Ferrante, [2]Serena Palmieri

[1]Department of Clinical Sciences and Community Health, University of Milan, Milan (Italy), [2]Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan (Italy)

Paraneoplastic Cushing's syndrome (CS) is a rare form of endogenous hypercortisolism due to ectopic ACTH secretion, reported in association with several neuroendocrine tumours. We describe a challenging case of ectopic CS caused by recurrent mediastinal paraganglioma (PGL). A 59-year-old male was recently referred to our clinic for CS. He reported a previous history of ectopic ACTH production from a mediastinal mass, surgically resected in 1986 and identified as a PGL. After surgery, he referred long-term remission until the current year, when he developed rapid weight-gain (8 kg in 2 months) along with new-onset hypertension, type 2 diabetes, dyslipidaemia, obstructive sleep apnoea, and hypokalaemia. Upon evaluation, he exhibited moon faces, truncal obesity, limb muscle atrophy, and generalized edema. Hormonal evaluation revealed severe ACTH-dependent hypercortisolism with cortisol after dexamethasone 1mg 29.4 mcg/dL, ACTH 148 pg/mL, urinary free cortisol (UFC) repeatedly >600 mcg/24h and elevated nocturnal salivary cortisol (mean 21.6 nmol/L; normal <2.8). There was no cortisol suppression with high-dose dexamethasone, no response to CRH, and pituitary MRI was normal, supporting an ectopic source. CT scan showed 18 mm mediastinal mass surrounding great vessels, likely consistent with PGL recurrence, and adrenal hyperplasia. Urinary fractionated metanephrines and chromogranin A were normal.
The case was further complicated by hypokalaemia (2.6 mmol/L) and drug-resistant hypertension, so treatment with Osilodrosat (2mg upon awakening + 5mg in the evening) was started in addition to spironolactone 50 mg and potassium supplementation (6 tablets/day). At follow-up, the patient showed clinical improvement, normalization of potassium levels, and stabilization of blood pressure. Osilodrostat dosage was subsequently increased to 5 mg twice daily achieving UFC normalization. Surgery was postponed due to occurrence of pneumonia, during which adrenal crisis, therefore hydrocortisone replacement therapy was added. Genetic testing for hereditary forms of PGL was negative. The patient is currently awaiting surgery and continuing block-and-replace therapy.

Comprehensive Visualization Dashboard for Longitudinal Cohort Analysis of Adrenal Tumor Progression

Masaki Uchihara [1,2], Hendrik Luecke-Tieke [3], Otilia Kimpel [1], Hanna Remde [1], Barbara Altieri [1], Martin Fassnacht [1], Akiyo Tanabe [2], Jörn Kohlhammer [3]

1) University Hospital Würzburg, Würzburg, Germany
2) National Center for Global Health and Medicine, Japan Institute for Health Security, Tokyo, Japan
3) Fraunhofer Institute for Computer Graphics Research IGD, Darmstadt, Germany

Pheochromocytoma, paraganglioma, and adrenocortical carcinoma present distinctive clinical challenges due to their dual characteristics as tumors and endocrine disorders. The considerable variability in patient progression complicates ongoing monitoring and treatment decisions. Despite extensive cohort data from initiatives like ENS@T, current tools inadequately capture the dynamic changes in disease states over time. Furthermore, understanding the rate of progression, which is critical for optimal treatment planning, remains poorly represented by existing visualization methods.

We developed an interactive visualization dashboard for longitudinal cohort analysis of adrenal tumors progression. Our platform employs advanced computer graphics and human-centered design principles, allowing clinicians to explore patient trajectories interactively. Users can dynamically align timelines based on treatments, hormone levels, genetic backgrounds, and patient-reported outcomes. Additionally, with another feature, clinicians can prioritize cohort patients at critical analysis time points—such as surgery, recurrence, or the initiation of systemic therapy—with a single click, providing an immediate overview of the time-series progression. Intuitive visual encoding, including color-coded signal-light indicators, effectively highlights key clinical parameters and treatment milestones.

Preliminary evaluations confirmed strong usability and clinical relevance, highlighting interactivity, streamlined outcome comparisons, and enhanced multidisciplinary communication. The dashboard facilitates precision medicine by improving decision-making and providing actionable insights from complex patient cohorts.

Classic clinical presentation versus incidental discovery of pheochromocytomas: a retrospective comparison

Messina Erika[1], Puglisi Soraya[1], Perotti Paola[1], Saba Laura[1], Guardia Anna[1], Brero Dalila[1], Gigante Antonio[1], Caramia Annalisa[1], Romanisio Martina[2], Giachino Daniela[1], Pia Anna[3], Reimondo Giuseppe[1], Terzolo Massimo[1]

[1]Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy
[2]Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy
[3]Department of Endocrinology, Diabetes and Metabolism, Santa Croce and Carle Hospital, Cuneo, Italy

Background
Pheochromocytomas are increasingly diagnosed incidentally (10–49%). This study aims to compare the clinical, biochemical, and imaging features at diagnosis between pheochromocytomas with a classical clinical presentation (CP) and those discovered incidentally (IP), and to explore potential differences in clinical evolution.

Patients and Methods
A retrospective analysis was conducted on patients diagnosed with pheochromocytoma between 2007 and 2024 at the San Luigi Hospital [median follow-up: 3.5 years (IQR 1–6)].

Results
Eighty-one patients (43 females, 38 males) were included, with a median age at diagnosis of 57 years (IQR 43–67). CP occurred in 41 cases (50.6%), IP in 40 (49.4%). Pheochromocytomas were predominantly located in the right adrenal gland (63%), with a median size of 35 mm (IQR 30–55). Catecholamine secretion was confirmed in 65 patients (86%), with a similar distribution in CP and IP patients (adrenergic: 41% vs. 33%; noradrenergic: 37% vs. 33%; non-secreting: 17% vs. 20%). Functional imaging showed uptake in (23/26) 88% of CP and (23/27) 85% of IP cases assessed.
Nearly all patients (76/81) underwent adrenalectomy, primarily with laparoscopic approach (91%). Genetic testing was completed in 50 patients: pathogenic germline mutations were found in 28% (14/50), with no significant differences in cluster distribution between groups.
A less aggressive clinical course was observed more frequently among IP patients. The mean age at diagnosis was higher in IP than CP patients (63.0 vs. 52.0 years, p=0.02). Pathological features traditionally considered “suspicious for malignant behaviour” were less frequent in IP patients (p=0.02). Additionally, no IP patients experienced recurrence (vs. 7/41 CP patients, p=0.01) or metastatic disease (vs. 6/41 CP patients, p=0.01).

Conclusion
Although reliable predictors of malignancy are lacking, incidentally discovered pheochromocytomas appear to have a lower tendency for recurrence and metastasis. These findings should be confirmed in prospective studies.

Laboratory study of pheochromocytoma in Albanian population

Brunilda Mezani[1,2], Anila Mitre[1,2]

[1]Department of Biology, University Tirana Albania,
[2]Department of Medical Laboratory Sciences and Imaging, Western Balkans University Tirana, Albania

brunilda.mezani@wbu.edu.al

Abstract

Background & aim:

Pheochromocytoma is a rare tumor that develops in the adrenal medulla, the inner part of the adrenal glands. This tumor leads to the excessive release of catecholamines, resulting in symptoms such as rapid heart rate, high blood pressure, anxiety, intense headaches, excessive sweating, and unintentional weight loss.

Methodology: This study studied Incidentaloma patient with the pheochromocytoma. Levels of catecholamines and their byproducts in the blood and urine are measured. The analysis includes measuring plasma-free metanephrines or fractionated metanephrines in urine. Additional tests, such as measuring total urinary metanephrines, plasma or urinary catecholamines, or urinary vanillylmandelic acid (VMA), will be used to confirm the presence of the tumor.

Results: The study was conducted on patients aged 20-100 years old. We detect elevated levels of catecholamines or their by products in the blood and urine of patients with pheochromocytoma . The most accurate tests include measuring plasma-free metanephrines or fractionated metanephrines in urine. Additional tests, such as measuring total urinary metanephrines, plasma or urinary catecholamines, or urinary vanillylmandelic acid (VMA), will be used in future steps to confirm the presence of the tumor.

Conclusion & Future Research: This study highlights the diagnostic value of plasma-free metanephrines and fractionated urinary metanephrines as the most accurate tests for detecting the tumor. Elevated levels of catecholamines and their by products provide critical evidence for diagnosis, while additional confirmatory tests, including urinary vanillylmandelic acid and total catecholamine measurements, can strengthen diagnostic accuracy.

Early and precise diagnosis is essential to prevent life-threatening complications such as hypertensive crises, arrhythmias, or stroke. Future research should focus on refining diagnostic algorithms, and incorporating novel biomarkers.

Keywords

Adrenal Tumors, Phaeochromocytoma, Metadrenalines, Catecholamines, Hypertension

Validation of prognostic biomarkers in adrenocortical carcinoma through Next-Generation Sequencing and pyrosequencing in real-life setting

Mohamed Abubaker[1], Tucci Lorenzo[2,3,4],De Biase Dario [5,6], Lippert Juliane [7], James Lisa[1], Chadderton Nicola[1], Fair Alice[1], Mulla Amina[1], O'Sullivan Brendan[1], Skordilis Kassiani [8], De Leo Antonio [4,6], Taniere Phillipe[1], Di Dalmazi Guido [3,4], Ronchi Cristina L[2,9].

1 Molecular Pathology Diagnostic Service, Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK
2 Department of Metabolism and Systems Science, University of Birmingham, Birmingham, UK
3 Division of Endocrinology and Diabetes Prevention and Care, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy.
4 Department of Medical and Surgical Sciences (DIMEC), Alma Mater Studiorum University of Bologna, Bologna, Italy.
5 Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy
6 Solid Tumor Molecular Pathology Laboratory, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
7 Institute of Human Genetics, University of Wuerzburg, Wuerzburg, Germany
8 Department of Histopathology, Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom
9 Department of Endocrinology, Queen Elizabeth Hospital Birmingham NHS Trust, Birmingham, UK

Background: Adrenocortical carcinoma (ACC) is a rare malignancy with heterogeneous outcomes. We previously showed that somatic variants and *PAX5* CpG methylation assessed on paraffin-embedded (FFPE) samples can improve prognostic classification.
Aim: To investigate the real-life feasibility of a Next-Generation Sequencing (NGS) and *PAX5* pyrosequencing service for ACC prognostic classification in two European centres.
Methods: 50 ACC patients (n=30 Birmingham/n=20 Bologna, 2002-2024) were investigated, including a retrospective cohort with known genetic background and an independent validation cohort. Tumour DNA was extracted from FFPE tissue and sequenced using a validated NGS panel covering 10 ACC-specific genes (*CTNNB1, APC, ZNRF3*, *TP53, RB1, CDK4, ATM, MEN1, NF1,* and *TERT* promoter). Variant allele frequency (VAF) thresholds were validated between 5%-10%. For *PAX5* methylation, 24 samples (2019-2024) were tested using bisulphite-pyrosequencing quantitative Qiagen assay to assess methylation across 7 CpG sites in the promoter region (threshold >25% indicating hypermethylation). Turnaround time (TAT) and cost-effectiveness were also assessed.
Results: After excluding 11 cases with low-quality DNA, 39 samples were analysed. In the retrospective cohort (n=6), previously identified somatic variants were confirmed, with two additional *TERT* promoter variants detected. In the validation cohort (n=33), 29 variants were found in 19 cases, including pathogenic/likely pathogenic variants (53.5% of total findings) in *CTNNB1* (9.3%, p.Ser45Pro, mean VAF 62.3%),*TP53* (23.3%, mean VAF 63.9%), *NF1* (16.3%, mean VAF 41.7%) and variants of uncertain significance in *TERT, APC, MEN1, NF1,* and *ZNRF3* (13.9%). For *PAX5* pyrosequencing, 9/24 samples (2022-2024) showed reliable results with one case being hypermethylated (mean methylation 68.5%). Average TAT was 21 days, with costs of €350-400/sample for NGS and €40/sample for pyrosequencing.
Conclusion: The ACC-specific NGS service was reliable, feasible, and cost-effective. *PAX5* pyrosequencing was only suitable for samples aged <2-years. Further investigations are required for prospective testing. This service could be implemented in clinical setting to improve prognostic classification and identify drug targets.

Metabolic and Pressor Effects of Metyrapone treatment in patients with mild hypercortisolism

[1]Valentina Morelli, [2]Antonio Musolino, [2]Vittoria Favero, [3]Chiara Parazzoli, [3]Carmen Aresta, [1,2] Luca Persani, [1,4]Sabrina Corbetta, [2, 3]Iacopo Chiodini

[1]Dept. of Endocrine and Metabolic Diseases, IRCCS Istituto Auxologico Italiano, Milan, Italy;
[2]Dept. of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy;
[3]Unit of Endocrinology, Grande Ospedale Metropolitano Niguarda, Milan, Italy,
[4]Department of Biomedical, Surgical and Dental Sciences, University of Milan;

Introduction: Mild Cushing's syndrome (mCS), is associated with type 2 diabetes (T2D) and hypertension (HT). In patients with adrenal incidentaloma (AI), surgery can lead to the improvement of these complications. In patients not candidate for surgery or who refuse it, or with bilateral AI a medical therapy has been advocated.
Patients and Methods: This prospective observational study (NCT05255900) aimed to evaluate the effect of treatment with metyrapone, a steroidogenesis inhibitor, on glycometabolic control and/or blood pressure (BP) in mCS. Inclusion criteria: i) AI and cortisol levels after 1mg-dexamethasone suppression test (1mgDST) >1.8 µg/dL; ii) mild signs and symptoms of hypercortisolism; iii) not candidate for surgery; iv) treatment with metyrapone 250 mg qd for less than one week; v) impaired fasting glucose and/or impaired glucose tolerance, and stable T2D or HT. Patients have been evaluated at study entry and followed-up for 24 weeks performing a 24-hour arterial BP monitoring (ABPM) and blood tests (HbA1c levels and 75g-OGTT in patients without T2D).
Results: 19 patients (6/13 M/F, mean age 66.8±7.7 yrs) with mean 1mgDST levels 4.1±3.6 µg/dl, have been enrolled, so far. After 24 weeks of metyrapone (mean 394±151 mg/day) we found a BP improvement in 36.8% of patients, a reduced prevalence of altered BP-dipping from 78.4% to 42.1% (P= 0.06), an improvement of glycemic control in 28.5% of patients. An improvement and worsening of BP and/or glycometabolic control occurred in 47.4% and 10.5% of patients, respectively. At the end of follow up, no patients showed symptoms or biochemical signs of hypocortisolism or hyperandrogenism. Ameliorated patients were mainly males (83.3% vs. 30.8%, p=0.03) and tended to have higher mean baseline BP values than not ameliorated patients (130±9.9 vs 115.9±10, P=0.07).
Conclusion: in our study about half of patients with mCS treated with low-dose metyrapone experienced an improvement of BP and/or glycometabolic control.

Rebound thymic Hyperplasia after recovery from severe hypercortisolism: a case report

Musolino Antonio (1), Cairoli Elisa (2), Palleschi Alessandro (3), Toja Paola (4), Chiodaroli Manuela (1), Corbetta Sabrina (4-5), Persani Luca (1-6), Morelli Valentina (4)

1. Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy.
2. SC Endocrinology-Diabetology Dietetics and Clinical Nutrition, ASST Lariana, Sant'Anna Hospital, San Fermo della Battaglia (CO)
3. Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy; S.C. Thoracic Surgery and Lung Transplantation, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Milan, Italy.
4. UOS Bone Metabolism Diseases and Diabetes, Scientific Research and Treatment Institute (IRCCS) Istituto Auxologico Italiano, Milan, Italy.
5. Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy.
6. Department of Endocrine and Metabolic Pathologies, IRCCS Istituto Auxologico Italiano, Milan, Italy.

Background: Rebound thymic hyperplasia (RTH) is a rare benign condition resulting from compensatory proliferation of thymocytes after remission from stressful conditions, including endogenous hypercortisolism of any origin. 19 cases of RTH are reported in literature and two of them occurred after adrenal Cushing syndrome remission. Its appearance can sometime be misleading, making the differential diagnosis with a thymic epithelial or neuroendocrine tumor challenging.

Case description: We report the case of a 31-year-old patient who referred to our outpatient clinic for worsening hirsutism, acne and asthenia. Blood tests showed increased androgens, high morning cortisol, free urinary cortisol x13 ULN and high ACTH values, suggesting an ACTH dependent hypercortisolism. Dynamic tests oriented toward an ectopic Cushing syndrome (ECS). An enhanced total-body CT scan was performed, revealing a 14 mm peribronchial nodule in the left upper pulmonary lobe. After pulmonary segmentectomy the nodule was proven to be a well-differentiated atypical bronchial carcinoid, with no signs of invasion and disease-free resection margins. Hypoadrenalism rapidly occurred after surgery proving hypercortisolism resolution and the patient started immediately glucocorticoid replacement therapy. Then, a radiological follow-up was started and 8 months after surgery an enhanced chest-abdomen CT scan revealed an anterior mediastinal enlargement of 17x30mm with greater density in the arterial phase compared to previous radiological examinations (65HU vs 12HU). To better characterize the lesion, the patient underwent an enhanced MRI, which showed typical radiological features of RTH: hyperintensity in T2 weighted images, signal intensity reduction in out of phase images, no signal restriction in diffusion weighted images.

Conclusions: RTH is a benign condition that goes in differential diagnosis with other anterior mediastinal masses. Thymus gland may also be the site of metastases or primitive neuroendocrine tumors; careful differential diagnosis is necessary. MRI may be useful to characterize RTH in a non-invasive way.

Evaluation of microRNAs as Liquid Biopsy Markers in Adrenocortical Tumours

Mytareli Chrysoula[1]*, Kalotychou Vassiliki[1], Lafioniatis Alexandros [1], Kaltsas Gregory [2], Zografos N. George[3], Markou Athina [4], Papanastasiou Labrini[4], Fountas Athanasios[4], Vasilliadi Dimitra Argyro[5], Kassi Evanthia[2,6],Mantzourani Marina[1], Angelousi Anna[1]

[1] First Department of Internal Medicine, European Reference Network on Rare Endocrine Conditions (ENDO-ERN), Laikon General Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece.
[2] Department of Propaedeutic and Internal Medicine, European Reference Network on Rare Endocrine Conditions (ENDO-ERN), Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece.
[3] First Department of Surgery, 'G. Gennimatas' General Hospital of Athens, Athens, Greece.
[4] Unit of Endocrinology, and Diabetes Center, 'G. Gennimatas' General Hospital of Athens, Athens, Greece.
[5] Department of Endocrinology, Diabetes and Metabolism, European Reference Network on Rare Endocrine Conditions (ENDO-ERN), Evangelismos Hospital, Athens, Greece.
[6] Department of Biological Chemistry, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Introduction: Liquid biopsy markers, including circulating micro-RNAs, hold promise for distinguishing malignant cases from the large number of adrenocortical tumours (ATs). However, their clinical application remains unestablished due to the scarcity of studies conducted so far, involving small patient populations.

Aim: This study aims to validate the diagnostic performance of selected circulating microRNAs (miR-483-5p, miR-210, miR-335), identified through microRNA profiling studies, as malignancy markers in a large cohort of patients with ATs.

Methods: Serum samples from 90 participants were collected, including 75 ATs patients and 15 controls. The ATs comprised 50 cases of adrenocortical adenomas (ACA) and 25 cases of adrenocortical carcinomas (ACC). In the ACC subgroup, 16 samples were obtained from active ACC cases (preoperative or recurrent), while the remaining from disease-free patients with a median follow-up of 55 months. MicroRNA expression was analyzed using quantitative real-time PCR.

Results: Circulating levels of miR-483-5p and miR-210 were significantly elevated in active ACC cases compared to both ACAs ($p<0.001$ and $p=0.004$, respectively) and controls ($p=0.002$ and 0.003, respectively). Notably, miR-483-5p serum levels were higher in the active vs. disease-free ACC cases ($p = 0.01$). MiR-483-5p demonstrated the highest diagnostic accuracy for distinguishing active ACC from ACA cases (AUC=0.869, 95%CI: 0.761–0.978, $p<0.001$), with 81.3% sensitivity and 88% specificity. It also differentiated active ACC from disease-free ACC patients (AUC=0.854, 95%CI: 0.672–1, $p=0.004$), achieving 81.3% sensitivity and 89% specificity. A sub-analysis suggested higher markers levels in preoperative vs. recurrent ACC cases, though not statistically significantly.

Conclusion: Our study suggests that circulating miR-483-5p and miR-210 are promising non-invasive biomarkers for distinguishing active ACC from ACA cases. The absence of miR-483-5p in disease-free ACC patients further suggests its potential in ACC monitoring. Future research should focus on analyzing serial serum samples to clarify miRNA dynamics throughout ACC progression and in response to treatment.

Cardiovascular Outcomes in Patients with Bilateral Adrenal Tumors and Cortisol Excess: Surgery vs. Conservative Management

Elisabeth Nowak[1], Charlotte Viëtor[2,3], Richard A. Feelders[2,4], Hans Hofland[2], Marta Araujo Castro[5,6], César Minguéz Ojeda[7], Eider Pascual-Corrales[5], Bahaa Salama[8], Irina Bancos[8], Rashi Sandooja[8,9], Martin Fassnacht[10], Barbara Altieri[10,11], Mario Detomas[10], Malgorzata Bobrowicz[12], Urszula Ambroziak[12], Adrianna Gladka[12], Roberta Giordano[13,14], Fabio Bioletto[13], Mirko Parasiliti-Caprino[13], Edelmiro Menéndez Torre[15,16,17], Diego Rivas-Otero[15,16], Tomás González-Vidal[15,16], Filippo Ceccato[18], Irene Tizianel[18], Marianna Torchio[18], Serena Palmieri[19], Alessandra Mangone[19,20], Giovanna Mantovani[19,20], Iacopo Chiodini[21], Vittoria Favero[21], Alessandro Prete[22,23,24], Onnicha Suntornlohanakul[22,25], Valentina Morelli[26], Manuela Chiodaroli[26], Małgorzata Trofimiuk-Müldner[27], Alicja Hubalewska-Dydejczyk[27], Soraya Puglisi[28], Giuseppe Reimondo[28], Krystallenia I. Alexandraki[29], Ariadni Spyroglou[29], Henrik Falhammar[30,31], Przemysław Kłosowski[32,33], Alexandra Chrisoulidou[34], Antoine Tabarin[35], Paola Loli[36], Anna Angelousi[37], Duarte Pignatelli[38], Felicia Hanzu[39], Sybille Fuld[40], Christina Pamporaki[40], Shobana Athimulam[41], Marcus Quinkler[42], Athina Markou[43], Troy Puar[44,45], Timo Deutschbein[10,46], Verena S. Hoffmann[47], Martin Reincke[1]

1Department of Medicine IV, LMU University Hospital, LMU Munich, Munich, Germany.

2Department of Internal Medicine, Division of Endocrinology, Erasmus Medical Center, 3015 GD Rotterdam; The Netherlands.

3Department of Surgical Oncology and Gastrointestinal Surgery, Erasmus MC Cancer Institute, Erasmus University Medical Center, 3015 GD Rotterdam, the Netherlands.

4Holman Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, New York University Langone Medical Center, New York, NY, United States.

5Department of Endocrinology and Nutrition, Hospital Universitario Ramón y Cajal [Ramon y Cajal University Hospital].

6Instituto de Investigación Ramón y Cajal (IRYCIS) [Ramon y Cajal Research Institute], Spain.

7Urology Department, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain.

8Division of Endocrinology, Mayo Clinic, Rochester, MN, 55905, USA.

9Division of Diabetes, Endocrinology, and Metabolism, Department of Medicine, University of Nebraska Medical Center, Omaha,68198 NE.

10Department of Internal Medicine I, Division of Endocrinology and Diabetes, University Hospital, University of Würzburg, Würzburg, Germany.

11Bavarian Cancer Research Center (BZKF), Würzburg, Germany.

12Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Poland Banacha 1a, 02-097, Warsaw, Poland.

13Division of Endocrinology, Diabetes and Metabolism, Department of Medical Sciences, University of Turin, Turin, Italy.

14Department of Clinical and Biological Sciences, University of Turin, Turin, Italy.

15Department of Endocrinology and Nutrition, Hospital Universitario Central de Asturias Oviedo, Spain.

16Instituto de Investigación Sanitaria del Principado de Asturias (ISPA) [Health Research Institute of Asturias], Oviedo, Spain.

17Department of Medicine, University of Oviedo, Spain; Centre for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III [Carlos III Health Institute], Madrid, Spain.

18Endocrine Unit, University-Hospital of Padova, Padova, Italy.

19Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

20Department of Medical Sciences and Community Health - Dipartimento di Eccellenza 2023-2027, University of Milan, Milan, Italy.

21Department of Biotechnology and Translational Medicine, University of Milan, Milan, Italy; Unit of Endocrinology, Grande Ospedale Metropolitano Niguarda, Milan, Italy.

22Department of Metabolism and Systems Science, College of Medicine and Health, University of Birmingham, United Kingdom.

23Department of Endocrinology, Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom.

24National Institute for Health and Care Research (NIHR) Birmingham Biomedical Research Centre, United Kingdom.

25Endocrinology and Metabolism Unit, Division of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Thailand.

26Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy.

27Department of Endocrinology, Jagiellonian University Medical College, Cracow, Poland.

28Internal Medicine 1, Department of Clinical and Biological Sciences, S. Luigi Gonzaga Hospital, University of Turin, Orbassano, Italy.

292nd Department of Surgery, Aretaieio Hospital, National and Kapodistrian University of Athens.

30Department of Endocrinology, Karolinska University Hospital, Stockholm, Sweden.

31Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.

32Department of Endocrinology and Internal Diseases, Faculty of Medicine, Medical University of Gdańsk, Gdańsk, Poland.

33Department of Endocrinology and Internal Diseases, University Clinical Center, Gdańsk, Poland.

34Department of Endocrinology, Theagenio Cancer Hospital, Thessaloniki, Greece.

35Department of Endocrinology, diabetes and nutrition, University Hospital of Bordeaux, 33604 Pessac, France.

36Endocrinology, Clinica San Carlo, Paderno Dugnano, Milano, Italy.

37Department of Endocrinology and Diabetes Center, G. Gennimatas Hospital, Athens, Greece.

38Department of Endocrinology, Centro Hospitalar e Universitário de S. João, Porto, Portugal.

39Endocrinology and Nutrition Department, Hospital Clínic of Barcelona, University Barcelona, Institut de Investigacions Biomediques Pi I Sunyer Barcelona (IDIBAPS), CIBERDEM, Barcelona, Spain.

40Department of Medicine III, University Hospital and Medical Faculty Carl Gustav Carus, Technische Universität, Dresden, Germany.

41Division of Endocrinology, Diabetes, Bone and Mineral Disorders, Henry Ford Health, Detroit, MI 48202, United States.

42Endocrinology in Charlottenburg, Berlin, Germany.

43 Endocrinology Unit and Diabetes Center, Athens General Hospital "G, Gennimatas", Athens, Greece.

44Department of Endocrinology, Changi General Hospital, Singapore.

45Duke-National University School (Duke-NUS) Medical School, Singapore.

46Medicover Oldenburg MVZ, Oldenburg, Germany.

47Institute for Medical Information Processing, Biometry, and Epidemiology, Ludwig-Maximilians-Universität, Marchioninistr. 15, 81377, Munich, Bavaria, Germany.

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Background: Treatment of patients with bilateral adrenal tumors and cortisol excess is not standardized and poses a therapeutic dilemma. Untreated cortisol excess is associated with cardiometabolic morbidity/mortality, but bilateral adrenalectomy (BADX) causes adrenal insufficiency and possibly life-threatening adrenal crises. Data on cardiovascular (CV) outcomes by treatment modality are lacking.

Methods: This retrospective ENSAT study (30 centers, 12 countries) included patients with bilateral adrenal tumors ≥10 mm, post-dexamethasone serum cortisol ≥50 nmol/l, and ≥36 months of follow-up. Primary endpoints were all-cause mortality and biochemical remission. Secondary endpoints included CV events, comorbidities, and adrenal crises.

Findings: Among 629 patients (17% Cushing's syndrome [CS], 83% mild autonomous cortisol secretion, [MACS]), median age was 62.0 years, and 68% were female. Most patients with CS underwent surgery (81%), while patients with MACS mainly received symptomatic treatment for comorbidities (79%). Over a median follow-up of 7.9 years, biochemical remission was achieved in 45% of patients with CS and 13% with MACS. In both groups, 7% died and 12% (CS) and 15% (MACS) had ≥1 CV event without significant differences across treatments. Uncontrolled cortisol excess, especially in MACS, was linked to increased CV events. Furthermore, smoking, prior CV events, and age were key mortality and CV risk factors in patients with MACS. In CS, BADX reduced hypertension rates by 34% with similar trends for unilateral adrenalectomy. In contrast, symptomatic therapy in MACS was associated with worsening of all investigated comorbidities. After BADX, adrenal crises occurred in 40% of patients with CS and MACS, of which none were fatal.

Interpretation: Although mortality and CV event rates were similar across treatments, surgery resulted in better biochemical control and more favorable comorbidity outcomes. As uncontrolled cortisol excess is associated with higher rates of CV events, therapeutic interventions should aim to normalize – not just reduce – cortisol concentrations.
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AZD1775: effect of monotherapy or EDP-M combination in the treatment of ACC preclinical models

Nozza Emma[1,2], Catalano Rosa[1], Di Muro Genesio[1], Di Bari Sonia[1,2], Lopez Gianluca[3,4], Vaira Valentina[3,5], Marra Giusy[1], Esposito Emanuela[1], Mangili Federica[6], Treppiedi Donatella[6], Battistin Michele[5,7], Di Dalmazi Guido[8,9], Palmieri Serena[6], Ferrante Emanuele[6], Mantovani Giovanna[1,6], Peverelli Erika[1,6]

[1]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy;
[2]PhD Program in Experimental Medicine, University of Milan, Milan, Italy;
[3]Pathology Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy;
[4]Department of Biomedical, Surgical, and Dental Sciences, University of Milan, Milan, Italy;
[5]Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy;
[6]SC Endocrinology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy;
[7]Center for Preclinical Research, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy;
[8]Division of Endocrinology and Diabetes Prevention and Care, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy;
[9]Department of Medical and Surgical Sciences, Alma Mater University of Bologna, Bologna, Italy.

Current therapy for advanced adrenocortical carcinoma (ACC) is represented by EDP-M, but its efficacy is limited and new therapeutic approaches are needed. Previous in vitro findings of our group showed that AZD1775, an inhibitor of the G2/M checkpoint gatekeeper Wee1, reduces proliferation and increases apoptosis in ACC cell lines and primary cultures. The compensatory upregulation of Myt1, normally redundant in CDK1 inhibition, has been involved in the mechanisms of acquired resistance to AZD1775 in other tumors.

Aim of this study was the investigation in vitro, in vivo, and ex-vivo of AZD1775+EDP-M therapy, as well as to validate AZD1775 antitumoral efficacy in NCI-H295R mouse xenograft, and to explore the onset of Myt1-depending resistance.

In vitro coincubation of NCI-H295R with AZD1775 and EDP-M showed synergistic effects in reducing both cell viability and cell proliferation, and additive effects in cortisol secretion control. In NCI-H295R xenografts, AZD1775 demonstrated an antitumor efficacy comparable to EDP-M, but no synergistic effects of combined treatment were observed. All treatments were well tolerated and able to decrease tumor vascularization and induce necrosis.

Although an upregulation of Myt1 was observed after 24-hours of AZD1775 *in vitro* treatment, no variations in Myt1 expression was found in mice tumors after 21-days treatment. However, *ex vivo* primary cultures derived from AZD1775+EDP-M treated xenograft showed a decreased sensitivity to monotherapies of AZD1755 and EDP-M compared to cells derived from untreated mice, whereas the cotreatment maintained the same antiproliferative efficacy.

In conclusion, we demonstrated the comparable efficiency of AZD1775 to EDP-M therapy on ACC xenografts. The combination of AZD1775+EDP-M controlled NCI-H295R growth in vitro, in vivo, and even upon ex vivo re-exposure. Thus, our data support AZD1775 as promising ACC therapeutic option, and its combination with EDP-M as useful strategy to enhance drug efficacy, prevent resistance and minimize side effects by reducing the therapeutic dosage.

FH gene mutations and cancer predisposition: elucidating the tissue-specific tumorigenesis mechanisms associated with hereditary paraganglioma

Sara Cajamarca Ocampo[1], Charlotte Lussey-Lepoutre[1,2], Judith Favier[1,] Alexandre Buffet[1,3]

[1] Centre de Recherche des Cordeliers Inserm UMR1138
15 rue de l'école de Médecine
75006 Paris France
[2]Sorbonne Université, AP-HP, Hôpital Pitié-Salpêtrière, Service de Médecine Nucléaire, 75013 Paris, France
[3]Département de Médecine Génomique des Tumeurs et Cancers, Hôpital Européen Georges Pompidou, Fédération de Génétique et de Médecine Génomique AP-HP Centre Université Paris Cité, F-75015 Paris, France

Germline mutations in the *FH* gene, which encodes fumarate hydratase, have been identified as the cause in rare cases of particularly aggressive paragangliomas and pheochromocytomas and of HLRCC syndrome, associating cutaneous leiomyomas, uterine leiomyomas, and papillary renal cell carcinomas (Fhd-RCC). FH deficiency has therefore been extensively studied in renal cancer models; however, it is still unclear how it leads to PPGL development and what explains its low penetrance.
We hypothesize that FH loss has tissue-dependent consequences and we aim to disclose the specificities that underpin tumoral development in PPGL compared to the tumorigenesis mechanisms described in Fhd-RCC.

To do so, we generated immortalized mouse chromaffin (imCC) in which *Fh* was inactivated using CRISPR-Cas9 technology. Homozygous gene inactivation was confirmed through multiple approaches: Sanger sequencing, protein detection *via* western blot, and enzymatic activity assessment through spectrophotometry.
Our ongoing comparative analysis between *Fh*-deficient chromaffin cells and renal carcinoma models has revealed both similarities and differences. Phenotypically, both *Fh*-deficient cell types demonstrated increased migratory capacity and reduced proliferation rates compared to their WT controls, suggesting shared basic cellular responses to FH loss.
In terms of metabolism, ^{13}C-Glucose and ^{13}C-Glutamine tracing experiments reveal that both cells rely on glutamine as their main source of carbon but only imCC *Fh* KO can do reductive carboxylation.
Both *Fh*-deficient models display high levels of mitochondrial ROS and activation of the NRF2-mediated antioxidant response pathway.
In terms of epigenetic deregulation, imCC and renal *Fh* -/- cells display different DNA methylation profiles as imCC *Fh* KO show both hypermethylation and hypomethylation in TSS and gene bodies respectively while renal cells show hypermethylation in all regions.

Further investigations are needed to characterize the transcriptomic consequences of these different methylation patterns. Additional analyses of protein succination and other molecular features are ongoing to fully identify the distinctive characteristics of FH-dependent tumorigenesis in PPGL compared to FHd-RCC and will be available in September.

Autophagy-Related Protein Expression in Adrenocortical Tumors

Oliveira Sofia B.[1,2,3,4], Sousa Diana[1,5], Santos Madalena[1], Rios Elisabete[6], Silva Tiago[7], Leite Valeriano[7], Pereira Sofia S.[1], Pignatelli Duarte[1,2,3,4,8]

[1]Unit for Multidisciplinary Research in Biomedicine (UMIB); School of Medicine and Biomedical Sciences (ICBAS); Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal
[2]Institute for Research and Innovation in Health (i3S), University of Porto, Porto, Portugal
[3]IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal and Immunology of the University of Porto, Porto, Portugal
[4]Departamento de Endocrinologia, Unidade Local de Saúde de São João (ULS de São João), Porto, Portugal
[5]Faculdade de Medicina Dentária – Universidade Católica Portuguesa (UCP), Viseu, Portugal
[6]Department of Pathology, Faculty of Medicine of the University of Porto, Porto, Portugal,
[7]Serviço de Endocrinologia, Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Portugal, Lisboa, Portugal
[8]Department of Biomedicine, Faculty of Medicine of the University of Porto, Porto, Portugal

Adrenocortical carcinomas (ACC) are rare and often highly aggressive tumors with heterogeneous clinical outcomes. This variability in ACC behavior is largely attributed to its complex and still incompletely understood molecular landscape. Autophagy, a dynamic cellular process that culminates in the degradation and/or recycling of cellular compartments, plays a dual role in tumorigenesis by promoting either cell survival or cell death. However, its role in adrenocortical tumors (ACT) remains poorly understood.

Therefore, we aimed to assess the expression of key autophagy-related proteins in ACT, to better understand the potential role of autophagy in ACC pathophysiology.

For that, the expression of autophagy related proteins - autophagy protein 5 (ATG5), microtubule associated protein 1 light chain 3 beta (LC3B), and sequestosome 1 (SQSTM1), also known as p62 - was evaluated by immunohistochemistry (IHC) in paraffin-embedded ACC (n=27) and adrenocortical adenoma (ACA) (n=20) samples. ATG5 and LC3 expression were quantified by counting positive stained cells in tumor hot spots, with a minimum of 1000 cells analyzed per case. For LC3, only dot-like staining patterns, indicative of alterations in autophagy dynamics, were considered. Quantification of p62 staining is currently ongoing.

Positive staining for ATG5 was observed in only 41% of ACC (11/27), whereas LC3 expression was detected in 89% (24/27) of malignant ACT. The percentage of LC3 positive cells was significantly higher in ACC when compared to ACA (15% *vs* 2%, $p<0.001$). Moreover, ACC without metastatic disease exhibited significantly higher LC3 expression than those with metastasis (5% *vs* 19%, $p<0.01$).

These preliminary findings suggest that autophagy may play a role in ACC pathophysiology. The dot-like LC3 staining pattern appears to be a marker of ACC, with higher expression associated with less aggressive malignant features, particularly the absence of metastasis. Further mechanistic studies will be performed to validate these findings.

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Does the 1.8 µg/dL cut-off in the 1 mg dexamethasone suppression test predict cardiometabolic risk in MACS?

Opalinska Marta[1], Kokoszka Joanna[2], Kolasa Magdalena[2], Sitarz Katarzyna[3], Banas Aleksandra[4], Pabian Gabriela[4], Bugajska Jolanta[5], Sztefko Krystyna[5], Hubalewska-Dydejczyk Alicja[1]

1. Chair and Department of Endocrinology, Jagiellonian University Medical
2. Department of Endocrinology, Oncological Endocrinology, Nuclear Medicine and Internal Medicine, University Hospital in Krakow, Kraków, Poland
3. Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland
4. Students Scientific Group of Endocrinology, Jagiellonian University Medical College, Kraków, Poland
5. Department of Clinical Biochemistry, Institute of Pediatrics, Jagiellonian University Medical College, Kraków, Poland

Introduction:

Approximately 20-50% of adrenal tumors in patients without overt Cushing's syndrome are associated with mild autonomous cortisol secretion (MACS), defined by 1mg dexamethasone suppression test (DST) showing cortisol levels >1.8 µg/dL. Clinical evidence suggests that individuals with MACS have a higher risk various cardiometabolic diseases including obesity, hypertension, hyperlipidemia, nonalcoholic fatty liver disease, cardiovascular diseases, prediabetes, type 2 diabetes, osteopenia, osteoporosis, and vertebral fractures.

Objectives:

This study aimed to evaluate whether a cortisol cut-off point of 1.8 µg/dL in the 1 mg dexamethasone suppression test can differentiate cardiometabolic risks associated with MACS in patients with adrenal incidentalomas.

Methods:

The cortisol suppression test results of 347 patients with adrenal incidentalomas were analyzed. Of these, 141 patients had confirmed adrenal adenomas following surgery, and 206 were non-operated individuals with homogeneous adrenal masses <10 HU. The cohort consisted of 100 men and 247 women, with a median age of 63.5 for men and 64 for women. The analysis compared those with negative (serum cortisol ≤1.8 µg/dL) and positive (serum cortisol >1.8 µg/dL) DST results in relation to the presence of diabetes, obesity, hypertension, ischemic heart disease, and chronic heart failure.

Results:

The disease prevalence in patients with negative (serum cortisol ≤1.8 µg/dL) versus positive (serum cortisol >1.8 µg/dL) DST results was as follows: Hypertension: 72.22% vs. 77.88% (p=0.02), Diabetes mellites: 23.93% vs. 32.74% (p=0.06), Ischemic heart disease: 11.54% vs. 18.58% (p=0.15), Chronic heart failure: 4.27% vs. 7.08% (p=0.16), Obesity: 32.49% vs. 31.69% (p=0.88). No significant differences in disease prevalence were found between males and females.

Conclusion:

A cortisol cut-off of 1.8 µg/dL in the 1 mg dexamethasone suppression test may be useful in differentiating patients with MACS at higher risk for hypertension and diabetes. This threshold appears to be an independent and significant factor in the development of these conditions.

Aldosterone, direct renin concentration and aldosterone-to-renin ratio in the screening of patients with primary aldosteronism by automated chemiluminescence immunoassays (LIAISON®XL, DiaSorin)

Ostrowska Magdalena[1,2], Szatko Alicja[1,2], Zgliczyński Wojciech[1], Glinicki Piotr[1,2]

[1] EndoLab Laboratory, Centre of Postgraduate Medical Education, Warsaw, Poland
[2] Department of Endocrinology, Centre of Postgraduate Medical Education, Warsaw, Poland

Introduction

Primary aldosteronism (PA) is the most common endocrine cause of secondary hypertension. It occurs in 5%-10% of hypertensive patients. PA is a group of disorders associated with semi-autonomous hypersecretion of aldosterone. Laboratory diagnosis in patients with suspected PA consists of screening tests aimed at determination of serum/plasma aldosterone concentration, direct renin concentration (DRC) and calculation of the aldosterone-to-renin ratio (ADRR).
The aim of the study was to assess the usefulness of aldosterone and DRC using the chemiluminescent immunoassay, and to use the ADRR ratio as a screening test in the biochemical diagnosis of patients with suspected primary aldosteronism.

Materials and Methods

Patients were divided into 2 groups:

1. Patients with primary aldosteronism (n=35)
2. Patients with adenoma + hypertension (n=200)

Serum aldosterone concentration, direct plasma renin concentration and aldosterone-to-renin ratio (ADRR) were determined in all patients.
Aldosterone and direct renin concentrations were determined by chemiluminescent immunoassay (CLIA) on a LIAISON®XL analyzer (DiaSorin, Italy). The ADRR ratio is shown as ng/dL / µIU/mL.

Results

The optimal cut-off for aldosterone was 13.8 ng/dL (AUC 0.8816; $p<0.0001$). Sensitivity and specificity for serum aldosterone concentration for patients with PA were 79% and 82%, respectively. The optimal cut-off for DRC in patients with PA was 4.22 µIU/mL (AUC 0.9466; $p<0.0001$). Sensitivity and specificity for DRC were 83% and 94%, respectively. The optimal cut-off point discriminating PA patients from adenoma hypertensive patients (ADRR ratio) was 3.85 ng/dL / µIU/mL (AUC 0.9843; $p<0.0001$), achieving 94% sensitivity and specificity.

Conclusion

Determination of aldosterone and DRC levels using the CLIA immunoassay and the use of the ADRR ratio show a high diagnostic value as a screening test for patients with PA.

A Rare Case of Paediatric Adrenal Rest Cortisol and Androgen-Producing Adenoma in the Renal Hilum

Palmieri Serena[1], Berrettini Alfredo[2], Lopez Gianluca[3], Mangone Alessandra[1,4], Pes Ilaria[4], Balzo Maria[1], Tabano Silvia[5,6], Muller Ilaria[1,4], Mantovani Giovanna[1,4]

[1]Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.
[2]Department of Paediatric Urology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.
[3]Division of Pathology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.
[4]Department of Clinical Sciences and Community Health, Department of Excellence 2023-2027, University of Milan, Milan, Italy.
[5] Laboratory of Medical Genetics, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.
[6]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy.

Background: Ectopic adrenal tissue is a rare congenital anomaly resulting from aberrant embryological migration of adrenocortical cells from the urogenital ridge. While often clinically silent and lacking adrenal medulla, ectopic adrenal rests may occasionally undergo neoplastic transformation. Fewer than 50 cases of ectopic adrenal tumours have been described in the literature to date, with hormonally active lesions being exceedingly uncommon, particularly in the paediatric population.

Case Presentation: We report a rare case of a 15-year-old female presenting with secondary amenorrhea, insomnia, striae rubrae, acne and progressive hirsutism (Ferriman-Gallwey score: 29) over the previous year. Her medical history was notable for idiopathic erythrocytosis. Her biochemical profile was consistent with ACTH-independent Cushing syndrome (ACTH < 5 ng/L, cortisol post-overnight 1 mg dexamethasone suppression test 16.9 µg/dl, mean urinary free cortisol 118 µg/24h - normal values 3-43 - mean nocturnal salivary cortisol of 11.2 nmol/L - normal values <2.8) with severe hyperandrogenism (total testosterone 225 ng/dl). Abdominal CT revealed a 30 mm heterogeneous, hypervascular, left renal hilum mass (unenhanced mean HU 22) with intense FDG-PET uptake (SUV 7.5). Both adrenal glands had regular morphology, size and location. Given the suspicion of ectopic adrenocortical carcinoma, an open radical left nephrectomy with lymphadenectomy was performed. Histopathological analysis demonstrated features of an ectopic adrenocortical adenoma, confirmed by immunohistochemistry (SF1+, alpha-inhibin+, Melan-A+, synaptophysin+), with a low proliferative index (Ki-67: 3%) and a Wieneke score of 0, excluding malignancy. Postoperative tests showed both low androgen and cortisol levels. Menstrual cycles normalized, and erythrocytosis resolved. Next-generation sequencing (clinical exome) focusing on ARMC5, KDM1A, TP53, PDE11A, PDE8B, and PRKAR1A genes was performed. No pathogenetic variants were identified.

Conclusions: This case highlights a rare paediatric presentation of hormonally active ectopic adrenocortical adenoma mimicking adrenal carcinoma, contributing to a better understanding of a lesser-known aspect of adrenal rest pathology.

Cardiometabolic Burden in Bilateral Macronodular Adrenal Disease with Primary Aldosteronism: A Multicenter, Retrospective Cohort Study

Annalisa Panarelli[1,2], Marta Araujo-Castro[3], Livia Mara Mermejo[4,5], Adina Turcu[4], Jung Hee Kim[6], Seung Shin Park[6], Fabio Bioletto[7], Mirko Parasiliti-Caprino[7], Masakatsu Sone[8], Athina Markou[9], Troy Puar[10], Piotr Kmieć[11], Paolo Mulatero[2], Norio Wada[12], Mika Tsuiki[13], Margarita González Boillos[14], Noemi Jimenez López[15], Takashi Yoneda[16], Filippo Ceccato[17], Margaret de Castro[4], Stephanie Espiard[18], Takamasa Ichijo[19], Shoichiro Izawa[20], Masanori Murakami[21], Serena Palmieri[22], Iacopo Chiodini[23,24], Takuyuki Katabami[25], Henrik Falhammar[26], Mitsuhide Naruse[27], Marcus Quinkler[28], Martin Reincke[1], Elisabeth Nowak[1]

[1]Department of Medicine IV, LMU University Hospital, LMU Munich, Germany.
[2]Internal Medicine and Hypertension Division, Department of Medical Sciences, University of Turin, Turin, Italy.
[3]Unidad de Neuroendocrinología, Servicio de Endocrinología, Hospital Universitario Ramón y Cajal. Madrid & Instituto de Investigación Biomédica Ramón y Cajal (IRYCIS) & Departamento de Medicina, Universidad de Alcalá de Henares, Madrid, España.
[4]Division of Metabolism, Endocrinology, and Diabetes, University of Michigan, Ann Arbor, MI, USA.
[5]Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil
[6]Department of Internal Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea.
[7]Division of Endocrinology, Diabetes and Metabolism, Department of Medical Sciences, University of Turin, Turin, Italy; Department of Clinical and Biological Sciences, University of Turin, Turin, Italy.
[8]Department of Metabolism and Endocrinology, St. Marianna University School of Medicine
[9]Department of Endocrinology and Diabetes Center, G. Gennimatas Hospital, Athens, Greece.
[10]Department of Endocrinology, Changi General Hospital, Singapore, Singapore.
[11]Department of Endocrinology and Internal Medicine, Medical University of Gdansk, Gdansk, Poland.
[12]Department of Diabetes and Endocrinology, Sapporo City General Hospital, Sapporo 060-8604, Japan.
[13] Department of Endocrinology and Metabolism, National Hospital Organization Kyoto Medical Center, Kyoto 612-8555, Japan.
[14]Endocrinology & Nutrition Department. Hospital Universitario de Castellón, Castellón, Spain.
[15]Endocrinology & Nutrition Department. Hospital Doce de Octubre, Madrid, Spain.
[16]Department of Health Promotion and Medicine of the Future, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Japan.
[17]Endocrinology Unit, Department of Medicine DIMED, Padova University Hospital, Via Ospedale Civile, 105, 35128, Padua, Italy.
[18]CHU Lille, Endocrinology, Diabetology and Metabolism, 59000 Lille, France; University Lille, 59000 Lille, France; Inserm U1190, 59000 Lille, France.
[19]Department of Diabetes and Endocrinology, Saiseikai Yokohamashi Tobu Hospital, Yokohama 230-0012, Japan.
[20]Division of Endocrinology and Metabolism, Tottori University Faculty of Medicine, Yonago 683-8504, Japan.
[21]Department of Molecular Endocrinology and Metabolism, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan.
[22] Department of Clinical Sciences and Community Health, University of Milan, Fondazione IRCCS Cà Granda-Ospedale Maggiore Policlinico, Milan, Italy.
[23]Department of Biotechnology and Translational Medicine, University of Milan, Milan 20010, Italy.
[24]Unit of Endocrinology, Ospedale Niguarda Cà Granda, Milan 20100, Italy.
[25]Department of Metabolism and Endocrinology, St. Marianna University Yokohama Seibu Hospital, Yokohama 241-0811, Japan.
[26]Department of Endocrinology, Karolinska University Hospital, Stockholm, Sweden.
[27]Department of Endocrinology, Clinical Research Institute, NHO Kyoto Medical Center and Endocrine Center, Ijinkai Takeda General Hospital, Kyoto, Japan.
[28]Endocrinology in Charlottenburg, Berlin, Germany.

Background: Bilateral macronodular adrenocortical disease (BMAD) is often linked to autonomous cortisol secretion but, more recently, has also been associated with primary aldosteronism (PA).

Methods: This international, multicenter, retrospective cohort study included adult patients from Europe, the US, Japan, and South Korea with radiological evidence of BMAD and biochemically confirmed PA. The primary endpoint was the prevalence of major adverse CV events (MACE). Secondary endpoints included cardiometabolic comorbidities and surgical outcomes per Primary Aldosteronism Surgical Outcome (PASO) criteria.

Results: Of 280 patients, 249 from 41 centers in 12 countries were eligible. Median age was 55 years; 62% were male. Median hypertension duration at PA diagnosis was 9.9 years. Among those tested, 52% had cortisol co-secretion; 48% had isolated PA. At baseline, 56% had metabolic comorbidities, and 16% had ≥1 MACE. Patients with a previous CV event were older, more often male, had longer durations of hypertension and higher rates of diabetes. Adrenalectomy was performed in 89 patients (36%; 83 unilateral, 6 bilateral); 180 (72%) received mineralocorticoid receptor antagonists (MRA). Over a median follow-up of 36 months (MRA) and 18 months (surgery), MACE occurred in 8% and 6%, respectively (p=1.000). Blood pressure control and target organ damage rates were similar, but more MRA-treated patients needed ≥3 antihypertensives (48% vs. 14%, p<0.001). Among surgically treated patients, complete clinical success was achieved in 26%, and biochemical success in 71%.

Conclusion:
BMAD with PA carries a high cardiometabolic burden. Early detection and precise subtyping are essential to guide therapy and prevent target organ damage.

Accuracy of Urinary Free Metanephrines Measured by Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) in the Diagnosis of Pheochromocytoma and Paraganglioma

Mirko Parasiliti-Caprino1,2, Federico Sibilia2,3, Elisa Febbraro1,2, Daniele Giuseppe Candela1,2, Matteo Procopio1,2, Stefano Arata1,2, Martina Bollati1,2, Chiara Lopez1,2, Federico Ponzetto2,4, FabioSettanni4, Roberta Giordano1,5, Giulio Mengozzi2,4, Ezio Ghigo1,2, Mauro Maccario1,2 and Emanuela Arvat2,3

1 Division of Endocrinology, Diabetes and Metabolism; University Hospital Città della Salute e della Scienza di Torino, Turin, Italy

2 Department of Medical Sciences; University of Turin

3 Division of Oncological Endocrinology; University Hospital Città della Salute e della Scienza di Torino, Turin, Italy

4 Clinical Biochemistry; University Hospital Città della Salute e della Scienza di Torino, Turin, Italy

5 Department of Clinical and Biological Sciences; University of Turin

BACKGROUND. Fractionated plasma or urinary metanephrines are the cornerstone of diagnosing pheochromocytoma and paraganglioma (PPGL). However, they yield a significant rate of false positives (up to 20%), partly due to the lack of properly defined reference intervals. Free urinary metabolites may more specifically reflect the metabolic activity of sympathetic PPGL (PPGLs), potentially offering greater diagnostic accuracy than total analytes.

OBJECTIVES. To define reference intervals for urinary free, deconjugated, and total metanephrines, and to assess their diagnostic accuracy in detecting sympathetic PPGL.

PATIENTS AND METHODS. We included all measurements of urinary total metanephrines performed at the Baldi and Riberi Laboratory of the Città della Salute e della Scienza University Hospital in Turin, between August 2020 and January 2025. Free metanephrines were measured on the same urine samples. All assays were conducted using liquid chromatography–tandem mass spectrometry (LC-MS/MS). Clinical and diagnostic data were collected for each patient.

RESULTS. A total of 3,188 urinary total metanephrine measurements were analyzed, along with corresponding free analyte levels. After excluding repeat samples, 832 patients followed at our institution were included. Among them, 35 PPGLs were diagnosed (24 sympathetic PPGLs), and 797 patients tested negative. Reference intervals were established for 24-hour urinary free, spot urinary free, 24-hour urinary deconjugated, and 24-hour total metanephrines, stratified by sex. Diagnostic accuracy was consistently high: 24-hour urinary free (AUC 0.929), deconjugated (AUC 0.942), and total metanephrines (AUC 0.943) showed excellent performance. Spot urinary free metanephrines were slightly less accurate but still valuable (AUC 0.895, $p = 0.052$). CONCLUSIONS. This study defined sex-specific reference intervals for multiple urinary metanephrine forms. The diagnostic performance of 24-hour urinary free metanephrines for sympathetic PPGLs was excellent. Adoption of this approach may reduce healthcare costs while maintaining high diagnostic accuracy.

Adrenal cortical carcinoma: retrospective study of “long survivor” patients

I. Pedrazzini[1], D.Cosentini[1], G.Mesetti[1], B.Trevisan[1], V.Cremaschi[1], D.Bettini[1], M.Marenzi[1], S.Rodella[1], F. Consoli[1], A. Esposito[1], S. Grisanti[1], M. Laganà[1], A.Berruti[1]

1. Medical Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, ASST Spedali Civili, Brescia, Italy.

BACKGROUND

The prognosis for patient with adrenocortical carcinoma (ACC) is usually considered poor: the average survival is around 3-4 years, dropping to 15 months in metastatic patients. However, the course of the disease is very heterogeneous and long survivors (LO) patients are described. Nevertheless data on these patients are limited.

METHODS

In this observational retrospective study, we evaluated all the metastatic patients consecutively seen from January 1994 to April 2025 at the Medical Oncology of ASST Spedali Civili of Brescia. The study aims to assess the number of metastatic LO patients (overall survival OS > 60 months), to describe their characteristics and to identify potential favourable prognostic factor.

RESULTS

Out of 346 ACC patients, 59 (17%) had metastatic disease with an overall survival higher than 60 months, including 4 with metastatic disease at diagnoses. The majority were women (58%) under the age of 55 (75%) and, considering the patients in which ki67 was available, more than 50% of them had a k67<20% with all patients having expression below 51%.
Almost all patients, 58 (98%), underwent an adrenalectomy, and 27 (49%) received adjuvant mitotane.
The most common site of metastasis was the lung 24 (41%), followed by locoregional recurrence, 22 (37%). 43 of patients (78%) received a subsequent metastasectomy, 19 (35%) loco-regional therapies and 35 (64%) at least one line of systemic treatment.
The median OS of this cohort was 7.8 years (range 5.1-24.6). Median OS from the first relapse was 5.3 years (range 0.4-18.4).

CONCLUSION

About 20% of the patients followed at our Institution had a survival higher than 5 years. Factors that appear to influence prognosis include age, hormone secretion, location of metastases. the ability to perform surgery on the primary tumour and subsequent metastasectomies.

Beyond the 50 nmol/L cut-off: improving 1mg-overnight dexamethasone suppression test accuracy with assay-specific thresholds

Pilloni Chiara[1,2,3,4], Lewis Laura[5], Mandal Sumedha[1], Lanzolla Giulia[3], Asia Miriam[2], Ronchi Cristina L.[1,2], Boi Francesco[3], Prete Alessandro[1,2,6]

[1]Department of Metabolism and Systems Science, School of Medical Sciences, College of Medicine and Health, University of Birmingham, Birmingham, UK.
[2]Department of Endocrinology, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK.
[3]Endocrinology Unit, Department of Medical Science and Public Health, University of Cagliari, University Hospital of Cagliari, Cagliari, Italy.
[4]Endocrinology, Diabetology and Internal Medicine Unit, Catholic University of the Sacred Heart, Rome, Italy.
[5]Department of Biochemistry, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK.
[6]National Institute for Health and Care Research (NIHR) Birmingham Biomedical Research Centre, Birmingham, UK.

Background: The 1mg-overnight dexamethasone suppression test (1mg-DST) is widely used to screen for autonomous cortisol secretion. Despite its simplicity, non-compliance and suboptimal dexamethasone exposure can affect the 1mg-DST accuracy. Furthermore, there is limited evidence on the impact of different serum cortisol assays on the widely used cut-off of 50 nmol/L.

Methods: All 1mg-DST with immunoassay (IA; Abbott Alinity) serum cortisol >50 nmol/L measured at the UHB NHS Foundation Trust between October 2018 and February 2025 had a corresponding measurement of cortisol and dexamethasone levels by liquid chromatography-tandem mass spectrometry (LC-MS/MS). A dexamethasone cut-off ≥3.3 nmol/L was used to establish adequate dexamethasone exposure. Linear regression and Bland-Altman analyses were used for IA vs. LC-MS/MS comparison.

Results: We analysed 576 1mg-DST results from 505 patients; 46 results (8.0%) had undetectable dexamethasone levels, suggesting non-compliance with the test, while 42 had suboptimal dexamethasone levels (7.3%). There was good agreement between IA and LC-MS/MS cortisol measurements (R^2 0.9113; $p<0.0001$); however, IA had a mean bias of -12.4 nmol/L (standard deviation [SD] 42.2), with mean 1mg-DST cortisol values of 88 nmol/L (SD 116) by IA compared to 99 nmol/L (SD 139) by LC-MS/MS (p-value 0.007). We identified 19 cases (3.3%) where 1mg-DST cortisol levels were abnormal when measured by IA (mean 91 nmol/L; SD 85) but <50 nmol/L when measured by LC-MS/MS.

Conclusions: By implementing routine dexamethasone measurement in patients with abnormal 1mg-DST results, we identified a false-positive rate of 15.3%. While there was generally good agreement between IA and LC-MS/MS measurements, IA shows a negative bias with the potential for a considerable number of abnormal test results remaining undetected when using the 50 nmol/L cut-off. We propose a 38 nmol/L cut-off during the 1mg-DST as more sensitive to detect autonomous cortisol secretion when measuring cortisol by IA Abbott Alinity.

A case of multiple cardiac paragangliomas in a SDHD germline mutation

Alessia Pinna 1, Simona Buralli 2, Hermann Dalpiaz 1, Giulia Mori Ubaldini 1, Stefano Masi 1, Stefano Taddei 1, Alessandra Bacca 2

1. Department of Surgical Pathology, Medicine, Molecular and Critical Area, Pisa, Italy
2. Medicine I, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy

Background
A small subset of paragangliomas (PGLs) arise as primary cardiac tumors, making primary cardiac paraganglioma (PCP) exceedingly rare. We report a case of multiple PCP in a patient with a previous diagnosis of head and neck (HN) and abdominal PGLs.

Design and method
A 35 year old woman, with a history of arterial hypertension (AH) and subsequent diagnosis of familiar paragangliomatosis syndrome type 1 in SDHD germline mutation, was hospitalized at our hypertension clinic for a general follow up. She was symptomatic for face flushing, palpitations and tachycardia. The patient underwent positron emission tomography (PET) with 68Ga-DOTATOC, and magnetic resonance (MR) of the neck, that confirmed presence of known PGLs. Specific heart imaging was also performed, including resting cardiac echocardiography and cardiovascular MR. She was diagnosed with three PCPs, localized in order at the bottom of the left atrium (26x24x27 mm), at the right atrioventricular groove (17x16x16 mm) and at the connective tissue between right atrium and right ventricle (5x5x5 mm). The masses did not affect cardiac contractile function (EF 68%). She presented elevated urinary normetanephrines (NorMT) values: 2741 mcg/24 h; and normal urinary metanephrines (MT) values: 159.4 mcg/24 ore.

Results
Blood pressure (BP) and heart rate (HR) showed satisfactory control in quadruple therapy (medium BP values 126/86 mmHg, medium HR 88 bpm). The patient was evaluated by the cardiac surgery unit to consider the possibility of resection of PCPs.

Conclusions
A young woman, affected by familiar paragangliomatosis syndrome type 1, was diagnosed for PCPs, and she was symptomatic for AH and catecholamines hypersecretion syndrome. She was treated with medical therapy. BP and HR were under control. Surgical eradication was considered as an option to exclude the risk of cardiac mechanical damage and potential adverse events caused by catecholaminergic hypersecretion.

Investigation of alternative hormones for evaluating selectivity of adrenal vein sampling in the subtype diagnosis of primary aldosteronism

Ponzetto Federico (1), Montesano Giulia (1), Procopio Matteo (2), Leoni Laura (1), Settanni Fabio (3), Ghigo Ezio (2), Mengozzi Giulio (1,3), Parasiliti-Caprino Mirko (2)

(1) Clinical Biochemistry Laboratory, Department of Medical Sciences, University of Turin, Turin, Italy
(2) Endocrinology, Diabetes and Metabolism, Department of Medical Sciences, University of Turin, Turin, Italy
(3) Clinical Biochemistry Laboratory, City of Health and Science University Hospital, Turin, Italy

International guidelines recommend adrenal venous sampling (AVS) for subtype diagnosis of primary aldosteronism (PA), which is crucial for suggesting surgical or medical treatment. Successful cannulation of adrenal veins is assessed using the cortisol (F) ratio between adrenal and peripheral veins (selectivity index, SI). Given the limitations associated with F measurements, this study aimed to evaluate whether the measurement of other hormones could improve SI calculation.

The study included 122 PA patients undergoing unstimulated and sequential AVS. Concentrations of F, catecholamines (noradrenaline, NA, adrenaline, AD), metanephrines (normetanephrine, NMT, metanephrine, MT, normetanephrine, NMT), and steroids (DHEA, DHEAS, androstenedione, A4, 11-deoxycorticosterone, DOC, 17-hydroxyprogesterone, 17OHP, and progesterone, P) were measured in both adrenal vein and peripheral vein samples using CLIA, HPLC, and LC-MS/MS, respectively. Hormone-specific SI cut-offs were calculated along with the percentage of selective procedures identified based on each marker. Differences in SI values of all hormones were compared between the selective and non-selective procedure groups.

Using as reference cut-off SI=3 for F, ROC curve analysis revealed the following SI cut-offs for MT (11.90, AUC=0.640), NMT (1.65, AUC=0.640), NA (1.40, AUC=0.598), AD (1.50, AUC=0.557), DHEA (1.10, AUC=0.850), DHEAS (22.00, AUC=0.831), A4 (16.66, AUC=0.825), DOC (4.49, AUC=0.950), 17OHP (4.62, AUC=0.944), and P (4.75, AUC=0.829). All markers, except P, improved the rate of selective procedures compared to F (74.1%). Notably, only steroid hormones exhibited significantly higher SI values in the group of selective procedures, with increases of up to 10-fold for DOC and 17OHP.

Monitoring alternative hormones and employing hormone-specific SI cut-offs increased the rate of selective AVS procedures, with steroids demonstrating the best diagnostic accuracy compared to metanephrines and catecholamines. Evaluating successful cannulations, the highest increases in SI were observed for DOC and 17-OHP, highlighting the latter as a promising additional marker due to its easily measurable levels in both adrenal and peripheral veins.

Urine Steroid Metabolomics and Timed Urine Steroid Profiling: A Novel Test for the Diagnosis and Differential Diagnosis of Cushing's Syndrome

Alessandro Prete[1,2,3,4], Abdi Lida[5,6], Bain Joshua T.[1,3], Shaheen Fozia[1,3], Suntornlohanakul Onnicha[1,3,7], Deflorenne Elisa[8,9], Blanchard Anne[10], Nestola Manuela[11], Pamporaki Christina[12], Tizianel Irene[13,14], Nowak Elisabeth[15], Bertherat Jérôme[16], Vassiliadi Dimitra A.[17], Bancos Irina[18], Dennedy M. Conall[19], Kastelan Darko[20,21], Fassnacht Martin[22,23], Mulatero Paolo[24], Ambroziak Urszula[25], Tabarin Antoine[26], Quinkler Marcus[27], Puglisi Soraya[28], Deinum Jaap[29], Ueland Grethe Å.[30], Lenzini Livia[31], Beuschlein Felix[32,15], Reincke Martin[15], Ceccato Filippo[13,14], Eisenhofer Graeme[33,12], Corsello Salvatore M.[11,34,35], Amar Laurence[8,9], Zennaro Maria-Christina[8], Karavitaki Niki[1,2,4], Taylor Angela E.[1,3], Arlt Wiebke[5,6]

[1] Department of Metabolism and Systems Science, School of Medical Sciences, College of Medicine and Health, University of Birmingham, Birmingham, UK.
[2] National Institute for Health and Care Research (NIHR) Birmingham Biomedical Research Centre, Birmingham, UK.
[3] Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham, UK.
[4] Department of Endocrinology, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK.
[5] Medical Research Council Laboratory of Medical Sciences, London, UK.
[6] Institute of Clinical Sciences, Imperial College London, London, UK.
[7] Endocrinology and Metabolism Unit, Division of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand.
[8] Université Paris Cité, PARCC, INSERM, F-75006 Paris, France.
[9] Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Unité Hypertension artérielle, Paris, France.
[10] Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Centre d'Investigations Cliniques 9201 Paris, France.
[11] Unit of Endocrinology, Università Cattolica del Sacro Cuore, Rome, Italy.
[12] Department of Medicine III, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany.
[13] Department of Medicine DIMED, University of Padova, Padua, Italy.
[14] Endocrine Disease Unit, University-Hospital of Padova, Padua, Italy.
[15] Department of Medicine IV, LMU University Hospital, LMU, Munich, Germany.
[16] Department of Endocrinology, French Reference Center for Rare Adrenal Disorders, Hôpital Cochin, Université Paris Cité, Institut Cochin, Inserm U1016, CNRS UMR8104, F-75014, Paris, France.
[17] Department of Endocrinology, Diabetes and Metabolism, Evangelismos Hospital, Athens, Greece.
[18] Division of Endocrinology, Diabetes, Metabolism and Nutrition, Mayo Clinic, Rochester, MN, USA.
[19] The Discipline of Pharmacology and Therapeutics, School of Medicine, National University of Ireland, Galway, Ireland.
[20] Department of Endocrinology, University Hospital Zagreb, Zagreb, Croatia.
[21] School of Medicine, University of Zagreb, Zagreb, Croatia.
[22] Department of Internal Medicine, Division of Endocrinology and Diabetes, University Hospital, University of Würzburg, Würzburg, Germany.
[23] Central Laboratory, University Hospital Würzburg, Würzburg, Germany.
[24] Division of Internal Medicine and Hypertension Unit, Department of Medical Sciences, University of Torino, Turin, Italy.
[25] Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Poland.
[26] Service d'Endocrinologie, Centre Hospitalier Universitaire, Hopital du Haut Leveque, Pessac, France.
[27] Endocrinology in Charlottenburg, Berlin, Germany.
[28] Department of Clinical and Biological Sciences, San Luigi Hospital, University of Turin, Turin, Italy.
[29] Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands.
[30] Department of Medicine, Haukeland University Hospital, 5021 Bergen, Norway.
[31] Internal & Emergency Medicine – ESH Specialized Hypertension Center, Department of Medicine-DIMED, University of Padua, Padua, Italy.
[32] Klinik für Endokrinologie, Diabetologie und Klinische Ernährung, UniversitätsSpital Zürich (USZ) und Universität Zürich (UZH), Zurich, Switzerland.
[33] Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany.
[34] Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore, Rome, Italy.
[35] Unicamillus-Saint Camillus International University of Health Sciences, Rome, Italy.

Introduction: Twenty-four-hour urinary free cortisol is used to diagnose Cushing's syndrome (CS); amongst its limitations is failure to differentiate ACTH-dependent CS (AD-CS) from ACTH-independent CS (AI-CS). We tested the performance of urine steroid metabolomics (USM), the computational analysis of 24-hour urine steroid metabolome data by machine learning, for the

diagnosis and differential diagnosis of CS. Given the physiological diurnal rhythm of cortisol secretion, we also hypothesised that glucocorticoid excretion should be higher during nighttime in CS compared to controls, thereby facilitating more sensitive detection of CS.

Methods: 264 subjects completed a 24-hour urine collection (40 AD-CS, 103 AI-CS due to adrenocortical adenoma/hyperplasia, 121 healthy controls). A subset of 52 individuals (13 CS, 39 healthy) provided a nighttime and daytime urine collection. Mass spectrometry-based multi-steroid profiling was used to quantify the urinary excretion of 27 steroid metabolites. Data were analysed by generalised matrix learning vector quantisation (a prototype-based supervised machine learning approach) and multiple linear & logistic regression models adjusted for age, sex, and BMI.

Results: Twenty-four-hour USM demonstrated very high accuracy in differentiating CS from controls (AUC-ROC 0.99, 95%CI 0.99-0.99), reflected by higher urinary excretion of glucocorticoid and glucocorticoid precursor metabolites in CS. USM yielded high accuracy in differentiating AD-CS from AI-CS (AUC-ROC 0.91, 95%CI 0.89-0.94), with androgen metabolites being the most discriminatory. Timed steroid excretion in controls reflected the diurnal pattern of adrenal steroidogenesis, with lower nighttime than daytime excretion of glucocorticoid metabolites. Nighttime glucocorticoid metabolite excretion (AUC-ROC 1.00, 95%CI 0.99-1.00) performed better than daytime (AUC-ROC 0.91, 95%CI 0.77-1.00) and 24-hour excretion (AUC-ROC 0.94, 95%CI 0.86-1.00) in separating CS cases from controls.

Conclusions: USM is a non-invasive, one-step candidate test for the accurate diagnosis and differential diagnosis of CS. Timed nighttime urine collection leverages cortisol circadian rhythmicity and improves the diagnostic accuracy of the current reference standard 24-hour collection.

Analysis of Predictive Factors of Malignancy in Adrenal Masses: findings from a Large Single-Center Surgical Cohort

Puglisi Soraya 1, Sarzani Elettra Vittoria 1, Romanisio Martina 2, Messina Erika 1, Parola Cinzia1, Cantore Lorenzo 1, Perini Anna 1, Reimondo Giuseppe 1, Berchialla Paola 1, Volante Marco 3, Porpiglia Francesco 3, Fiori Cristian 3, Terzolo Massimo 1

1 Department of Clinical and Biological Sciences, University of Turin, Orbassano (Italy)
2 Department of Translational Medicine, University of Eastern Piedmont, Novara (Italy)
3 Department of Oncology, University of Turin, Orbassano (Italy)

Introduction
Risk stratification for malignancy in adrenal masses is crucial to either avoid unnecessary surgery or reduce the risk of missing aggressive lesions. This study aims to investigate predictors of malignancy in patients with adrenal masses.
Methods
Retrospective single-center study on 573 adult patients who underwent adrenalectomy at S.Luigi Hospital (2005–2023 period). Exclusion criteria: biochemical diagnosis of pheochromocytoma (plasma/urinary metanephrines >2 times the upper normal limit), bilateral adrenalectomy for ACTH-dependent Cushing syndrome, and local recurrence of adrenocortical carcinoma (ACC).
The final cohort included 191 adrenal masses, preoperatively classified as indeterminate [if ≥1 of the following: i) >10 HU density on unenhanced CT or loss of signal intensity on out-phase MRI; ii) radiological heterogeneity; iii) diameter ≥40 mm; iv) ≥30% growth at follow-up; v) FDG-PET uptake] or likely-benign (none of the above).
Results
Among the 191 masses, 56 (29.3%) were considered likely-benign, and 135 (70.7%) indeterminate. Finding an indeterminate lesion was associated with older age (61 vs 51 years, $p<0.001$), more frequent history of extra-adrenal cancer (24% vs 11%, $p=0.039$) and elevated DHEAS (14% vs 0%, $p<0.001$). Histopathology revealed 33 malignancies (17.3%, 21 ACC and 12 metastases), all in the indeterminate group. Multivariate analysis identified tumor diameter [OR 1.27 per mm, 95%CI 1.14–1.51, $p=0.001$], density >10 HU and/or heterogeneity (OR 363.44, 95%CI 68.59–3757.09, $p<0.001$), and age (OR 1.11 per year, CI 1.03–1.20, $p=0.008$) as independent predictors of malignancy. ROC analysis identified 54.5 mm as the best diagnostic cut-off for size (specificity 84%, sensitivity 74%, AUC 0.77). Elevated DHEAS was associated with malignancy in univariate (OR 17.7, CI 4.23–93.4, $p<0.001$), but not in multivariate analysis.
Conclusions
Radiological features are the strongest independent predictors of malignancy in adrenal masses, with age as an additional factor. DHEAS may play a role in risk stratification, particularly when ACC is suspected.

Role of Succinate and its receptor in modulating the migration of human pheochromocytoma tumor cells

Francesca Amore[1], Silvia Nigro[2], Matilde Tavanti[2], Jiri Neuzil[3,4,5], Mario Maggi[1,6], Letizia Canu[1,6], Elena Rapizzi[2,6]

[1] University of Florence, Dept. Experimental and Clinical Biomedical Sciences, Florence, Italy;
[2] University of Florence, Dept. Experimental and Clinical Medicine, Florence, Italy;
[3] Institute of Biotechnology, Czech Academy of Sciences, Prague-West, Czech Republic;
[4] School of Pharmacy and Medical Science, Griffith University, Southport, Qld, Australia;
[5] Faculty of Science and 1st Medical Faculty, Charles University, Prague, Czech Republic;
[6] ENS@T Center of Excellence, Florence, Italy;
[7]Department of Medical Sciences, Section of Experimental Medicine and Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy.

Loss of function of succinate dehydrogenase (SDH) due to mutations in the gene encoding the SDHB subunit, is associated with a more aggressive tumor phenotype and a high risk of metastasis. SDH deficiency leads to intracellular accumulation of succinate, an oncometabolite that alters cellular metabolism and modulates epigenetic regulation, promoting tumor growth and progression. Recently, succinate has also been identified as a ligand for the G protein-coupled receptor SUCNR1, however its role is still not fully understood. SUCNR1 expression is ubiquitous and the G proteins to which it is coupled vary depending on the tissue considered. In this study, we compared two human pheochromocytoma cell lines: hPheo1 Parental, with functional SDHB and characterized by a lower aggressiveness, and hPheo1 SDHB deficient, lacking the SDHB subunit.

SDHB deficient cells exhibit high levels of intracellular succinate, upregulation of the SUCNR1 receptor, increased phosphorylation of ERK1/2, lower proliferation, but higher migratory capacity than Parental cells, confirming their more aggressive and metastatic phenotype. Interestingly, treatment of Parental cells with methylated succinate, a form that is permeable to cells, induced epigenetic modifications similar to those observed in SDHB deficient cells, including increased K-succinylation and H3K9me3 methylation, but only the treatment with unmethylated succinate, which acts only extracellularly, induced increased SUCNR1 expression in Parental cells.

Moreover, we demonstarted that unmethylated succinate induced ERK1/2 activation and increased cell migration in both the cell lines, suggesting that succinate-SUCNR1 binding may influence tumor progression through the activation of ERK1/2 pathway. Indeed, treatment with the selective ERK1/2 inhibitor (SCH772984) reduces cell migration. These data demonstrate that succinate-SUCNR1 binding may promote a more invasive and migratory, rather than proliferative, tumor growth strategy, opening new therapeutic perspectives for the treatment of aggressive PPGL.

Development of MODMAP (Multi-Omics Data Management Platform) for Enhancing Hypertension Treatment Efficacy Through Machine Learning-Based Personalized Decision Support: The HT-Advance Project

Reel Parminder Singh[1,2], Marshall Pete[2], Jones Claire[2], Baird Jennifer[2], Umagiliya Bandara Hasithi[3], McKenzie Andrew[3], Ismail Dhekra[4], Riddell Alexandra[5], MacKenzie Scott M.[5], Bain Joshua[6], Lintelmann Jutta[7], Baron Stéphanie[8], Taylor Angela[6], Witting Michael[7], Peitzsch, Mirko[9], Davies Eleanor[5], Hogarth Fiona[3], Deinum Jaap[10], Zennaro Maria-Christina[4], Cole Christian[1,2]

[1]Division of Population Health & Genomics; [2]Health Informatics Centre; [3]Tayside Clinical Trials Unit}, University of Dundee, UK [4]Université de Paris, INSERM, PARCC, Paris, France [5]School of Cardiovascular and Metabolic Health, BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow G12 8TA, UK [6]Institute of Metabolism and Systems Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK [7]Metabolomics and Proteomics Core, Helmholtz, Munich, Germany [8]Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Centre d'Investigations Cliniques 9201 Paris, France [9]Institute of Clinical Chemistry and Laboratory Medicine, University Hospital and Faculty of Medicine Carl Gustav Carus, TU Dresden, Dresden, Germany [10]Radboud UMC, Nijmegen, Netherlands.

MODMAP (Multi-Omics Data MAnagement Platform) is a secure, end-to-end digital infrastructure developed within the HT-Advance project (https://ht-advance.eu/) to enable machine learning (ML)-driven personalised decision support in hypertension care. Building on the clinical and scientific foundation established by the Horizon 2020 ENS@T-HT program, which identified key biomarkers for endocrine hypertension (EHT) subtypes—such as primary aldosteronism, pheochromocytoma, Cushing's syndrome, and primary hypertension (PHT) —MODMAP has enabled the streamlined capture, integration, and interpretation of large-scale multi-omics data. It supports clinical trials that explore effectiveness of the pre-trained ENS@T ML model vs usual clinical care and the treatment response of PHT medication. MODMAP connects clinicians and omics laboratories through a unified platform that facilitates sample tracking, omics data collection (plasma/urinary steroids, catecholamines, microRNAs, genotypes) and disease prediction delivery. Data are securely stored and managed in an ISO27001-accredited environment in Health Informatics Centre (HIC) at University of Dundee.

MODMAP ensures a seamless data flow from patient blood and urine collection (via Castor integration) to ML-based EHT predictions, which are returned to clinicians via a user-friendly interface. The platform incorporates a validated trained model from ENS@T-HT, optimized for accuracy, specificity, and complexity, and deployed using R-based wrappers. Built using agile software development practices—including version control, continuous integration, and testing frameworks—MODMAP maintains high standards for reliability, reproducibility, and model performance. It also ensures that all software and ML models are properly versioned and validated.

As an early adopter of ML methodologies in clinical practice with compliance to the new EU AI Act, MODMAP exemplifies how robust digital tools can translate biomarker research into actionable healthcare insights. It serves not only as a technical solution for personalised hypertension management but also as a model for securely and effectively integrating machine learning into clinical workflows.

CHEK2 as a potential susceptibility gene for pheochromocytomas and paragangliomas

Renda Riccardo[1,2], Contarino Andrea[2,3], Mantovani Giovanna[1,2], Palmieri Serena[2]

[1]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy
[2]Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[3]Department of Health Sciences, University of Milan, Milan, Italy

Introduction: The Checkpoint Kinase 2 (*CHEK2*) gene plays a crucial role in DNA damage repair. Loss of function mutations increase the risk of malignancy, most notably breast, colorectal, prostate, thyroid and kidney cancers. Recently, a potential association between *CHEK2* and multiple endocrine tumours has emerged, including pheochromocytomas (PHEO) and paragangliomas (PGL).

Case presentation and review: A 51-year-old male was hospitalized for fever and abdominal pain. Abdominal CT revealed an 85x75x75 mm mass in the right adrenal gland, with suspicious radiological features of malignancy (baseline HU > 20, central necrosis and calcifications, possible infiltration of the inferior vena cava and hepatic hilum) and increased uptake on FDG-PET/CT scan (SUVmax 20). An excess of adrenal hormonal secretion was excluded, except for elevated urinary normetanephrines. The patient underwent right adrenalectomy and right hepatectomy, and histology confirmed a PHEO completely confined to the adrenal gland, with PASS score 8/20 and Ki67 5% (SDHB expression was maintained). Pre-surgery FDG-PET/CT scan incidentally detected also a thyroid uptake. Subsequent ultrasound and cytological investigations led to total thyroidectomy, which confirmed the concurrent presence of papillary and follicular thyroid carcinomas. There was no family history of PHEO/PGL or other tumours, but genetic analysis revealed a pathogenic heterozygous variant of the *CHEK2* gene (NM_007194.4):c.908+1G>T. To date, 8 patients with PHEO/PPGL and *CHEK2* mutations have been reported in literature, with a median age at first diagnosis of 41 years. Unilateral PHEO occurred in 5 cases, extra-adrenal sympathetic and head-and-neck PGLs in 1 case each and multifocal disease (exclusively abdominal) in another case. When available, the biochemical phenotype was adrenergic in 2 cases, noradrenergic and non-secreting in 1 case each. Distant metastases were reported in 50% of patients.

Conclusions: *CHEK2* mutations may predispose to the development of PHEO/PGL, which may exhibit more aggressive features. Larger studies are needed to strengthen current evidence.

Evening Metyrapone Therapy in Mild Hypercortisolism: A Case Report

[1,2]Risio Alessandro, [2,3]Favero Vittoria, [2]Aresta Carmen, [2,3]Chiodini Iacopo

[1]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy,
[2]Unit of Endocrinology, Ospedale Niguarda Cà Granda, Milan, Italy.
[3]Department of Biotechnology and Translational Medicine, University of Milan, Milan, Italy

Introduction: The management of patients with primary bilateral macronodular hyperplasia and mild cortisol excess should be individualized. Although adrenalectomy is the treatment of choice, bilateral adrenal lesions complicate the surgical approach and may necessitate a medical strategy to avoid the risks of bilateral adrenalectomy and lifelong steroid dependence. Steroidogenesis inhibitors such as metyrapone have been recently proposed in this setting and the optimal timing of administration is still debated.

Case Presentation: We describe a 51-year-old woman with an already known right adrenal adenoma (identified in 2018), who was referred in 2024 for recent-onset weight gain. The computed tomography revealed benign bilateral adrenal lesions (right: 25×15 mm; left: 25×19 mm). Hormonal evaluation demonstrated inadequate cortisol suppression after 1 mg overnight dexamethasone suppression test (16 µg/dL) and elevated late-night salivary cortisol (4.29 µg/L; <2.08 µg/L), while 24-hour urinary free cortisol was in range. ACTH was suppressed (<1.5 ng/L). Treatment with metyrapone was started with one single evening dose (a 250 mg tablet at 4 PM), showing an improvement of salivary cortisol (8 AM:2.1 µg/L; 12 PM:2.1 µg/L; 4 PM:0.99 µg/L; 11 PM:1.63 µg/L). The dose was subsequently titrated to 2 tablets/day (4:00 PM and 8:00 PM) to better align with the circadian rhythm of endogenous cortisol secretion. At follow-up, salivary cortisol levels showed a restored diurnal profile (8 AM:2.33 µg/L; 12 PM:2.36 µg/L; 4 PM1.05 µg/L; 11 PM:0.75 µg/L) and a normalization of ACTH values (20.6 ng/L), and the patient reported significant clinical improvement.

Discussion: This case highlights the potential benefits of chrono-modulated metyrapone therapy in ACTH-independent Cushing's syndrome. While metyrapone is usually administered in multiple daily doses, exclusive evening dosing may be a valid strategy in selected patients. Adjusting drug timing to support the restoration of physiological cortisol circadian rhythm may improve both biochemical control and patient symptoms.

The outcomes of chemotherapy in the treatment of patients with malignant paraganglioma and pheochromocytoma

Laganà Marta[1], Marenzi Marta[1], Rodella Sara[1], Bettini Davide Lorenzo[1], Ranallo Nicoletta[2], Oldani Stefano[2], Morano Federica[3], Cosentini Deborah[1], Trevisan Benedetta[1], Cremaschi Valentina[1], Berruti Alfredo[1]

[1]Università degli Studi di Brescia - ASST Spedali Civili, Brescia, Italy
[2]IRST "Dino Amadori", Meldola, Italy
[3]IRCCS, Milan, Italy

Chemotherapy (Cht) regimens have been considered for the treatment of progressive or symptomatic metastatic pheochromocytomas and paragangliomas (mPPGLs), although objective responses have been observed in only a minority of patients. However, due to the rarity of these tumors, it has been challenging to assess the true efficacy of chemotherapy.

We conducted a retrospective, multicenter study including 44 patients with mPPGLs treated with chemotherapy - either the CVD regimen (cyclophosphamide, vincristine, and dacarbazine) or temozolomide - at the Oncology Units of ASST Spedali Civili of Brescia, IRCCS of Milan, and IRST "Dino Amadori" of Meldola. The primary endpoint was overall survival (OS), while secondary endpoints included progression-free survival (PFS), response rate, safety, and identification of potential predictive factors.

In total, 52% of patients had metastatic pheochromocytoma, while the remaining had metastatic paragangliomas. Fifteen patients received CVD as first-line therapy, 20 were treated with temozolomide, and the remaining 9 received other systemic chemotherapy regimens. Nineteen patients received a second-line therapy. The median OS (mOS) for patients receiving first-line chemotherapy was 20.8 months (95% CI: 1.83-93.10), and the median PFS (mPFS) was 9.7 months (95% CI: 0.9-78.8). The most frequent grade 3/4 adverse events were hematologic toxicities. No clinical or molecular parameters showed statistically significant prognostic or predictive value. However, the use of chemotherapy regimens other than CVD was associated with worse OS from the start of first-line treatment, suggesting a poorer prognosis. The absence of additional predictive factors may be attributed to the small sample size of the cohort.

Chemotherapy remains one of the main therapeutic options for patients with rapidly progressive mPPGL. Our study describes one of the largest cohorts of patients with these rare tumors. Further studies with larger populations are needed to identify and validate potential prognostic and predictive markers.

Integrated liquid biopsy using ccfDNA and urinary steroid metabolomics for early detection of recurrence in adrenocortical carcinoma: a preliminary study

[1]David S. Tourigny, [2,3]Lorenzo Tucci, [4]Balqees Shaaban, [2,5]Meng Jie Xu, [6]Yasir S Elhassan, [7]Kassiani Skordilis, [6]Miriam Asia, [2,6,8]Alessandro Prete, [2]Ana Crastin, [2]Ezra Leander Santosh David Onesimu, [2]Angela E Taylor, [2,6]Vasileios Chortis, [2,6]Cristina L Ronchi

1 School of Mathematics, University of Birmingham, Birmingham, UK.
2 Department of Metabolism and Systems Science, University of Birmingham, Birmingham, UK.
3 Medical and Surgical Sciences Department, Alma Mater Studiorum University of Bologna, Bologna, Italy.
4 MSc. Bioinformatics Programme, University of Birmingham, Birmingham, UK.
5 Department of Endocrinology, Taihe Hospital, Hubei University of Medicine, Shiyan, P. R. China.
6 Department of Endocrinology, Queen Elizabeth Hospital Birmingham NHS Trust, Birmingham, UK.
7 Department of Pathology, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom.
8 NIHR Birmingham Biomedical Research Centre, University of Birmingham and University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK.

Adrenocortical carcinoma (ACC) is a rare cancer with heterogeneous prognosis. Close disease monitoring is essential but relies on frequent radiological imaging. Circulating cell-free DNA (ccfDNA) and urine steroid metabolomics (USM) may represent non-invasive tools for post-operative surveillance.
We aimed to evaluate the role of combined ccfDNA sequencing and urine steroid metabolomics (USM) to monitor disease recurrence in ACC.
We investigated 6 patients (1M/5F, median age 37.5yrs) with histologically confirmed ACC. Plasma and 24-hour urine samples were collected before adrenalectomy (baseline), early post-operatively (28-42 days) and on 3-monthly follow-ups. ccfDNA, germline DNA (gDNA) and tumour DNA (tDNA) were isolated using commercially available kits and sequenced by customised ACC-specific panel and shallow (0.1x) whole genome sequencing (sWGS). Genetic alterations were called following standard bioinformatic protocols. gDNA was used to discriminate somatic variants. 32 steroid metabolites were quantified using gas chromatography/mass spectrometry and a previously developed generalised matrix learning vector quantisation algorithm was used to diagnose ACC and detect recurrence.
At tDNA level, 3/6 cases (50%) presented point mutations while all cases (100%) had an altered copy number variation pattern at sWGS. tDNA-derived somatic alterations were detected in baseline ccfDNA from 4/6 patients (71%). Baseline USM demonstrated ACC-specific steroid profiles in 5/5 patients.
Three patients developed radiological recurrence within 6 months, which coincided with the detection of somatic alterations in follow-up ccfDNA samples in 2/3 cases (67%). In one case, sWGS gave a clear signal for recurrence that would otherwise be missed by targeted sequencing alone. USM detected ACC-specific steroids at recurrence in all cases, one 3 months before radiological evidence of relapse.
In conclusion, integrating ccfDNA signatures and USM profiles could complement radiological surveillance in the monitoring of ACC patients. sWGS showed an additional value beyond targeted sequencing alone. Validation in a larger cohort is required to confirm these findings.

Artificial Intelligence in Adrenal Imaging: Detection and Segmentation of Adrenal Glands

Roszkowska Zuzanna, Bobrowicz Małgorzata, Ambroziak Urszula

Department of Internal Medicine and Endocrinology, Medical University of Warsaw
Warsaw, Poland

Background:
Accurate visualization of the adrenal glands is crucial for the evaluation of adrenal pathology. However, their small size, variable shape, and proximity to other retroperitoneal structures make automated analysis challenging. Artificial intelligence (AI) tools capable of detecting and segmenting adrenal glands could significantly improve workflow efficiency and lay the groundwork for future tumor characterization.

Objective:
At the current stage of the project, we aim to develop and validate an AI-based algorithm for automated detection and 3D segmentation of adrenal glands in non-contrast CT scans. This constitutes a foundational step for subsequent modules that will analyze adrenal lesions.

Methods:
A multidisciplinary team from the Medical University of Warsaw and Warsaw University of Technology is collaborating to build a comprehensive dataset of anonymized abdominal CT scans. Using state-of-the-art deep learning architectures, we are training models to precisely identify and segment both adrenal glands, generating accurate three-dimensional reconstructions. The performance of the model is evaluated based on segmentation accuracy and anatomical consistency.

Conclusion:
The AI algorithm successfully detects and segments adrenal glands in three dimensions, offering a robust and scalable tool for future applications in adrenal imaging. This stage represents a critical foundation for further development of AI systems aimed at lesion detection and classification in adrenal pathology.

Keywords:
Artificial intelligence, adrenal glands, 3D segmentation, CT imaging, deep learning, radiology, endocrine diagnostics

Vitamin D receptor expression in adrenal medulla and pheochromocytoma cells

Rzepka Ewelina[1], Skalniak Anna[2], Ulatowska-Białas Magdalena[3], Lech Martyna[3], Opalińska Marta[1], Gilis-Januszewska Aleksandra[1], Przybylik-Mazurek Elwira[1], Hubalewska-Dydejczyk Alicja[1]

[1]Chair and Department of Endocrinology, Jagiellonian University Medical College, Cracow, Poland
[2]Center for the Development of Therapies for Civilization and Age-Related Diseases, Jagiellonian University Medical College, Cracow, Poland.
[3]Department of Pathomorphology, Jagiellonian University Medical College, Cracow, Poland

The expression of the vitamin D receptor (VDR) and the functional role of 1,25-dihydroxyvitamin D3 [1,25$(OH)_2D_3$] within the human adrenal medulla remain insufficiently characterized.

To date, no genomic data have been published concerning VDR mRNA expression in either human pheochromocytoma or normal adrenal medullary tissue.

The objective of this study was to characterize VDR expression in the normal human adrenal medulla and in pheochromocytoma cells, and to explore a potential correlation between VDR expression and the clinicopathological features of pheochromocytoma.

A total of 31 pheochromocytoma cases with available tissue samples from surgical resection, 4 samples of adrenal cortex and 4 samples of adrenal medulla as control group were analysed. The expression of VDR at the mRNA level was assessed by digital PCR in all pheochromocytoma cases and the control group.

VDR mRNA expression levels in adrenal medulla and pheochromocytoma cells were analysed relative to expression in control adrenal cortex, which was designated as the reference value. Among the examined tissues, pheochromocytoma specimens exhibited the lowest median VDR mRNA expression in comparison to both unaltered adrenal medulla and adrenal cortex. No statistically significant associations were identified between VDR mRNA expression in pheochromocytomas and the majority of clinical parameters assessed. However, a negative correlation was observed between VDR expression in tumour tissue and somatostatin receptor 2 (SSTR2) levels, after adjustment for confounding variables.

In this study, we report for the first time the expression of VDR in human adrenal medulla and pheochromocytoma tissue. The adrenal cortex exhibited the highest median VDR expression, followed by the adrenal medulla, with the lowest levels observed in pheochromocytoma specimens. No significant associations were found between VDR mRNA expression and the key clinical characteristics of the disease.

Exploration of *UBTF-MAML3* regulatory networks in pheochromocytoma and paraganglioma: a cellular model-based multi-omic approach

Milton E. Salazar-Hidalgo[1], Clara Reglero[1], Alberto Díaz-Talavera[1,2], Ester Arroba[1], María Monteagudo[1], Bruna Calsina[1], Cristina Montero-Conde[1,2], Luis J. Leandro-García[1], Marta Isasa[3], Ana Cuadrado[4], Ana Losada[4], Alberto Cascón[1,2], Mercedes Robledo[1,2]

[1] Hereditary Endocrine Cancer Group, Human Cancer Genetics Programme, Spanish National Cancer Research Center (CNIO), Madrid, Spain
[2] Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Institute of Health Carlos III (ISCIII), Madrid, Spain
[3] Proteomics Core Unit, Biotechnology Programme, Spanish National Cancer Research Center (CNIO), Madrid, Spain
[4] Chromosome Dynamics Group, Molecular Oncology Programme, Spanish National Cancer Research Center (CNIO), Madrid, Spain

MAML3 fusions are present in approximately 4% of pheochromocytomas and paragangliomas (PPGLs), with more than half of these cases developing metastasis. Our previous study identified distinct vulnerabilities in *MAML3* tumors beyond dysregulation of the Wnt signaling pathway[1]. These tumors exhibit elevated expression of PD-L1, neuroendocrine-to-mesenchymal transition markers, *MYC*-targets, and angiogenesis-associated genes, resulting in a unique tumor microenvironment characterized by specialized vasculature and immune profiles. However, the molecular targets directly regulated by the fusion remain unknown.

Within this framework, we generated a doxycycline-inducible cellular model expressing the hemagglutinin tagged *UBTF-MAML3* fusion. Subcellular fractionation showed predominant localization of the protein to nuclear and chromatin fractions, consistent with its putative role in transcriptional regulation. To assess the downstream transcriptional impact of *UBTF-MAML3*, we conducted a time course RNA-seq experiment. Differential expression and gene set enrichment analysis (GSEA) revealed progressive activation of gene programs that closely mirror those observed in patient tumors, reinforcing the model's utility for *in vitro* investigation of *UBTF-MAML3*-associated PPGLs.

ChIP-seq and global proteomic analyses have identified fusion-specific binding sites, complementing transcriptomic data and providing a detailed molecular profile of the fusion-driven regulatory network.

Together, these findings extend the characterization of this PPGL subtype and establish a robust model for interrogation of fusion-dependent programs, metastatic progression, and potential therapeutic vulnerabilities.

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Role of the tumor microenvironment in SDHx mutated Paraganglioma and Pheochromocytoma development

Sanceau Julie.[1], Westbrook Rebecca.[1], Favier Judith,[1]
[1] INSERM UMRS1138 – Centre de Recherche des Cordeliers, Paris, FRANCE

Pheochromocytoma and Paraganglioma (PPGL) are often driven by germline mutation (40%) in one of the 20 PPGL predisposing genes identified so far. Mutation in the SDHx genes, encoding the four subunits of succinate dehydrogenase, are the most common in PPGL and SDHB mutated tumours are at high risk of becoming metastatic.
SDHB tumour cells shows increased migratory capacities, which may not only be the result of their specific morphology but may also be due to their capacity to promote a pro-metastatic environment by influencing the different cells types in the tumor micro-environment (TME). SDHB patients present an increase in circulating succinate, a known oncometabolite, that could be responsible for TME reshaping. We have established a thorough protocol to dissociate fresh human tumours and gently sort the different cell types to either keep them in culture or do further molecular analysis. Bulk RNAseq from those enriched population will allow us to dig deeper in the subtle but essential changes induced by the SDHB mutated environment that could be missed by scRNAseq. As of today, 11 different tumors have been dissociated and CD31-positive or negative cells have been sequenced. Around 20 additional tumors samples are available to complete this study including patient mutated for SDHx, VHL, SLC25A11 or NF1 genes. Changes in the behavior of endothelial and fibroblast cells co-cultured with primary tumour cells (with and without SDHx mutations) is assessed. Preliminary data are in favor of an enhance migration of endothelial cells while cocultured with Custer 1 tumor cells that could illustrate a more efficient recruitment of cells to the TME. Several omics-based investigations are underway to further shed light on this mechanism.

Machine learning-based Survival Prediction in adrenocortical carcinoma: A Web Tool

Emre S Saygili[1,2], Yasir S Elhassan[2,3], Alessandro Prete[2,3,4], Juliane Lippert[5], Barbara Altieri[6,7], Cristina L Ronchi[2,3]

[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey
[2]Department of Metabolism and Systems Science, College of Medicine and Health, University of Birmingham, Birmingham, UK
[3]Department of Endocrinology, Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK
[4]NIHR Birmingham Biomedical Research Centre, University of Birmingham and University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK
[5]Institute of Human Genetics, University of Wuerzburg, Wuerzburg, Germany
[6]Division of Endocrinology and Diabetes, Department of Internal Medicine I, University Hospital, University of Wuerzburg, Wuerzburg, Germany
[7]Bavarian Cancer Research Center (BZKF), University Hospital of Würzburg, Würzburg, Germany

Background and Aim: Adrenocortical carcinoma (ACC) is a rare but aggressive malignancy characterized by heterogeneous clinical outcomes. Prognostic classification in ACC remains challenging due to its variable disease course. The S-GRAS score—comprising tumor stage, grading, resection status, age, and symptoms—has proven valuable in predicting patient outcomes. This study aimed to apply advanced machine learning (ML) methodologies to improve prognostic classification and support personalized clinical decision-making in ACC.

Methods: We utilized the S-GRAS patient dataset (Elhassan 2021, n=942) as a training set and an independent external cohort (n=152) for validation to construct and test ML models aimed at predicting patient outcomes. Sixteen ML algorithms were developed using individual clinical predictors. Endpoints were 5-year overall mortality (OM) and 1- and 3-year disease progression (DP). The top-performing models were integrated into a user-friendly, web-based application for individualized risk estimation.

Results: Quadratic Discriminant Analysis, Light Gradient Boosting Machine, and AdaBoost Classifier models achieved the best performance in predicting 5-year OM and 1- and 3-year DP. These models yielded F1 scores of 0.79, 0.63, and 0.83 respectively in the training dataset, and 0.72, 0.60, and 0.83 in the validation dataset. For 5-year OM prediction, the sensitivity and specificity were 77% and 77% in the training set, and 65% and 81% in the validation set. The web-based tool (https://acc-survival.streamlit.app) addressed to clinical staff was developed to provide accessible, individualized risk predictions for mortality and disease progression.

Conclusion: We could demonstrate that S-GRAS parameters can efficiently predict outcome in patients with ACC, even using a robust ML model approach. Our already available, free-to-use web app instantly estimates the risk of mortality and disease progression in patients with ACC, serving as an accessible tool to support personalized management decisions in clinical practice.

Glucocorticoid inhibition enables mono- and bispecific CAR-T cells to overcome immunotherapeutic resistance in adrenocortical carcinoma

Marc P. Schauer[1,2], Justus Weber[2], Laura-Sophie Landwehr[1], Andrea Stabile[2], Peter Spieler[2], Barbara Altieri[1], Silviu Sbiera[1], Hermann Einsele[2], Martin Fassnacht[1,3], Michael Hudecek[2,3,4]

[1] Department of Internal Medicine I, Division of Endocrinology & Diabetes, University Hospital Wuerzburg, GER
[2] Department of Internal Medicine II, Chair for Cellular Immunotherapy, University Hospital Wuerzburg, GER
[3] National Center for Tumoral disease (NCT) WERA, University of Wuerzburg, GER
[4] Fraunhofer Institute for Cellular Immunotherapy (IZI), Branch Site Wuerzburg, GER

Glucocorticoids (GC) are secreted by ~60% of adrenocortical carcinoma (ACC) lesions and negatively affect immune-recognition and –therapy. Despite our previous observation that ROR1 expression is tightly linked to and triggered by GC secretion in ACC, GC inhibition induced a significant reduction of ROR1 making combinatorial CAR-T cell therapies with GC inhibitors obsolete.

Hence, we applied transcriptomic and proteomic target screens and identified B7-H3 as additional CAR target that is negatively regulated to ROR1, significantly upregulated in ACC (n=90) and strongly associated with poor survival prognosis. When evaluating this resistance mechanism, molecular analyses revealed a strong increase of B7-H3 upon GC inhibition driven by improved intratumoral STING pathway signaling that is usually abrogated upon glucocorticoid receptor (GR) activation. We show that GC inhibition upregulates B7-H3 by disrupting GR/NF-κB complex tethering while enabling nuclear translocation of Stat3 and NF-κB. With this observation, and while observing potent CAR-T cell functionality alone, a significant enhancement of mono- and bispecific CAR-T cell efficacy in ACC was observed when combined with GC inhibitors. However, single transcriptomic data indicated B7-H3 expression in some healthy tissues increasing the risk of on-target off-tumor toxicity. Hence, we exploited the role of co-opted intracellular proximal T cell signaling molecules that can be repurposed into surface receptors to engineer a synthetic logic gate CAR-T cell platform. By switching target binding domains, we were able to create an asymmetric **F**unctionally **L**ayered and **U**nequal e**X**pression (FLUX)-CAR system which enables gradient-level AND-gating through spatially defined adaptor placement, achieving tunable logic behavior beyond binary control. Finally, when testing FLUX-CAR-T cells alone and together with GC inhibitors, we could selectively eliminate high antigen expressing ACC while obviating CAR-T cell killing in low single antigen positive tissues and inducing complete and persistent ACC tumor eradication indicating a broad and safe application potential for GC-producing ACC in the clinic.

Untargeted metabolomics of human serum samples by UHPLC-HRMS as a tool for endocrine hypertension identification and patients stratification: a pilot study

Sénard Thomas[1,3], Perrone Alessandro[2,3], Bissi Valentina[2,3], Gennai Alessia[2,3], Galante Greta[2,3], Francesca Donnarumma[1,2], Coscia Kimberly[1,2], Pagotto Uberto [1,2], Di Dalmazi Guido[1,2] and Fanelli Flaminia [2,3]

[1]Division of Endocrinology and Diabetes Prevention and Care, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy.
[2]Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy.
[3]Center for Applied Biomedical Research, Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy.

Background. Prevalence of endocrine forms of hypertension (EHT), like pheochromocytoma/paraganglioma (PPGL) and hypercortisolism, might be cumbersome.
Aim. To apply an untargeted metabolomic workflow to identify potential biomarkers to discriminate EHT from primary hypertension.
Methods. For this pilot study, we collected serum samples from patients with EHT, taken at the time of first diagnosis (hypercortisolism, n=7 and PPGL, n=6). Serum from patients with non-secreting adrenal tumors (n=9) and those with primary hypertension (n=9) were used as controls. The samples were extracted via protein precipitation before being run on our liquid chromatography – mass spectrometry (LC-MS) platform comprised of a Vanquish Flex UHPLC coupled to a Orbitrap Exploris 240 high-resolution mass spectrometer (Thermo Scientific™). Metabolites separation was performed on a Hypersil GOLD™ aQ C18 Polar column (Thermo Scientific™) using a 26 minutes gradient at 350 µL/min flow rate. Mobile phases consisted of H2O and acetonitrile, both added with 0.1% formic acid. The MS was used in data-independent acquisition mode in both positive and negative polarity. To improve identification, MS/MS spectra were acquired at different collision energies within a same *m/z* window. Raw data were processed using MS-DIAL software referencing MSP spectral libraries.
Results. A robust identification was performed for 320 metabolites. Preliminary data, through principal component analysis (PCA) and T-tests, show clear distinctions between the control group and other groups. Methionine, lysophosphatidylcholines (LPC 18:3, 18:1, 16:0) and lysophosphatidylethanolamine (18:2, 16:0) were the main discriminatory compounds, followed by bile acids (glycocholic acid, deoxycholic acid, glycochenodeoxycholic acid) and amino acids (valine, tyrosine, isoleucine). Group separation was obtained between essential hypertension and PPGL with LPC 18:1 (P<.001) and LPC 18:0 (P<.002). Following these promising results, we will focus on the analysis of larger cohorts adding patients with primary hyperaldosteronism and hypercortisolism to detect potential differences in their metabolic profiles.

Uncharted Territory: A novel SDHC mutation in a young patient with paraganglioma

Siampanopoulou Vasiliki[1], Linardaki Emmanouela[2], Papalexis Petros[1], Lafioniatis Alexandros-Aristeidis[1], Tsirou Georgia[1], Chalmantzi Varvara[1], Stratakis Constantine[2], Angelousi Anna[1]

[1]Endocrinology Unit, First Internal Medicine Department, Laiko General Hospital of Athens, Endo-ERN Centre of Excellence of Adrenal Tumours, National and Kapodistrian University of Athens, Athens, Greece
[2]Human Genetics & Precision Medicine, DIGENIA & EDIMO, Institute of Molecular Biology and Biotechnology of the Foundation for Research and Technology Hellas (IMMB-FORTH), Heraklion, Crete, Greece.

INTRODUCTION: Paragangliomas are neuroendocrine tumours of the sympathetic or parasympathetic ganglia that have a strong genetic predisposition. Mutations of the succinate dehydrogenase (SDHx) genes are most commonly involved and transmitted in an autosomal dominant manner.

OBJECTIVE: A 23-year-old female patient was referred to our department for a palpable mass in the neck. Magnetic resonance imaging (MRI) revealed a 37x17x36 mm lesion involving the right carotid space, while the 68-Gallium-DOTATATE scan unveiled an enhancement of the mass (SUV_{max}=18, SUV_{liver}=7). Urine and plasma metanephrines/normetanephrines levels were negative for catecholamine excess. The lesion was removed surgically, and the histology report confirmed the presence of a right carotid body paraganglioma.

METHODS: Germline testing was pursued through a 50-cancer gene panel following genetic counselling.

RESULTS: Genetic testing revealed a *SDHC* likely pathogenic variant (c.2T>G p.Met1?) in a heterozygous state, causing a substitution in exon 1 of the *SDHC* gene, which disrupts the initiation (start) site of the coding for the *SDHC* RNA that probably leads to not expressing the corresponding protein from the mutant allele, consistent with *SDHC* haploinsufficiency. This specific variation (chr1:161284197) has not been referred before, however, five other variants in the same coding area are listed as pathogenic and related to paragangliomas. This patient was also a carrier of an *SPG11* gene mutation (c.1471-1472delCT) which is associated with hereditary spastic paraplegia (HSP) in the homozygous states. Currently, no direct link has been found between these two gene mutations, as the first involves disruption of central to mitochondrial energy metabolism and the latter disorders in axonal maintenance and endosomal-lysosomal transport of neurons. The genetic analysis of the parents is pending.

CONCLUSIONS: Genetic analysis plays a vital role in the diagnosis and management of patients with paragangliomas. Clinically relevant variants can not only help establish genotype-phenotype correlations but also may identify asymptomatic family members.

Familial bilateral macronodular adrenocortical disease with multicentric renal cell carcinoma showing double hit ARMC5 mutations

Cardili L, Silva JTC, Ledesma FL, Chambo JL, Almeida MQ, Hoff A, Mendonca BB, Bertherat J, Fragoso MCBV

(1) Leonardo Cardili, MD, PhD. Department of Pathology, Instituto do Câncer do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brasil. Email: leonardo.cardili@hc.fm.usp.br
(2) João Tomás de Camargo Silva, Department of Endocrinology, Laboratorio de Hormonios e Genética Molecular, LIM42, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brasil
(3) Felipe Lourenco Ledesma, MD, PhD. Department of Pathology, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brasil
(4) Jose Luiz Chambo, MD, PhD. Department of Urology, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brasil
(5) Madson Queiroz de Almeida, MD, PhD. Department of Endocrinology Hospital das Clínicas and Department Of Clinical Oncology Instituto do Cancer da Faculdade de Medicina da Universidade de São Paulo, Brasil.
(6) Ana Hoff, MD PhD. Department of Endocrinology Instituto do Câncer do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brasil.
(7) Wilian Nahas, MD, PhD. Department of Urology, Instituto do Câncer do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brasil.
(8) Berenice Bilharinho Mendonca, MD, PhD. Department of Endocrinology Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Brasil.
(9) Jerome Bertherat, MD, PhD, Department of Endocrinology and Center for Rare Adrenal Diseases, Hôpital Cochin, Assistance Publique Hôpitaux de Paris, Paris, France.
(10) Maria Cândida Barison Villares Fragoso, MD, PhD. Department of Endocrinology Hospital das Clínicas and Department Of Clinical Oncology Instituto do Cancer da Faculdade de Medicina da Universidade de São Paulo, Brasil. Email: maria.villares@hc.fm.usp.br

Background: ARMC5 is a tumor suppressor gene implicated in the pathogenesis of several proliferative adrenocortical disorders, including Bilateral Macronodular Adrenocortical Disease (BMAD). Germline ARMC5 mutations are found in more than half familial BMAD cases, and are associated to higher risk for solid tumors, such as meningioma. Concurrent RCC have not been reported to date.
Clinical Case: A 47-year-old white man presented a nodule in the right kidney incidentally found during radiological follow up after bilateral adrenalectomy performed two years earlier due to Bilateral Macronodular Adrenocortical Disease (BMAD). During a decade-long follow up, ensuing CT scans of abdominopelvic region revealed the arise of new multiple hypovascularized solid lesions throughout both kidneys, the largest measuring 3.1 cm. No signs of metastatic disease were found to date. Multicentricity and bilaterality are strong markers of genetic predisposition in Renal Cell Carcinoma (RCC). We have assessed three surgical specimens of these kidney tumors resected due to progressive growth. All tumors presented similar histopathological features, non-usual for clear cell RCC, remarkably the leiomiomatous stroma. We were able to find ARMC5 mutations in both germline and tumor somatic samples. In
germline samples (peripheral blood and adrenal gland direct primary culture), we detected a pathogenic variant in exon 6 (c.2423A>C, p.H808P). In somatic samples (formalin-fixed, paraffin-embbeded RCC tumor tissue), we also found genomic alterations in ARMC5, including pathogenic variants in exon 3 (c.1090C>T, p.R364*) and exon 6 (c.2047_2048insAGTA, p.L683Qfs*56). Our findings expand the spectrum of solid tumors reported alongwith familial ARMC5-related BMAD, and endorses the need for close monitoring and extended clinical follow up for carriers due to possible higher risk for development of non-adrenocortical neoplasms.
Conclusion: This is the first case report showing both germline and somatic ARMC5 mutations in familial BMAD with multicentric and bilateral RCC.

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Histological Differences Between Adrenal Cushing's Syndrome and MACS with regards to Steroidogenic Enzymes and Cell Morphology

Dimitra Sinni[1], Sofia Avgousti[2], Panagiotis Karageorgiou[1], Marinela Tzanela[1,] Celso E. Gomez-Sanchez[3], Georgios Kyriakopoulos[2], Dimitra A Vassiliadi[1],

[1]Department of Endocrinology "D. Ikkos"- Diabetes Center- EndoERN-Evangelismos General Hospital, Athens, Greece,
[2]Department of Pathology, Evangelismos General Hospital, Athens, Greece
[3]Endocrine Section, G.V. (Sonny) Montgomery VA Medical Center, USA, Endocrinology, University of Mississippi Medical Center, USA

Introduction: Mild autonomous cortisol secretion (MACS) and overt Cushing syndrome (CS) represent a spectrum of adrenal cortisol excess. While both conditions share hormonal features, progression from MACS to overt CS is uncommon, suggesting distinct underlying regulatory and pathological mechanisms. To better characterize these differences, we investigated adrenal tumor morphology and steroidogenic enzyme expression in relation to clinical and biochemical phenotypes.

Objective: To compare adrenal tumor cell morphology and immunohistochemical expression of key steroidogenic enzymes (CYP11B1, HSD3B2, and CYP17A1) in patients with MACS and overt Cushing syndrome, and to assess their associations with clinical and hormonal profiles.

Methods: Adrenal tissues from patients with clinically and biochemically confirmed MACS or CS who underwent adrenalectomy were retrospectively analyzed. Histopathological evaluation included quantification of compact versus clear cells. Immunohistochemical staining for CYP11B1, HSD3B2, and CYP17A1 was performed. Findings were correlated with clinical presentation, cortisol levels, and dexamethasone suppression test results.

Results: Tumors from patients with overt CS demonstrated a higher proportion of compact cells, while clear cell predominance was more common in MACS. CYP11B1 expression was significantly higher in CS-associated tumors. CYP17A1 showed a trend toward increased expression in cortisol-secreting adenomas overall. HSD3B2 was strongly expressed across all samples, with no significant difference between groups.

Conclusion: Adrenocortical tumors associated with MACS and CS exhibit distinct histopathological and enzymatic profiles that align with clinical severity. These findings highlight the relevance of tumor cell composition and steroidogenic enzyme activity in modulating cortisol secretion, and support the utility of morphological and immunohistochemical characterization in the diagnostic and pathophysiological assessment of cortisol-producing adrenal lesions.

Refining Therapeutic Timing and Fertility Strategies in Female Patients with Adrenocortical Carcinoma

Antoan Stefan Šojat[1,2], Miomira Ivović[1,2], Milan Jovanović[2,3,] Natalija Antić[1], Kristina Saravinovska[1,2], Svetlana Vujović[1,2], Ljiljana Marina[1,2]

[1] Clinic of Endocrinology, Diabetes and Metabolic Diseases, University Clinical Centre of Serbia, Belgrade, Serbia
[2] Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[3] Clinic of Endocrine Surgery, University Clinical Centre of Serbia, Belgrade, Serbia

A 25-year-old woman was admitted for post-operative staging following right adrenalectomy for adrenocortical carcinoma (ACC). She had been referred from a regional hospital after incidental discovery of a large adrenal mass during routine follow-up for a sleeve gastrectomy performed abroad. Subsequent CT imaging revealed a 52 × 46 × 42 mm hyperintense right adrenal lesion with suspected extension into the adrenal vein. Hormonal evaluation excluded excess secretion, and retroperitoneoscopic adrenalectomy was completed. Histopathological analysis confirmed ACC (pT2NxMx, Ki-67 = 5%, mitotic rate: 10/50 HPF, Weiss score 4, ENSAT stage 3). Due to personal circumstances, staging was delayed until six weeks postoperatively. Biochemical and radiologic restaging were unremarkable. With a focus on fertility preservation, cryopreservation was performed for the first time in Serbia through collaboration with an oncofertility team, although the procedure was complicated by ovarian hyperstimulation syndrome, which was effectively managed. Following multidisciplinary review, adjuvant mitotane therapy was not initiated as the three-month postoperative threshold had been surpassed. The patient remains clinically stable under surveillance.

Conclusion

This case underscores the clinical complexities in managing ACC in young women, highlighting the need for refined classification algorithms for borderline cases, improved coordination for timely fertility preservation, and reconsideration of criteria for adjuvant mitotane initiation. It contributes to evolving frameworks for balancing oncologic efficacy with reproductive outcomes in female patients.

A rare cause of bilateral adrenal masses

Souteiro Pedro, Gil-Santos Sara, Calheiros Raquel, Oliveira Joana, Inácio Isabel, Santos Ana Paula

[1]Endocrinology Department, Portuguese Institute of Oncology of Porto (IPO Porto) / Porto Comprehensive Cancer Center (P.CCC), Porto, Portugal

Introduction: Bilateral adrenal masses can be caused by metastatic disease, adrenal congenital hyperplasia, primary tumors or infections.

Clinical case: 47-year-old woman, without relevant past medical history. Due to persistent fever and fatigue an abdominal ultrasound was requested showing bilateral adrenal masses. The abdominal CT confirmed the presence of large bilateral lesions (129x63mm on the right and 113x63mm on the left) with a non-adenoma density. The patient was then referred to Endocrinology clinic, where she presented no signs or symptoms suggestive of pheochromocytoma, hypercortisolism or hyperandrogenism and physical examination showed only cutaneous hyperpigmentation. Bloodwork revealed: ACTH 1964 (7.2-63.3) pg/mL, plasma cortisol 6.03 (5.0-25.0) ug/dL, immeasurable levels of testosterone and estradiol and normal urinary metanephrines, 17-OH progesterone, sodium and potassium. Due to impending primary adrenal insufficiency, she was started on 20mg of hydrocortisone daily. At that time, adrenal metastasis of a primary occult tumor was the main diagnostic hypothesis and so an ^{18}F-FDP-PET/CT was performed, showing bilateral malignant involvement of the adrenal glands as well as pelvic and axillary lymph node metastasis but no primary tumor. Considering this, the absence of hormonal hypersecretion, and taking into account the rarity of bilateral adrenal carcinomas, primary adrenal lymphoma was considered as a possible diagnosis. Additional bloodwork to pursue that hypothesis showed elevated β2-microglobulin [6.03 (<3) ug/dL], LDH [421 (67-248) U/L] and ferritin [293 (10-120) ug/L] suggesting and hematological neoplasia. After a first non-diagnostic histology, a second left adrenal biopsy confirmed a large cell diffuse B lymphoma. The patient was started on chemotherapy, with significant clinical improvement, but no adrenal functional recovery, being still supplemented with hidro- and fludrocortisone.

Conclusion: Although rare (1% of the cases of non-Hodgkin lymphoma), primary adrenal lymphoma should not be overlooked as a possible cause of bilateral adrenal masses with suspicious characteristics on imaging studies.

Functional Oncocytic Adrenal Adenoma Presenting with Cushing's Syndrome: A Case Report

Suchmiel Mateusz[1], Rzepka Ewelina[2], Hubalewska- Dydejczyk Alicja[2]

1 Department of Endocrinology, Oncological Endocrinology, Nuclear Medicine and Internal Medicine, University Hospital in Krakow, Poland
2 Chair and Department of Endocrinology, Jagiellonian University School of Medicine, Krakow, Poland

Oncocytic adrenal adenomas are rare neoplasms, often non-functioning, characterized by cells with eosinophilic, granular cytoplasm due to increased mitochondrial content. Functional variants, particularly those causing Cushing's syndrome, are rare and diagnostically challenging.

A 41-year-old female was admitted to the Department of Endocrinology for evaluation of a right adrenal incidentaloma detected on abdominal CT, measuring 30x28x26 mm. Physical examination revealed clinical signs of hypercortisolism, including central obesity, supraclavicular fat accumulation, facial rounding, lower limb edema, and purple striae on the abdomen. Laboratory investigations showed elevated late-night salivary cortisol, increased urinary free cortisol excretion, suppressed ACTH and DHEA-S levels, and lack of cortisol suppression on the 1 mg overnight dexamethasone suppression test, consistent with ACTH-independent Cushing's syndrome.

The patient underwent laparoscopic adrenalectomy without complications. Histopathological examination revealed an adrenocortical tumor with an oncocytic appearance, marked nuclear pleomorphism, and positive immunohistochemistry for MelanA. The tumor was not classified as malignant, with a Ki-67 proliferation index of 4%. Although the tumor was completely excised, capsular disruption was noted.

Oncocytic adrenal adenomas are rare lesions that can occasionally present with hormonal activity, leading to conditions such as Cushing's syndrome. This case emphasizes the importance of a comprehensive diagnostic approach, including endocrine evaluation, imaging, and detailed histopathological assessment. Laparoscopic adrenalectomy remains the treatment of choice for functioning adrenal tumors. Long-term follow-up is recommended, particularly in cases with atypical features such as capsular disruption, elevated proliferative index, or associated comorbidities like osteoporosis and anemia, due to the uncertain biological behavior of some oncocytic neoplasms.

Determination of plasma neuropeptide INSM-1 levels in adrenal tumors: a preliminary report

Szatko Alicja*[1,2], Leszczyńska Dorota[2], Ambroziak Urszula[3], Bobrowicz Małgorzata[3], Ostrowska Magdalena[1,2], Łomiak Michał[2], Zgliczyński Wojciech[2], Glinicki Piotr[1,2]

[1]Centre of Postgraduate Medical Eduction, EndoLab Laboratory, Warsaw, Poland
[2]Centre of Postgraduate Medical Eduction, Department of Endocrinology, Warsaw, Poland
[3]Medical University of Warsaw, Department of Internal Medicine and Endocrinology, Warsaw, Poland

Introduction
Adrenal tumours are often unexpected findings on radiological examinations and are then referred to as incidentalomas. In recent years, the incidence of adrenal incidentalomas has increased tenfold, paralleling the rise in the number of imaging studies performed. The diagnostic algorithm for adrenal incidentalomas involves the integration of clinical, laboratory, and imaging findings. However, in many cases, routine diagnostics are not sufficient to accurately determine the type of adrenal neoplasm.
INSM-1 (Insulinoma-associated protein 1) is a neuropeptide produced by endocrine and neuroendocrine cells, with potential applications in oncological endocrinology.
Aim of the study
The aim of this study was to evaluate the usefulness of INSM-1 in the differential diagnosis of adrenal tumors and extra-adrenal paragangliomas (PGLs).
Materials and methods
The study included 104 patients with adrenal tumors and PGL (mean age 52 years, 94 females): 30 mild autonomous cortisol secretion (MACS), 12 Cushing syndrome (CS), 3 adrenocortical cancer (ACC), 9 primary aldosteronism (PA), 10 myelolipoma, 7 pheochromocytoma (PHEO), 30 nonfunctional adenomas (NFAA) and 3 paragangliomas (PGL). Diagnosis of individual tumors was made based on the current ECE / ENSAT guidelines. Plasma INSM-1 levels were determined using ELISA immunoassay in all subjects.
Results
In the studied groups (MACS, CS, ACC, PA, myelolipoma, PHEO and PGL), the sensitivity and specificity rates of the biomarker INSM-1 were determined. In MACS, the sensitivity and specificity rates were 77% and 43%, respectively, while in CS they were 58% and 63%. In PA, the sensitivity and specificity of INSM-1 were 56% and 71%, while in ACC: 67% and 53%. In catecholamine-secreting tumours, the respective sensitivity and specificity rates were: PHEO 57% and 77%, while in PGL they were 67% and 60%. In myelolipoma, both rates were: 50% and 57%.
Conclusion
In the preliminary study, INSM-1 demonstrated moderate usefulness in distinguishing adrenal tumors (MACS, ACC) and PGL.

Effects of progesterone on microRNA expression in ACC cell lines

Szeredás Bálint Kende[1,2‡], Nyirő Gábor[1,2,3‡], Abate Andrea[4], Fassi Marco[4], Tamburello Mariangela[4], Vékony Bálint[1,2], Hantel Constanze[5,6], Berruti Alfredo[7], Igaz Péter[1,2*], Sigala Sandra[4*]

‡ These two authors contributed equally to this work
*These two authors should both be regarded as last authors
[1] Department of Endocrinology, Faculty of Medicine, Semmelweis University, Budapest, Hungary
[2] Department of Internal Medicine and Oncology, Faculty of Medicine, Semmelweis University, Budapest, Hungary
[3] Department of Laboratory Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary
[4] Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy
[5] Department of Endocrinology, Diabetology and Clinical Nutrition, University Hospital Zurich (USZ) and University of Zurich (UZH), Zurich, CH-8006, Switzerland.
[6] Medizinische Klinik und Poliklinik III, University Hospital Carl Gustav Carus Dresden, 01307, Dresden, Germany.
[7] Medical Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, ASST Spedali Civili, Brescia 25123, Italy

Introduction: Adrenocortical carcinoma (ACC) is a rare and aggressive tumor, which is hormone-producing in at least 50% of cases. Its prognosis is poor, with a 5-year survival rate below 35% in advanced cases. The only adrenal cortex-specific drug available for treatment is mitotane, but its use is challenging due to its toxicity and narrow therapeutic index. Among new potential therapeutic agents, the hormone progesterone has emerged, previously described as having antitumor effects.

Aim: We aimed to explore the effect of progesterone on microRNA expression in human adrenocortical carcinoma cell lines in vitro.

Method: We studied three cell lines (MUC1, TVBF-7, NCI-H295R). RNA was isolated from both cell pellets and the supernatants, and changes in the microRNA profile were examined using Qiagen miRNAseq next-generation sequencing on an Illumina MiSeq sequencer. Among the microRNAs showing significant changes, three were selected for further validation by quantitative RT-qPCR: *hsa-miR-424-5p* (MUC1), *hsa-miR-98-5p* (TVBF-7), and *hsa-miR-7-5p* (NCI-H295R). DeltaCT values from the RT-qPCR data of treated and untreated cells and supernatants were normalized to the geometric mean of RNU48 and cel-miR-39. Statistical analysis was performed by t-test with Welch's correction.

Results: According to the sequencing data, *hsa-miR-424-5p* in the MUC1 cell line and *hsa-miR-98-5p* in the TVBF-7 cell line showed significantly increased expression, while *hsa-miR-7-5p* in the NCI-H295R cell line showed significantly decreased expression after progesterone treatment both in the cell pellets and in the supernatants. These results were confirmed by RT-qPCR validation, except for the supernatant of the TVBF-7 line (*miR-424* in supernatant: p=0.05; *miR-424* in pellet: p=0.003; *miR-98* in supernatant: p=0.303; *miR-98* in pellet: p=0.0002; *miR-7* in supernatant: p<0.0001; *miR-7* in pellet: p=0.022).

Conclusions: There are some data on the potential oncogenic or tumor-suppressing roles of the miRNAs examined, but further studies are needed to uncover their pathophysiological relevance.

Preclinical Investigation of FGF/FGFR Pathway Inhibition in Adrenocortical Carcinoma

Tamburello Mariangela [1,2], Kappenstein Lena [1], Paul Alexander [1], Altieri Barbara [1], Landwehr Laura-Sophie [1], Sigala Sandra [2], Berruti Alfredo[3], Kiseljak-Vassiliades Katja [4], Fassnacht Martin [1], Sbiera Silviu [1]

1 Division of Endocrinology, Department of Internal Medicine I, University Hospital, University of Würzburg, Würzburg, Germany
2 Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy
3 Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia and ASST Spedali Civili di Brescia, Brescia, Italy
4 Division of Endocrinology, Metabolism and Diabetes, Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, USA.

Adrenocortical carcinoma (ACC) is a rare but aggressive endocrine malignancy with limited treatment options. The Fibroblast Growth Factor/Fibroblast Growth Factor Receptor (FGF/FGFR) signaling axis, known for its roles in adrenal development and oncogenesis, has emerged as a potential therapeutic target. Previous work identified FGFR1–4 upregulation in ACC, correlating with poor prognosis. In this study, we investigated the therapeutic relevance of FGFR inhibition in ACC using both 2D monolayers and 3D spheroid cultures across five ACC cell lines, with or without co-cultured adrenal fibroblasts. Selective FGFR inhibitors—erdafitinib (pan-FGFR), rogaratinib (FGFR1–3), and fisogatinib (FGFR4)—were tested to assess effects on cell viability, FGFR isoform expression, signaling activity, and secretion of FGF2 and FGF21.

All ACC models predominantly expressed FGFR1-IIIc, with TVBF-7 uniquely exhibiting high FGFR2 and FGFR4 levels. Erdafitinib and rogaratinib reduced cell viability in a concentration-dependent manner, with TVBF-7 being the most sensitive. Fisogatinib had minimal effects, except in NCI-H295R and JIL-2266. In 3D cultures, FGFR inhibition variably affected spheroid viability; TVBF-7 remained highly responsive, while JIL-2266 showed fibroblast-mediated resistance. FGFR inhibitors modulated receptor isoform expression—TVBF-7 notably upregulated FGFR1 and FGFR3 and downregulated FGFR4. Basal FGFR expression shifted significantly in 3D versus 2D, in a model-specific manner. Immunohistochemistry revealed modest reductions in proliferation and DNA damage markers, with limited apoptosis. FGFR signaling (pFGFR, pERK) was only partially suppressed. Notably, untreated spheroids accumulated FGF2 over time, an effect reversed by FGFR-inhibitors in monocultures but buffered in co-culture, highlighting the influence of stromal components. FGF21 responses were variable across models.

Overall, these findings demonstrate that FGFR signaling supports ACC cell viability and paracrine interactions within the tumor microenvironment. FGFR inhibitors show context-dependent efficacy and influenced by tumor-stroma interactions, underscoring the utility of 3D co-culture systems to evaluate drug responses.

Neuropsychiatric Burden, Frailty, and Quality of Life in Patients with Adrenal Incidentalomas: A Case-Control Study

Tasouli Elisavet-Charikleia[1], Andreadaki Evangelia†[1], Siampanopoulou Vasiliki[1], Mytareli Chrysoula[1], Lafioniatis Alexandros[1], Papalexis Petros[1], Bourgioti Charis[2], Diamantopoulos Ioannis[2], Angelousi Anna[1]

[1] Unit of Endocrinology, First Department of Internal Medicine, Laikon General Hospital, National and Kapodistrian University of Athens, Athens, Greece
[2] Department of Radiology, School of Medicine, National and Kapodistrian University of Athens, Aretaieion Hospital, Athens, Greece

Objective:
Neuropsychiatric manifestations and quality of life (QoL) outcomes in patients with adrenal incidentalomas (AIs) remain incompletely characterized. We aimed to comprehensively evaluate QoL, affective symptoms, and frailty in patients with benign AIs, and to examine the impact of mild cortisol excess on psychological and functional outcomes in the absence of overt hypercortisolism.

Methods:
In this case-control study, we included 120 patients with adrenal incidentalomas (50 with non-functioning adrenal incidentalomas [NFAIs] and 70 with mild autonomous cortisol secretion [MACS]) and 100 age- and sex-matched healthy referent controls. Patients with MACS were stratified by cortisol levels after the 1 mg overnight dexamethasone suppression test (DST): <3 μg/dL and ≥3 μg/dL. Exclusion criteria included active malignancy, glucocorticoid therapy, uncontrolled thyroid disease, and pre-existing neurocognitive disorders.

All participants underwent hormonal evaluation, including baseline and post-DST cortisol, ACTH, plasma renin activity, aldosterone, and adrenal androgens. QoL was assessed using the WHOQOL-BREF (physical, psychological, social, environmental domains). Depressive and anxiety symptoms were evaluated with the Beck Depression Inventory (BDI) and the Depression Anxiety and Stress Scale (DASS-21). Frailty was assessed using a standardized frailty index.

Results:
Among all groups, patients with MACS and post-DST cortisol ≥3 μg/dL exhibited the most significant impairments across all WHOQOL-BREF domains, with the highest levels of depressive symptoms and frailty ($p < 0.001$). Patients with MACS <3 μg/dL exhibited milder but still measurable impairments. Notably, NFAI patients reported significantly lower QoL in the psychological and social domains, comparable depression scores, but increased anxiety and stress levels compared to controls ($p < 0.05$). The highest frailty burden was observed in patients with MACS ≥3 μg/dL.

Conclusion:
Benign adrenal incidentalomas, even in the absence of overt cortisol excess, are associated with reduced QoL, increased psychological burden, and greater frailty. Routine assessment of these parameters is warranted in clinical practice.

Diagnosis of Pheochromocytoma in a patient with recurrent reverse Takotsubo Cardiomyopathy

Foteini Thanasoula[1]*, Foteini Petrea[1], Marina Mitropoulou[1], Ioannis Petropoulos[2], Anna Dimoula[2], Evangelos Repasos[2], Vasiliki Vasileiou[1]

[1]Alexandra General Hospital, Endocrinology Department and Diabetological Centre, Athens, Greece
[2]Alexandra Hospital of Athens, Department of Clinical Therapeutics, Athens, Greece

Background: Background: Pheochromocytomas (PHEO) are rare neuroendocrine tumors that secrete catecholamines, causing serious cardiovascular complications. While typically presenting with hypertension and tachycardia, their association with Takotsubo syndrome (TTS), especially the reverse variant, is less commonly recognized.

Objectives: To increase clinical awareness regarding the association between Takotsubo cardiomyopathy and catecholamine secreting pheochromocytoma.

Case Presentation: A 40-year-old woman with history of reverse TTS presented with palpitations, chest pain and vomiting. On admission, she had hypertensive crisis (BP 220/110 mmHg), sinus tachycardia (HR 120 bpm) and ischemic changes on ECG. Elevated troponin and hyperglycemia were noted. Echocardiography showed hypokinesia of the left ventricle, leading to urgent coronary angiography, which was normal. The patient was treated in the ICU with labetalol and intravenous nitrate, leading to significant improvement, suggesting a possible reverse Takotsubo syndrome. Due to the clinical picture, the patient's young age and medical history, pheochromocytoma was suspected.

Results: A computed tomography (CT) scan revealed a nodular mass in the left adrenal gland with heterogeneous contrast uptake. Hormonal screening confirmed elevated plasma catecholamines levels. No metastases or paragangliomas were found on staging. After 15 days of alpha-blockade with doxazosin, laparoscopic adrenalectomy was performed. Histology confirmed pheochromocytoma (3 cm, PASS score = 1, Ki67 <1%). Genetic testing was negative. Postoperatively, hormonal levels normalized, imaging showed no recurrence and the patient remained asymptomatic without antihypertensive therapy.

Conclusion: Pheochromocytomas can manifest as reverse Takotsubo syndrome due to catecholamine excess. Clinicians should consider pheochromocytoma in young patients with unexplained TTS, as timely diagnosis and treatment are crucial to prevent long-term cardiac damage

The prognostic role of microRNA expression in Pheochromocytomas and Paragangliomas: A Retrospective Analysis

Foteini Thanasoula[1, 5], Maria Yavropoulou[2], Athanasios Papatheodorou[3,] Georgios Kyriakopoulos[4], Vasiliki Vasileiou[5], Eva Kassi[2,6], Gregory Kaltsas[2], Anna Angelousi[1]

[1] 1st Department of Internal Medicine, Unit of Endocrinology, Center of Excellence of Rare Endocrine Diseases (ENDO-ERN), Laikon Hospital, National and Kapodistrian University of Athens, Greece
[2] 1st Department of Propaedeutic and Internal Medicine, Unit of Endocrinology, Center of Excellence of Rare Endocrine Diseases (ENDO-ERN) and Neuroendocrine Tumours (NETs) (EURACAN), Laikon Hospital, National and Kapodistrian University of Athens, Greece
[3] Department of Medical Research,251 Hellenic Air Force & VA General Hospital, Athens, Greece
[4] Department of Pathology, Evangelismos Hospital, Athens, Greece
[5] Endocrinology Department and Diabetological Centre, Alexandra General Hospital, Athens, Greece
[6] Department of Biochemistry, National and Kapodistrian University of Athens, Greece

Introduction: Pheochromocytomas (PHEOs) and paragangliomas (PGLs) are rare neuroendocrine tumors with an overall 5-26% incidence of metastasis. Identifying predictive markers of their potential clinical behavior is challenging.

Objectives: This study aims to investigate the association between the expression of specific miRNAs with the histopathological and clinical parameters of patients with PHEOs/PGLs.

Methods: Clinical data of 48 patients operated for PHEOs/PGLs (n=21 PHEOs, n=27 PGLs) were retrospectively collected. Germline mutations were found in n=10 out of 22 screened patients (n=5 with SDHX, n=1 NF1, n=3 RET, n=1 EPAS). The expression of 5 miRNAs (miRNA15, miRNA16, miRNA101, miRNA183, miRNA483) was assessed using RT-qPCR on 48 formalin-fixed paraffin-embedded tissue samples. Statistical analysis included t-tests for normally distributed data and Mann-Whitney tests for non-normal data.

Results: Median size of the primary tumor was 6.1cm. Median Ki 67% index levels were 3%, median PASS was 6 for PHEOs and GAPP score 4 for PGLs. Metastatic disease was found in 10/48 patients (20.8%). The survival rate was 89.6% during a median follow-up of 79 months. Our analysis revealed that miRNA483 was significantly overexpressed in PHEOs/PGLs with Ki-67 >4% compared to those with Ki-67%<4%(p=0.05). MiRNA483 was also significantly overexpressed in metastatic tumors compared to the nonmetastatic ones (p=0.05), in Head and Neck (HN) PGLs (p=0.03) compared to non-HNPGLs and in patients who were deceased compared to those who survived (p=0.03). MiRNA183 was overexpressed in PHEOs with PASS >4 (p=0.02), while miRNA101 was overexpressed in PHEOs with PASS >6 (p=0.04). MiRNA15 was downregulated in PHEOs/PGLs with tumor size >5cm (p=0.03). Both miRNA15 and miRNA16 were overexpressed in HNPGLs compared to non-HNPGLs (p=0.0001 and p=0.01, respectively).

Conclusions: Our findings suggest that specific microRNAs, such as miRNA483 and miRNA101 could serve as potential biomarkers for assessing metastatic behavior in PHEOs/PGLs. Further studies are needed to validate their clinical utility.

Fluctuations in diurnal plasma cortisol cycle in patients treated with Metyrapone or Osilodrostat for endogenous Cushing Syndrome

Thomeret Louis[a], Bonnet-Serrano Fidéline[a,b], Blondel Juliette[c], Vaduva Patricia[a], Bouys Lucas[a,d], Violon Florian[a,e], Ragazzon Bruno[a], Berthon Annabel[a], Perlemoine Karine[a], Gouttefarde Antonin[b], Noblin Mathéo[b], Zientek Corinne[b], Guibourdenche Jean[b,f], Bricaire Léopoldine[d], Guignat Laurence[d], Libe Rossella[a,d], Groussin Lionel[a,d], Assié Guillaume[a,d], Jouinot Anne[a], Puszkiel Alicja[c,f], Blanchet Benoit[c,f], Bertherat Jérôme[a,d]

[a] Team « Genomic and Signaling of Endocrine Tumors », Institut Cochin, INSERM U1016- CNRS UMR8104-Université Paris-Cité, Paris, France
[b] Hormonology department, Hôpital Cochin, AP-HP, Paris, France
[c] Drug Biology and Toxicology department, Hôpital Cochin, AP-HP, Paris, France
[d] Endocrinology department, Hôpital Cochin, AP-HP, Paris, France
[e] Pathology department, Hôpital Cochin, AP-HP, Paris, France
[f] Université Paris-Cité, Paris, France

Introduction: Steroidogenesis inhibitors represent an efficient pharmacological option in the treatment of endogenous Cushing Syndrome (CS). Metyrapone and Osilodrostat are both 11 beta-hydroxylase inhibitors but impact differently global steroidogenic profile according to previous clinical and *in vitro* studies. The aim of our work was to investigate diurnal fluctuations of plasma steroids concentrations in patients treated by either Metyrapone or Osilodrostat.

Material and Methods: Plasma steroids and drug concentrations diurnal profiles (6 samples a day) were determined using UPLC-tandem mass spectrometry (UPLC-MS/MS) for patients treated with Metyrapone (n=14; median posology 1250mg/day; median treatment duration 246 days) or Osilodrostat (n=21; 10mg/day; 242 days) for various causes of CS, as well as in 14 non-treated patients with CS and 16 controls. Concentrations fluctuations in a 24-hour cycle were expressed by MIN-MAX range (MMR) and Coefficient of Variation (CV, %) (median [10-90 percentiles]).

Results: Cortisol plasma levels were significantly more stable during 24-hour cycle in patients treated with Osilodrostat than Metyrapone (MMR=100.9 nmol/L [7.3-283.8] vs 291.7 [122.5-482.6], *p=0.0002*). This difference was observed whatever hypercortisolism was strongly controlled (normal Urinary Free Cortisol + late-night plasma cortisol < 100nmol/L) or not.11-deoxycortisol plasma fluctuations were also higher in Metyrapone cycles compared to Osilodrostat (MMR=78.2 nmol/L [24.0-327.8] vs 14.5 [0.5-77.8], *p<0.0001)*, and compared to normal cycles and non-treated cycles. In our cohort, Osilodrostat plasma 24-hour concentrations were also more stable than Metyrapone concentrations (CV 51.2% [34.6-107.6] vs. 123.0% [53.8-191.4], *p=0.0002*).

Discussion: Our results show a higher stability in plasma cortisol levels in patients treated by Osilodrostat compared to Metyrapone, as well as for 11-deoxycortisol. These observations could result from differences in pharmacokinetics between the two drugs. An insight into other steroids (precursors and androgens) concentrations and analyses of correlations with pharmacodynamic parameters will be performed.

Paraganglioma Mimicking Thyroid Pathology: A Rare Case Report

Tkacz Edyta[1], Rzepka Ewelina[2], Hubalewska-Dydejczyk Alicja[2]

1 Department of Endocrinology, Oncological Endocrinology, Nuclear Medicine and Internal Medicine, University Hospital, Krakow, Poland
2 Chair and Department of Endocrinology, Jagiellonian University Medical College, Krakow, Poland

We report the case of a 66-year-old female diagnosed with paraganglioma of the thyroid gland. The patient was initially qualified for thyroidectomy due to a suspicious lesion in the left thyroid lobe, identified on ultrasound as potentially malignant.
Preoperatively, the patient did not exhibit hypertension or other symptoms suggestive of hormonal activity. Unintentional weight loss was the only reported symptom was. During thyroid surgery, intraoperative complications occurred, including bleeding and tracheal perforation, necessitating placement of a tracheostomy tube and admission to the Intensive Care Unit.
In initial histopathological examination identified a follicular tumor of uncertain malignant potential (FT-UMP). Re-evaluation of the histological slides revealed a paraganglioma in the left thyroid lobe, with two additional foci outside the main tumor capsule, measuring 9 mm and 5 mm. In immunohistochemical evaluation: chromogranin(+), S100(+ in sustentacular cells), GATA-3(+), calcitonin(-). No histological evidence of necrosis, marked nuclear atypia, elevated mitotic activity, or angioinvasion/neuroinvasion was observed.
Additionally, three microscopic foci of paraganglioma were identified in the tissue samples obtained from the trachea. Postoperative biochemical evaluation, including 24-hour urinary methoxycatecholamines, revealed metabolite levels within normal limits.
Three years following the surgery, a follow-up ^{68}Ga-DOTATATE PET scan showed increased expression of somatostatin receptors in a lesion located in the lower cervical, paratracheal region (Krenning score 4), raising suspicion of local recurrence. The patient is currently awaiting magnetic resonance imaging of the neck for further evaluation.
The patient's medical history includes right-sided breast cancer, surgically treated in 2023, followed by adjuvant letrozole therapy, as well as a diagnosis of chronic lymphocytic leukemia. Genetic testing for RET, SDHB, SDHD, MAX, MEN1, AIP, and VHL gene mutations was negative. A likely pathogenic variant was identified in one allele of the CHEK2 gene.
The patient remains under regular multidisciplinary follow-up at oncology, hematology, and endocrinology clinics.

Tumoral glucocorticoids induce a phagocytic CD68+/CD163+/C1Q+ macrophage phenotype primed for IFNγ-driven CXCL9 secretion

Triebig Alexandra[1], Maier Tanja[1], Schwarzlmueller Paul[1], Atici Sena[2], Kirmaier Martin E.[3], Gottschlich Adrian[3], Riols Fabien[4], Landwehr Laura-Sophie[5], Bechmann Nicole[6], Peitzsch Mirko[6], Paramasivam Nagarajan[7], Haid Mark[4], Theurich Sebastian[3], Fröhling Stefan[8], Glimm Hanno[9], Hübschmann Daniel[10], Fassnacht Martin[5], Reincke Martin[1], Friedmann-Angeli Jose Pedro[2], Weigand Isabel[1], Kroiss Matthias[1]

(1) Department of Internal Medicine IV, LMU University Hospital, LMU Munich, Munich, Germany
(2) Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Wuerzburg, Germany
(3) Department of Medicine III, LMU University Hospital, LMU Munich, Munich, Germany
(4) Metabolomics and Proteomics Core, Helmholtz Zentrum München, Munich, Germany
(5) Department of Internal Medicine I, Division of Endocrinology and Diabetes, University Hospital, University of Würzburg, Würzburg, Germany
(6) Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Carl Gustav Carus, Medical Faculty Carl Gustav Carus, Technische Universität Dresden
(7) Computational Oncology Group, Molecular Precision Oncology Program, National Center for Tumor Diseases (NCT), NCT Heidelberg, a partnership between the German Cancer Research Center (DKFZ) and Heidelberg University Hospital, Heidelberg, Germany
(8) Division of Translational Medical Oncology, German Cancer Research Center (DKFZ), Heidelberg, Germany
(9) Translational Medical Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, TUD Dresden University of Technology, Dresden, Germany
(10) Pattern Recognition and Digital Medicine Group, Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM GmbH), Heidelberg, Germany

Objective: Glucocorticoids (GCs) play a complex and multifaceted role in modulating the immune system in cancer. Adrenocortical carcinoma (ACC) produces an excess of GCs in approximately 60 % of cases serving as a valuable model to explore intra-tumoural GC activity. While our current understanding of the tumor immune microenvironment in ACC has advanced, the functional role of tumor-associated macrophages (TAMs) and their responsiveness to GCs still remain poorly defined in this context.
Methods: We here conducted tissue metabolomics and steroid hormone profiling on frozen ACC tumour samples and cell culture supernatants. Tissue immunohistochemistry and immunofluorescence microscopy was used to assess tumoural macrophages. Human PBMC derived monocytes and the monocyte cell line THP-1 was polarized *in vitro* and characterized using Nanostring nCounter RNA analysis, western blotting, and ELISA. Phagocytic activity of MΦ was measured via flow cytometry. CXCL9 was measured in plasma of ACC patients before and after the initiation of anti-PD1 immunotherapy by ELISA.
Results: The ACC tumour microenvironment is densely populated with $CD68^+/CD163^+$ "M2-like" MΦ irrespective of tumoural cortisol secretion. *In vitro*, human monocytes exposed to conditioned medium (CM) from both steroidogenic and non-steroidogenic ACC cells expressed markers indicative of an "M2-like" polarization. Exposure to GCs (dexamethasone, hydrocortisone; steroidogenic ACC cell CM) induced a $C1Q^+$ MΦ phenotype, marked by enhanced C1Q dependent phagocytic activity and increased CXCL9 secretion upon IFNγ stimulation. The presence of $C1Q^+$ MΦ was correlated with increased T cell infiltration and improved overall and progression-free survival in ACC patients. In ACC patients receiving PD-1 inhibitor therapy, plasma CXCL9 concentrations were significantly increased after the initiation of immunotherapy compared to baseline, especially in those with a higher percentage of intratumoral macrophages.
Conclusion: Tumoural GC production promotes the polarization of MΦ towards a phagocytic $CD68^+/CD163^+/C1Q^+$ subset. Their high phagocytic capacity and IFNγ induced cytokine secretion may enhance T cell recruitment and support immunotherapy efficacy in ACC.

Radiogenomics pilot study in adrenocortical carcinoma: assessing the relationship between genetic background and computerized tomography texture

Tucci Lorenzo [1,2,3,] Vara Giulio[4,5], De Leo Antonio[1,6], Skordilis Kassiani[7], James Lisa[7], Mosconi Cristina [8], Dhesi Balraj[9], De Biase Dario[10], Lippert Juliane[11,12], Mohamed Abubaker [7], Di Dalmazi Guido [1,2], Ronchi Cristina L.[3,13]

1 Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy
2 Division of Endocrinology and Diabetes Prevention and Care, Sant'Orsola Malpighi Polyclinic, Bologna, Italy
3 Department of Metabolism and System Sciences, University of Birmingham, Birmingham, United Kingdom
4 Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy
5 Casa di cura madre fortunata Toniolo, Radiology Unit, Bologna, Italy
6 Solid Tumor Molecular Pathology Laboratory, Sant'Orsola Malpighi Polyclinic, Bologna, Italy
7 Department of Histopathology, Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom
8 Radiology Unit, Sant'Orsola Malpighi Polyclinic, Bologna, Italy
9 Department of Radiology, Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom
10 Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy
11 Division of Endocrinology and Diabetes, Department of Medicine, University Hospital, University of Wuerzburg, Wuerzburg, Germany
12 Institute of Human Genetics, University of Wuerzburg, Wuerzburg, Germany
13 Department of Endocrinology, Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom

Background. Some somatic mutations have prognostic relevance in adrenocortical carcinoma (ACC). Radiomics has been explored for mutation prediction but not in ACC.

Aims. 1. To predict the presence of key pathogenic gene variants in ACC with radiomics. 2. To compare the ability of radiomics vs β-catenin nuclear staining (as reported from two previous papers) to predict mutations in β-catenin pathway genes (*CTNNB1, ZNRF3, APC*).

Methods. We enrolled 46 patients with ACC (32 females) from Birmingham (UK) or Bologna (Italy). Targeted Next-Generation Sequencing was performed on DNA extracted from formalin-fixed paraffin-embed samples using a customised panel with 10 ACC-specific genes. Radiomics was performed on portal-phase computerized tomography. We formulated deep learning model (DLM) to predict overall presence of variants and logistic regression models (LRM) to predict mutations in β-catenin pathway genes, tumour suppressor genes (*TP53, RB1, CDK4*), chromatin remodeling genes (*MEN1, TERT*), *NF1* and *ATM*. All models were based on radiomics features. Only pathogenic/likely pathogenic variants (P/LPv) and variants of unknown significance (VUS) were included in the analysis.

Results. P/LPv and VUS were detected in 26 cases (56.5%), including β-catenin pathway genes (n=9 (19.6%): *CTNNB1*=7 (15.2%), *ZNRF3*=2 (4.3%), *APC*=1 (2.2%)), tumour suppressor genes (n=14 (30.4%): *TP53* =12 (26.1%), *RB1*=2 (4.3%)), *MEN1*=4 (8.7%), *TERT*=3 (6.5%), *NF1* (n=8 (17.4%)). DLM predicted overall presence of variants with F1-score=0.70. LRM predicted P/LPv and VUS in β-catenin pathway genes overall (F1-score=0.6), and in *CTNNB1* (F1-score=0.55) *TP53* (F1-score=0.64), chromatin remodeling genes (F1-score=0.66), and *MEN1* (F1-score=0.5), specifically. In previous publications, β-catenin nuclear staining showed F1-score=0.52, F1-score=0.42 in predicting *CTNNB1* mutations, and F1-score=0.73, F1-score=0.45 in predicting mutations in β-catenin pathway genes.

Conclusions. Radiomics could be useful for early recognition of P/LPv and VUS in ACC and could be an alternative to β-catenin nuclear staining. Further studies on wider cohort are required to improve current findings.

Measurement of circulating cell-free DNA concentrations for differential diagnosis of adrenal masses: a pilot study

Tucci Lorenzo[1,2,3], Crastin Ana[1], Onesimu Ezra Leander Santhosh David [1], Prete Alessandro [1,4,5], Asia Miriam[5], Elhassan Yasir S. [5], Chortis Vasileios[1,5], Di Dalmazi Guido[2,3], Ronchi Cristina L.[1,5]

1 Department of Metabolism and Systems Science, University of Birmingham, Birmingham, United Kingdom
2 Medical and Surgical Sciences Department, Alma Mater Studiorum University of Bologna, Bologna, Italy
3 Endocrinology and Diabetes Prevention and Care Unit, IRCCS Sant'Orsola-Malpighi Polyclinic, Bologna, Italy
4 NIHR Birmingham Biomedical Research Centre, University of Birmingham and University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom
5 Department of Endocrinology, Queen Elizabeth Hospital Birmingham NHS Trust, Birmingham, United Kingdom

Background. Circulating cell-free DNA concentrations (ccfDNA-C) are higher in patients with adrenocortical carcinoma (ACC) compared to healthy subjects (HS). However, ccfDNA-C have not been compared among different types of adrenal masses (AM).

Objectives. To assess the usefulness of ccfDNA-C for AM differentiation.

Methods. We enrolled 110 adult patients (66 women) with a diagnosis of adrenocortical adenoma (ACA, n=64), other benign AM (OB, n=9), ACC (n=19), pheochromocytoma (n=8) or adrenal metastases from other cancers (MET, n=10). Blood samples, clinical, hormonal and radiological data were collected at first referral. AM with indeterminate radiology in plain computerised tomography and with adrenal hormone secretion not conclusive for a definite diagnosis were labelled as 'undefined AM' (n=46/110, 22 ACA, 5 ACC, 9 OB, 10 MET). ccfDNA was isolated using Cell3™ Xtract kit (Nonacus) and ccfDNA-C were measured by fluorometry. Age, sex and tumour size-adjusted univariate analysis was conducted to assess ccfDNA-C distribution among groups. We tested the diagnostic performance of our previously published HS-derived ccfDNA-C cut-off (>0.146 ng/μL) with logistic regression, positive (PPV) and negative predictive value (NPV) for ACC recognition.

Results. ACC showed higher ccfDNA-C compared to other groups, both in the entire cohort (p=0.007 vs ACA, p=0.001 vs OB, p=0.002 vs MET, p=0.018 vs pheochromocytoma) and in the undefined AM cohort (p<0.001 vs ACA, OB and MET). In the entire cohort, the ccfDNA-C HS-derived cut-off showed an odds ratio of 11.8 to predict ACC (95% confidence interval: 3.5-39.2; p<0.001). This cut-off predicted ACC with PPV=41.7% and NPV=95.8% in the entire cohort and with PPV=41.7% and NPV=100% in the undefined AM cohort. Through receiver-operating characteristic curve analysis, a new cut-off (≥0.193 ng/μL) was identified, with similar diagnostic performances over the entire cohort (sensitivity=73.7%, specificity=83.5%, PPV=48.3%, NPV=93.8%).

Conclusions. High ccfDNA-C in AM is ACC-specific, and our ccfDNA-C HS-derived cut-off is useful for ACC discrimination.

Delayed diagnosis of pheochromocytoma after takotsubo cardiomyopathy

Tywanek Ewa [1], Cabanek Agnieszka [1], Zwolak Agnieszka [1,2]

1-Center of Oncology of the Lublin Region St. Jana z Dukli in Lublin, Lublin, Poland
2- Department of Internal Medicine and Internal Medicine in Nursing, Medical University of Lublin, Lublin, Poland

Pheochromocytoma is a rare neuroendocrine tumor which typically presents with hypertension and heart rhythm disturbances. Takotsubo cardiomyopathy is a stress-induced non-ischemic heart failure that develops in response to an intense emotional or physical experience.
Extremely rare, mentioned above ,,the broken heart syndrome" may be caused by catecholamine secretion by the tumor arising from cells of the adrenal medulla.

We would like to report the case of a patient diagnosed with tacotsubo cardiomyopathy and delayed by a few years diagnosis of pheochromocytoma of the right adrenal gland.

A 50-year-old female patient with unstable hypertension and right adrenal gland tumor was admitted to Endocrinology Department for diagnosis and determining the further course of clinical and therapeutic procedures. 7 years earlier the patient was hospitalized in Cardiology Department due to hypertensive crisis and chest pain. Due to negative results of coronary angiography and akinesia of the cardiac apex confirmed in echocardiography, tacotsubo cardiomyopathy was diagnosed. At that time, CT scanning of the abdomen revealed well-demarcated right adrenal mass with dimensions of 40x30x35mm with basal density of 30-40HU. Hormonal assessment wasn't performed then. Endocrinology consultation was obtained after the suggestion of enlargement of adrenal tumor. Diagnosis revealed very high serum methoxycatecholamines levels. After pharmacological preparation with alpha- and beta-blockers, the patient was referred to right adrenalectomy. The operation was complicated by cardiorespiratory failure requiring treatment in an intensive care unit.

Pheochromocytoma is a rare neuroendocrine tumor, which may result in life threatening conditions such as hypertensive crisis or takotsubo cardiomyopathy.
Adrenal tumors, especially with an ambiguous imaging phenotype, should undergo direct clinical and biochemical assessment.

Constitutional duplication of *PRKACA* causes Primary Pigmented Nodular Adrenocortical Disease (PPNAD) and generates new chromatin interactions

Vaduva Patricia 1 (Dr), Violon Florian 1 (Dr), Chansavang Albain 2-3 (Dr), Hadjadj Djihad 3 (Dr), Raverot Gerald 4 (Pr), Espiard Stephanie (Dr), Attia Aminata 1 (Mrs), Bouys Lucas 1 (Dr), Thomeret Louis 1 (Dr), Weber Kuhn Aliny 7 (Dr), Perlemoine Karine 1 (Mrs), Chevalier Nicolas 8 (Pr), Vantyghem Marie-Christine 5 (Pr), Polak Michel 9 (Pr), Fragoso Maria 7 (Dr), Berthon Annabel 1 (Dr), Ragazzon Bruno 1 (Dr), Jouinot Anne 1 (Dr), Pasmant Eric 2 (Pr), Bertherat Jerome 1,6 (Pr)

1. Genomic and signaling of endocrine tumors team, INSERM U1016, CNRS UMR8104, Cochin Institute, Paris Cité University, 75005 Paris, France
2. Department of genomic medicine of tumors and cancers, Cochin Hospital, APHP, 75014 Paris, France
3. Genomic and epigenetics of rare tumors team, INSERM U1016, CNRS UMR8104, Cochin Institute, Paris Cité University, 75005 Paris, France
4. Department of Endocrinology, Groupement Hospitalier Est, Hospices Civils de Lyon, 69677 Bron, France
5. Department of Endocrinology, Diabetology, Metabolism and Nutrition, Lille University Hospital, 59000 Lille, France
6. Department of Endocrinology, Reference center for rare adrenal diseases, Cochin Hospital, APHP, 75014 Paris, France
7. Department of Endocrinology, Adrenal Unit, University of Sao Paulo, Sao Paulo, Brazil
8. Department of Endocrinology, Diabetology and Reproductive medicine, Nice University Hospital, 06200 Nice, France
9. Department of Endocrinology, Gynecology and Pediatric diabetology, Necker Hospital, APHP, Paris, France

Objective: *PRKACA* constitutional duplications have been described in rare cases of Cushing's syndrome due to bilateral nodular adrenocortical diseases (BNAD). The objective here was to evaluate the results of their systematic screening in BNAD and to specify the associated phenotype.
Methods: Between 2020 and 2024, 781 BNAD index cases with bilateral macronodular adrenal disease (BMAD) (n=693) or primary pigmented nodular adrenocortical disease (PPNAD) (n=88) were genotyped with a targeted Next Generation Sequencing (NGS) panel including the exonic and intronic flanking regions of *ARMC5,KDM1A,MEN1,PRKAR1A* and *PRKACA*, or by Whole Genome Sequencing(WGS). Familial screening was offered to relatives. *In situ* Hi-C libraries were generated from three patients' tumors and chromatin conformation analysis were performed.
Results: *PRKACA* constitutional duplications were identified in 8 index cases and 7/11 screened relatives, supporting the involvement of the *PRKACA* oncogene through a constitutional copy gain mechanism. The WGS performed on 4 index cases did not find any other gene involved in human pathology in the duplicated region, nor any other alteration in genes implicated in adrenal pathology. *PRKACA* tandem duplications generated neo-Topologically Associating Domains (TADs), self-interacting genomic regions, in patient derived tumor Hi-C maps compared to Micro-C data from human embryonic stem cell line.
All index cases and 6/7 relatives had ACTH-independent hypercorticism and underwent bilateral adrenalectomy. The resected adrenals contained micronodules, separated by atrophic cortex, making the diagnosis of PPNAD. PRKACA and PRKAR1A antibodies' staining was similar in these nodules and adjacent cortex, whereas PRKAR1A staining was decreased in patients' nodules with PRKAR1A alteration.
Conclusion: PRKACA constitutional duplication is causing PPNAD (9% of index cases with PPNAD here), but no other forms of BNAD. Immunohistochemistry differentiates the genetic background of PPNAD. *PRKACA* tandem duplications generate neo-TADs in patient derived tumor Hi-C maps, pointing to specific regions of gene expression dysregulation induced by *PRKACA* duplication in BNAD.

Pheochromocytoma organoids: a novel in vitro model

van den Berg Marit[1], Timmermans-Sprang Elpetra[1], Kusters Benno[2], Zethof Jan[3], Olthaar Andre[4], van Herwaarden Antonius[4], van Wolferen Monique[1], Timmers Henri[5], Kooistra Hans[1], Dona Margo[5,6†], Galac Sara[1†]

[1] Department Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
[2] Department of Pathology, Radboud University Medical Center, Nijmegen, The Netherlands.
[3] Department of Plant and Animal Biology, RIBES, Radboud University, Nijmegen, the Netherlands
[4]Department of Laboratory Medicine, Radboud University Medical Center, Nijmegen, The Netherlands.
[5] Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands
[6] Institute of Biology Leiden, Leiden University, Leiden, The Netherlands
[†] These authors share senior authorship

Given the lack of effective medical treatment for pheochromocytomas (PCCs) in both human and veterinary medicine, a reliable *in vitro* model is urgently needed to explore new treatment options. Organoids are three-dimensional, self-renewing structures that exhibit key features of their tissue of origin, providing valuable platforms for disease modeling and drug screening. In this study, we aimed to establish and characterize PCC organoid cultures derived from human and canine patients.

In a unique collaboration between human and veterinary medicine, tumor tissue was obtained from human and canine PCC patients following surgical resection. Primary cell suspensions were seeded in a three-dimensional matrix (basement membrane extract) and cultured in optimized medium enriched with specific growth factors. Primary tumor tissue and organoids at successive passages were characterized using histology, immunohistochemistry, immunofluorescence, and qPCR analysis, while metanephrine production in the culture supernatant was measured by liquid chromatography-tandem mass spectrometry.

Two human and nine canine PCC organoid cultures were successfully established. Similar results were obtained for the human and canine PCC organoids. These organoids expressed both differentiation markers (chromogranin A, synaptophysin) and stem/progenitor markers (nestin, SOX10), and retained key functional traits, as indicated by measurable metanephrine levels. A decline in differentiation marker expression and metanephrine production over time was observed, suggesting potential dedifferentiation or selective loss of differentiated chromaffin cells. For most organoid lines, expansion was limited beyond passage 2. Ongoing research focuses on optimizing culture conditions for sustained expansion and enhanced differentiation toward a chromaffin phenotype, with the ultimate goal of supporting future high-throughput drug-screening.

The establishment of these first human and canine PCC organoid lines marks a significant advancement in the field. While further optimization is needed, these organoids have great potential as an *in vitro* research tool, paving the way towards identification of new treatment modalities for this difficult-to-treat tumor type.

Drawbacks of circulating microRNA-based diagnostics of adrenocortical carcinoma

Vékony Bálint[1, 2], Nyirő Gábor[1, 2, 3], Butz Henriett[3, 4, 5], Szeredás Bálint Kende[1, 2], Tóth Viktória[6,7], Ferdinandy Peter[6,7], Patócs Attila[3, 4, 5], Igaz Peter[1, 2]

[1]Semmelweis University, Department of Internal Medicine and Oncology, Budapest, Hungary,
[2]Semmelweis University, Department of Endocrinology, Budapest, Hungary,
[3]Semmelweis University, Department of Laboratory Medicine, Budapest, Hungary,
[4]HUN-REN-SU, Hereditary Tumours Research Group, Budapest,
Hungary,
[5]National Institute of Oncology, Department of Molecular Genetics and the National Tumour Biology Laboratory, Budapest, Hungary,
[6]Semmelweis University, Department of Pharmacology and Pharmacotherapy,
Budapest, Hungary
[7]Center for Pharmacology and Drug Research & Development, Semmelweis University, Budapest, Hungary

Introduction: Differentiating benign and malignant adrenocortical tumors is clinically paramount, as benign tumors are common, but rare malignancies have bad prognosis. Circulating miRNAs show promise for diagnosing adrenal cortical carcinoma (ACC), though many factors, not limited to standardization hinder their utilization. This study aims to assess the interchangeability of real-time PCR (qPCR) and digital PCR (dPCR) in measuring circulating miRNAs and also to evaluate whether K2 or K3 EDTA anticoagulants could influence results.

Methods: Peripheral blood from 20 individuals was collected simultaneously into K2-EDTA and K3-EDTA tubes. Three ACC-associated miRNAs (hsa-miR-483-5p, -210-3p, -21-5p) and two controls (miR-16-5p, cel-miR-39-3p) were analyzed using both qPCR and dPCR. Statistical comparisons included Spearman's rank correlation, paired t-tests, and Bland-Altman analysis.

Results: In K2-EDTA samples, qPCR and dPCR showed significant correlations (ΔCt values vs. copy numbers; $p = 0.0072–0.049$), but proportional biases emerged for low/high miRNA levels. qPCR results in K3-EDTA samples exhibited higher variability (average SD: 1.1 vs. 0.91 for K2). Between the EDTA groups, raw Ct values differed significantly only for miR-483-5p, while ΔCt values showed differences across all miRNAs except miR-483-5p. dPCR results were unaffected by anticoagulant type.

Conclusions: qPCR and dPCR are not easily interchangeable, particularly for low or highly abundant miRNA levels, complicating cross-platform validation. The choice of EDTA may influence qPCR variability, underscoring the need for standardized protocols in miRNA-based biomarker studies.

Primary human adrenocortical spheroids as a novel *in vitro* model for adrenocortical carcinoma

Vietor Charlotte[1], van Koetsveld Peter[1], Dreijerink Koen[2], van der Vaart Jelte[3], Steenaard Rebecca[4], Feelders Richard[1], Hofland Leo[1]

[1]Erasmus Medical Center, Rotterdam, The Netherlands
[2]Amsterdam University Medical Center, Amsterdam, The Netherlands
[3]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences and University Medical Centre Utrecht, Utrecht, The Netherlands
[4]Maxima Medical Center, Veldhoven, The Netherlands

Introduction: Adrenocortical carcinoma (ACC) is a rare but aggressive malignancy with limited treatment options. Possible therapies are typically tested in monolayer cell cultures, though 3D models like spheroids—better mimicking tumor architecture and cell-cell interactions—are preferable. While ACC cell lines have successfully formed spheroids, those derived from human primary ACC tissue have not yet been reported.

Methods: Primary adrenocortical tissues (n=17) were collected from four Dutch centers. 5,000–20,000 tumor cells were seeded on low-adherence plates and incubated under shaking conditions at 37°C for 72 hours. Spheroids were cultured for 7 days with/without mitotane or for several weeks with/without ACTH. Spheroid size was monitored, and immunohistochemistry for SF-1 (adrenocortical marker) and Ki67 (proliferation) was performed. When available, comparisons with primary tumor tissue were made.

Results: Of 17 tissues, 13 were confirmed ACC, 1 had uncertain malignant potential, and 3 were adenomas. Spheroids formed successfully in 94.1% (16/17). Long-term cultures (n=12) persisted a median of 6.3 weeks (range 3–22). Spheroid size decreased (n=5), remained stable (n=4), or increased (n=1); two were not assessed. All spheroids showed SF-1 and Ki67 positivity, consistent with their respective primary tumors. Mitotane caused variable structural disintegration, yet Ki67 expression persisted, suggesting it affects cell integrity more than proliferation. ACTH treatment increased spheroid size in 6 patients compared to untreated spheroids, while spheroid size markedly decreased in one case. Moreover, in one case morphological destruction was observed upon ACTH treatment, whereas the other spheroids remained intact.

Conclusion: Primary human adrenocortical spheroids are a viable *in vitro* model, retaining adrenocortical identity and tumor-like proliferative capacity. Response to mitotane and ACTH varies across spheroids derived from different patients. Further research should confirm genetic concordance with primary tumors, assess RNA expression dynamics over time, and develop quantitative tools to evaluate treatment effects, enhancing this model's translational potential.

Contribution of immunohistochemistry and in situ hybridization in BMAD and correlations with patient genotype

Violon Florian[1,2], Bouys Lucas[1,3], Berthon Annabel[1], Ragazzon Bruno[1], Jouinot Anne[1], Vaduva Patricia[1], Vaquier Louis[2], Perlemoine Karine[1], Terris Benoit[1,2], Bertherat Jérôme[1,3], Sibony Mathilde[1,2]

1 Université Paris-Cité, Institut Cochin, INSERM U-1016, CNRS UMR-8104, F-75014, Paris, France
2 Pathology Department, AP-HP Hôpital Cochin, Paris, France
3 Endocrinology Department, AP-HP Hôpital Cochin, Paris, France

Introduction

Bilateral macronodular adrenocortical disease (BMAD) is an uncommon disease responsible for 2% of Cushing's syndrome. Our team has described four microscopic subtypes based on macronodule architecture and cell type proportion (clear, eosinophilic and oncocytic). Subtypes 1 and 2 correlate respectively with the presence of a pathogenic variant in *ARMC5* and *KDM1A* genes. *ARMC5* inactivation leads to accumulation of the A subunit of RNA polymerase II (POLR2A). We have also shown that bi-allelic inactivation of *KDM1A* occurs via loss of heterozygosity with deletion of the short arm of chromosome 1. The aim of this study is to describe the immunohistochemical and in situ hybridization characteristics of microscopic BMAD subtypes.

Patients and Methods

Immunohistochemistry and in situ hybridization were performed on 4 patients of subtypes 1 to 4 from the cohort of 35 patients previously described at our center. We used anti-alpha inhibin, anti-DAB2, HSD3B2, CYP11B2, CYP17A1, KDM1A and POLR2A antibodies. Fluorescent in situ hybridization (FISH) was performed with 1p36/1q25 commercial probe.

Results

In each subtype, HSD3B2 preferentially stains clear cells whereas CYP17A1 stains eosinophilic cells. HSD3B2 uniformly stains clear cells in patients with a pathogenic variant of *ARMC5*. In patients with a pathogenic variant of *ARMC5*, all nodular cells strongly express POLR2A compared to non-nodular adrenal. In subtype 2 (*KDM1A*), alpha inhibin is highly expressed in eosinophilic cells, and KDM1A immunoexpression is lower than in the adjacent adrenal. Similarly, this subtype is the only one showing a 1p deletion.

Discussion

The absence of HSD3B2 and CYP17A1 co-expression could distinguish BMAD from cortisol-producing adenomas. HSD3B2 and POLR2A are strongly correlated with ARMC5 pathogenic variants and, KDM1A immunohistochemistry and 1p36/1q25 FISH probe distinguish *KDM1A* altered BMAD. These markers can be used to guide genetic investigations or to confirm the pathogenic nature of germline variants of undetermined significance.

Assessment of [^{18}F]DOPA Positron Emission Tomography Imaging in genetic subtypes of Pheochromocytomas and Paragangliomas

Dahlmann Paul[1], Onkes Meike[1], Geuß Maria[1], Schweizer Júnia R. O. L.[2], Stüfchen Isabel[2], Lottspeich Christian[2], Wang Katharina[2], Schmid-Tannwald Christine[3], Auernhammer Christoph J.[2], Nölting Svenja[2,4], Werner Rudolf A.[1], Reincke Martin[2], Kroiss Matthias[2], Völter Friederike[1,2]

[1]Department of Nuclear Medicine, LMU University Hospital, LMU Munich, 81377 Munich, Germany
[2]Department of Medicine IV, Endocrinology, Diabetes and Metabolism, LMU University Hospital, LMU Munich, 80336 Munich, Germany
[3]Department of Radiology, LMU University Hospital, LMU Munich, 81377 Munich, Germany
[4]Department of Endocrinology, Diabetology and Clinical Nutrition, University Hospital Zurich (USZ) and University of Zurich (UZH), 8091 Zurich, Switzerland

Introduction:

Pheochromocytomas and paragangliomas (PPGLs) are malignancies with significant heritability. PPGLs are categorized into genetic clusters based on underlying pathogenic variations (PVs), which exhibit distinct clinical and biochemical tumor features and varying metastatic risks. [^{18}F]DOPA-PET/CT is recommended for the diagnosis of cluster 1B and cluster 2 PPGLs. This study aimed to assess differences in radioligand uptake of different genetic clusters on [^{18}F]DOPA-PET/CT.

Methods:

All patients with histologically verified PPGLs who underwent [^{18}F]DOPA-PET/CT between 03/2012 and 11/2023 as well as genetic testing at LMU Klinikum were included. Metabolic tumor volume (MTV) and total lesion uptake (TLU = SUVmean x MTV) were assessed semi-automatically using a threshold of SUVmax 4.0. Imaging parameters (SUVmax, MTV, TLU) were compared with the genetic cluster using the Kruskall-Wallis-test and the Mann-Whitney-U-test.

Results:

70 patients (mean age 52.7 years) were included in the study, 57 with Pheo, 13 with PGL, 27 sporadic PPGL, 10 cluster 1A PPGL, 7 cluster 1B PPGL and 25 cluster 2 PPGL. Radioligand uptake was significantly increased in Cluster 1a (14.1 (7.5 – 35.2)), Cluster 1b (14.3 (9.6 – 30.3)) and sporadic PPGL (16.0 (8.8 – 21.5)) compared to Cluster 2 PPGL, respectively (p = 0.002-0.021). The radioligand uptake of Cluster 1A, cluster 1 B and sporadic PPGL didn't show a significant difference. Additionally, Cluster 2 PPGL presented with significantly lower MTV and TLU compared to sporadic PPGLs and Cluster 1B PPGLs.

Conclusion:

There are significant differences on the uptake behaviour of different genetic clusters of PPGL on [^{18}F]DOPA-PET/CT with a decreased radioligand uptake and decreased MTV and TLU of Cluster 2 PPGLs compared to Cluster 1B PPGLs, Cluster 1A and sporadic PPGLs.

Single pembrolizumab dose as a successful salvage therapy for metastatic ACC

Vrčkovnik Rok[1], Kocjan Tomaž[1,2]

[1]Department of Endocrinology, Diabetes and Metabolic Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia
[2]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Background: Adrenocortical carcinoma (ACC) is a rare and aggressive malignancy with an incidence of 1–2 cases per million and abysmal prognosis when not amenable to complete resection.

Clinical case: A 63-year-old female presented on July 4, 2024 with progressive dyspnea, hypertension, and abdominal bloating. CT scan revealed multiple pulmonary metastases and an 11 cm right adrenal mass with inferior vena cava tumor thrombus. Hormonal evaluation was consistent with combined cortisol and androgen tumoral secretion. The tumor was deemed inoperable, and mitotane with first line chemotherapy (etoposide, doxorubicin, cisplatin) were initiated.

However, three months later the follow-up imaging showed the disease progression with growth of the primary tumor and metastases. Pembrolizumab as a salvage therapy was initiated on December 11th, while mitotane was continued. On December 17th, the patient developed severe autoimmune hepatitis, prompting initiation of high-dose glucocorticoids and mycophenolic acid, with discontinuation of both pembrolizumab and mitotane. Subsequently, the patient developed resistant hyponatremia due to autoimmune adrenalitis with complete mineralocorticoid insufficiency, and fludrocortisone was started.

Follow-up imaging on January 1st showed a significant shrinkage of primary tumor and metastases, which allowed for radical resection. On histopathology report the primary tumor was only 45 mm in size, mostly necrotic, with Ki67 over 50 % in the vital tissue, Weiss score 8/9.

Discussion: Pembrolizumab, a PD-1 inhibitor, may serve as a salvage therapy option in selected patients with advanced ACC. In our case, a single dose resulted in marked regression of both the primary tumor and metastases. However, clinicians should remain vigilant for potentially severe immune-related adverse events that may accompany treatment.

Oncocytic adrenal carcinoma- a case study

Węgrzyn Karolina [1], Sokołowski Grzegorz[2], Hubalewska- Dydejczyk Alicja[2],

1 Department of Endocrinology, Oncological Endocrinology, Nuclear Medicine and Internal Medicine, University Hospital in Krakow, Poland
2 Chair and Department of Endocrinology, Jagiellonian University School of Medicine, Krakow, Poland

Adrenal oncocytic neoplasms are rare entities, typically hormonally non-functional, most often discovered incidentally and usually involving the left adrenal gland. We present a case of a patient diagnosed with an adrenal oncocytic neoplasm exhibiting malignant potential.
A 22-year-old female was referred to the Department of Endocrinology for evaluation of progressive clinical signs of hyperandrogenism, including hirsutism, androgenic alopecia, voice deepening, and secondary amenorrhea. Hormonal assays revealed markedly elevated serum levels of testosterone, dehydroepiandrosterone (DHEA), androstenedione, and 17-hydroxyprogesterone. Circadian rhythm of cortisol secretion was disrupted, with a low morning serum cortisol level. An adrenocorticotropic hormone (ACTH) stimulation test confirmed primary adrenal insufficiency. Computer tomography (CT) revealed a well-demarcated, solid mass within the left adrenal gland, measuring 58 × 40 × 42 mm. The patient underwent a laparoscopic adrenalectomy. Histopathological examination identified an oncocytic adrenocortical neoplasm with features suggestive of malignant potential. Major criteria included vascular invasion, and minor criteria included focal necrosis. The Ki-67 proliferation index was approximately 7%.
Immunohistochemical analysis showed positivity for Melan A, inhibin, calretinin, and synaptophysin, and negativity for chromogranin A. Postoperatively, complete normalization of serum androgen concentrations was observed. A follow-up abdominal CT performed approximately six months after surgery demonstrated no evidence of local recurrence or metastatic disease. The patient did not attend subsequent follow-up appointments and is currently lost to endocrinological surveillance.
This case underscores the necessity of maintaining a high index of suspicion when evaluating adrenal lesions associated with hormonal disturbances. Oncocytic adrenocortical neoplasms, although rare, can exhibit malignant behavior and require diligent histopathological assessment and long-term follow-up. Standardized surveillance protocols, including serial imaging, are crucial for optimal patient outcomes.

Stable isotope tracing techniques in the study of PPGL

Westbrook Rebecca, Sanceau Julie, Favier Judith

INSERM UMRS1138 CRC, Paris, France

Paraganglioma and pheochromocytoma are tumours often associated with an inherited predisposing mutation. Many of the genes which have, to date, been identified as susceptibility genes are implicated in mitochondrial metabolism. Around half of hereditary cases are caused by mutations in genes encoding proteins directly involved in the tricarboxylic acid cycle. These include; succinate dehydrogenase and its assembly protein, fumarate hydratase and malate dehydrogenase. Such significant alterations to central carbon metabolism necessitate extensive rewiring in order to maintain energy production and anabolism. In this context, the altered metabolism of these cells is directly linked to their oncogenic phenotype. Understanding exactly how these cells adapt their metabolism may uncover vulnerabilities which could be exploited as therapy targets.

Stable isotope tracing, using carbon sources (primarily glucose and glutamine), which contain carbon 13, in the place of carbon 12. This heavy form of carbon is stable and can be detected by a mass shift of one by mass spectrometry. We have developed methods to use this technique to study the metabolism of primary cell cultures *in vitro*, and can utilise this set-up to investigate potential metabolite exchange between these cell types using coculture methods. We can derive populations of tumour cells, fibroblasts and endothelial cells from fresh tumour samples by serial plating and bead-associated sorting. So far, we have been able to generate cultures in this way from several tumours, and continue to receive samples with different genotypes to study.

We have also optimised an *in vivo* tracing model, using continuous isotope perfusion, which allows us to study cancer cell metabolism in the context of the whole organism. We are thus able to compare the metabolic phenotypes of cells and tumours with different mutations.

Delphi process regarding treatment recommendation in paediatric ACC

Verena Wiegering, Maria Riedmeier on behalf of the ENSAT-kids group

Background:

Paediatric Adrenocortical Tumours (pACTs) are extremely rare but potentially devastating neoplasms of the adrenal cortex. The incidence of pACTs is about 0.2-0.3 cases per million children per year in most countries, with a 15-fold higher incidence in Brazil due to a TP53 founder mutation. Over the last years, there have been great efforts to bring experts together to establish networks (as ENSAT kids, ExPert or ICPACT) to improve knowledge regarding pACTs. However, treating guidelines for pediatric patients have not been established yet.

Aim:

To seek international consensus on the surgical and medical treatment in children and young people with pACTs.

Method:

A three-step Delphi consensus method was used to finalise the statements from an international group of pACT experts with >30 experts from three continents (Europe, the Americas, and Asia). All members of the panel and steering committee took part in the voting rounds of the Delphi process. Members of the Delphi panel were selected based on their peer-reviewed publications in the field of pACTs. Electronic survey was created by the steering committee (ENSaT kids) and circulated to the Delphi panel. Level of agreement and disagreement was rated on a six-point Likert response scale with the opportunity to give controlled feedback. After each round, conflicting results were discussed within the ENSaT-KIDS group and questions are re-defined, if no consensus is reached based on feedback by the Delphi panel. Consensus was defined *a priori* as agreement by at least 70%.

Results

Results of this recommendation will be presented on the conference.

The Role of Cortisol Secretion in Pheochromocytomas and Paragangliomas: Clinical and Perioperative Implications

Karolina Zawadzka[1], Jan Calissendorff[2,3], Ewelina Rzepka[4], Michał Pędziwiatr[5], Alicja Hubalewska-Dydejczyk[4], Henrik Falhammar[2,3]

1 Doctoral School of Medical and Health Sciences, Jagiellonian University Medical College, Krakow, Poland
2 Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden
3 Department of Endocrinology, Karolinska University Hospital, Stockholm, Sweden
4 Chair and Department of Endocrinology, Jagiellonian University Medical College, Krakow, Poland
5 2nd Department of General Surgery, Jagiellonian University Medical College, Krakow, Poland

Background
Phaeochromocytomas and paragangliomas (PPGLs) are tumours characterised by excessive catecholamine secretion. Patients with phaeochromocytomas may also exhibit elevated plasma glucocorticoid concentrations. This study aimed to assess the prevalence, clinical implications, and perioperative outcomes of autonomous cortisol secretion in patients with PPGLs.

Methods
This retrospective cohort study was conducted across two tertiary endocrinology centres and included patients with PPGLs who underwent adrenalectomy. Patients were categorised into suppressive and non-suppressive groups based on the results of the 1 mg dexamethasone suppression test (DST). Clinical characteristics, biochemical markers, tumour features (including size and location), perioperative outcomes (such as intraoperative blood loss, conversion to open surgery, and length of hospital stay), and follow-up data (including recurrence and survival) were collected and analysed to evaluate potential differences between the two groups.

Results
Among 106 patients, 24.5% demonstrated non-suppressive cortisol concentrations post-DST. These patients were older (median age: 66 vs. 56 years, $P<0.001$), predominantly female (84.6% vs. 48.8%, $P=0.001$), and had larger tumours (5.2 vs. 4.0 cm, $P<0.05$). Diabetes was more prevalent in the non-suppressive group both pre-adrenalectomy (50.0% vs. 26.8%, $P<0.05$) and post-adrenalectomy (33.3% vs. 12.7%, $P<0.05$). This group also exhibited higher urinary and plasma metanephrine concentrations and a greater incidence of cardiovascular disease. Perioperative complications, including increased blood loss, conversion to open surgery, and prolonged hospitalisation, were more frequent in the non-suppressive group ($P<0.05$).

Conclusions
Approximately one-quarter of patients with PPGLs exhibit autonomous cortisol secretion, which is associated with larger tumours, a higher prevalence of diabetes, and increased perioperative risk. Routine DST screening may enhance preoperative assessment and provide further insight into the influence of cortisol on PPGL outcomes.

Safety of biopsy in pheochromocytoma/paraganglioma: a multicenter cohort study

Zhang Liang[1], Tisserand Gautier[2,] *, Shen Yanting[3,] *, Salehi Mahdi[4,] *, Amar Laurence[5], Cui Yunying[6], Świątkowska-Stodulska Renata[7], Moog Sophie[2], Liu Jun[3], Gubbi Sriram[8], Kocjan Tomaz[9], Igaz Péter[10], Hadoux Julien[2], Young William[4], Remde Hanna[11], Babińska Anna[7], de Miguel Valeria[12], Beuschlein Felix[13], Liu Longfei[14], Palmieri Serena[15], Jiang Jingjing[3], Fischer Alessa[13], Varghese Jeena[16], Carreira Anna[17,18], Nölting Svenja[13,19],Zhou Yue[6], Prodanov Tamara[8], Bancos Irina[4], Fassnacht Martin[11], Jimenez Camilo[16], Alkaissi Hussam[8], Tong Anli[6], Pacak Karel[20, 21,] *, Crona Joakim[1,] *
*Contributed equally

1) Department of Medical Sciences, Uppsala University, Uppsala, Sweden
2) Service d'Oncologie Endocrinienne, Département d'Imagerie, Institut Gustave Roussy, Villejuif, France
3) Department of Endocrinology and Metabolism, Zhongshan Hospital Fudan University, Shanghai, China
4) Division of Endocrinology, Diabetes, and Nutrition, Mayo Clinic, Rochester, Minnesota, USA
5) Assistance Publique-Hôpitaux de Paris (AP-HP) Centre, Hôpital Européen Georges Pompidou, Unité Fonctionnelle d'Hypertension Artérielle, Centre de référence des maladies de la surrénale, Université Paris Cité, F-75015 Paris, France
6) Department of Endocrinology, Key Laboratory of Endocrinology, National Health Commission of the People's Republic of China, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730, China
7) Department of Endocrinology and Internal Medicine, Faculty of Medicine, Medical University of Gdańsk, Gdańsk, Poland
8) National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA
9) University Medical Centre Ljubljana, Ljubljana, Slovenia
10) Department of Endocrinology, Faculty of Medicine, Semmelweis University, Korányi Str. 2/a, 1083 Budapest, Hungary
11) Department of Medicine, Division of Endocrinology and Diabetes, University Hospital Würzburg, University of Wuerzburg, Oberdürrbacherstrasse 6, 97080 Wuerzburg, Germany
12) Servicio de Endocrinología, Hospital Italiano de Buenos Aires, Ciudad de Buenos Aires, Argentina
13) Department of Endocrinology, Diabetology and Clinical Nutrition, University Hospital Zurich (USZ), and University of Zurich (UZH), Zurich CH-8091, Switzerland
14) Department of Urology, Xiangya Hospital, Central South University, 87 Xiangya Road, Changsha, 410008, Hunan, China
15) Endocrinology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122, Milan, Italy
16) Department of Endocrine Neoplasia and HormonalDisorders, The University of Texas MD Anderson Cancer Center, 1400 Pressler Street, Houston, TX 77030, USA
17) Université Paris Cité, AP-HP, Hôpital Européen Georges Pompidou, DMU Carte, Unité Hypertension Artérielle, Centre de références en maladies rares de la surrénale, Paris, France
18) Department of Endocrinology, Diabetes and Metabolism, Hospitais da Universidade de Coimbra, Unidade Local de Saúde de Coimbra, Coimbra, 3004-561, Portugal
19) Department of Medicine IV, LMU University Hospital, LMU Munich, 80336 Munich, Germany
20) Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA
21) Center for Adrenal Endocrine Tumors, AKESO, Prague 5, Czech Republic 15500

Background: Biopsy of confirmed or suspected pheochromocytoma/paraganglioma (PPGL) is generally discouraged due to the anticipated risk of complications from provocation of sudden catecholamine excess. However, a recent systematic review highlighted a lack of robust evidence supporting this recommendation, as most available data were limited to case reports without reported biopsy-related complications.

Methods: This multicenter retrospective cohort study evaluated patients with thoracoabdominal PPGL who underwent biopsy via percutaneous core needle or fine needle aspiration. Demographic data, tumor characteristics, and biopsy outcomes were collected according to a

standardized protocol. The primary outcome was biopsy-related mortality (95% Confidence Interval [CI]) for initial biopsies performed locally at participating institutions. To minimize selection bias, externally performed biopsies were excluded from this analysis. Secondary outcomes included the incidence (95% CI) of serious (A) catecholamine-related and (B) non-catecholamine-related complications observed after biopsy procedures performed at any institution.
Results: Across 17 centers, 209 patients underwent 221 biopsies (101 performed locally, 120 externally). Among 188 biopsies with available biochemical data, 72% (132/188) showed elevated catecholamines. No biopsy-related deaths occurred among the 96 local biopsies analyzed for the primary outcome (0%, 95% CI: 0–3.7%). Serious catecholamine-related complications occurred after 1.4% (3/220, 95% CI: 0.3–4.0%) of all biopsies. This included two cardiogenic shocks and one hypertensive crisis, all were treated in the intensive care unit. Serious non-catecholamine-related complications were reported in 4.1% (9/220, 95% CI: 1.9–7.6%). Transient episodic hypertension was noted in 13 cases.

Conclusion: In this preliminary analysis, biopsy of PPGL was not associated with mortality, and the incidence of serious catecholamine-related complications was 1.4%. Further analyses—including the evaluation of outcomes in functional PPGLs and the identification of risk factors—are ongoing and will be reported upon completion of the full cohort.

Immunotherapy – induced exudative pleuritis in adrenocortical cancer patient. A case report and literature review

Barbara Ziółkowska[1], Agnieszka Kotecka-Blicharz[2], Urszula Ambroziak[3]

[1] 2nd Department of Radiotherapy and Chemotherapy, Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice, Poland
[2] Department of Nuclear Medicine and Endocrinology Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice, Poland
[3] Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Poland

Immune checkpoint inhibitors (ICIs) have emerged as a pivotal therapeutic modality in the treatment of various malignancies and have recently been investigated for their efficacy in adrenocortical carcinoma (ACC). Despite their clinical benefits, ICIs are associated with a spectrum of immune-related adverse events (irAEs), including pneumonitis, thyroiditis, and colitis. Among these, pleural effusion is an uncommon manifestation.

We present a case of a 26-year-old female diagnosed with hormonally active, metastatic adrenocortical carcinoma (ENSAT stage IV). Following adrenalectomy, the patient received first-line chemotherapy with etoposide, doxorubicin, cisplatin (EDP), and mitotane. Disease progression was observed six months after completion of chemotherapy, prompting initiation of second-line immunotherapy with pembrolizumab, an anti-PD-1 agent. Partial remission was achieved as the best response. However, after 14 cycles (11 months of treatment), the patient developed progressive bilateral pleural effusions, necessitating hospitalization due to clinical deterioration and suspected pneumonia with concomitant pericardial effusion. Thoracentesis revealed exudative effusion without malignant cells.

Immunotherapy was discontinued, and empirical antibiotic therapy along with corticosteroids was initiated. Despite these interventions and subsequent pleurodesis, the pleural effusion recurred. Prolonged high-dose corticosteroid therapy led to temporary stabilization, but tapering efforts were complicated by recurrent effusion, hypoalbuminemia, and severe peripheral edema. Given the limited response to steroids, second-line immunosuppression with mycophenolate mofetil was commenced, with treatment ongoing at the time of reporting.

After excluding alternative etiologies, the pleural effusion was attributed to an immune-related adverse event secondary to immunotherapy. To the best of our knowledge, reports of pleural effusion as a manifestation of ICI-induced toxicity in ACC are exceedingly rare in the existing literature.

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