The Official Newsletter of the COST Action CA20122

Harmonis@tion News

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Working together for our better future

In this issue

Editorial Board

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Editor's Note

The Harmonis@tion Newsletter starts the New Year by looking back on the most important event of 2022: The ENS@T/Harmonis@tion meeting in Warsaw. This Newsletter has the spotlight on ENS@T/Harmonis@tion awardees and their adrenal tumor research. Also, the organizers of the Adrenal Tumor Masterclass as well as the ENS@T/Harmonis@tion meeting will share their reflections. Furthermore, you'll be able to read about the progress of the Harmonis@tion project and finally, some future perspectives and the ENS@T/Harmonis@tion meeting in Dubrovnik 2023!

Happy New 2023 from the Harmonis@tion Newsletter team!

Joakim Crona

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Harmonis@tion's 2022 recap and 2023 announcements

The first year of the Harmonis@tion life has ended and it is the right time to assess what has been done, as well as to plan what needs to be done in the following years.

Looking back at the goals we set before the start of the Action, we have succeeded in building a pan-European, multidisciplinary network of experts in the field of the adrenal gland. This network brings together not only clinicians and basic researchers, but also experts in information technology, ethics and law. Accordingly, the number of members of the Action increased from about thirty at the proposal stage, to 190 from 33 different countries. Since the involvement of early-career researchers is at the heart of the Harmonis@tion mission and policy, I am happy to say that young people make up more than 50% of the Harmonis@tion members.

A series of meetings were held during the first year of the Action, including Management Committee meetings, Working Groups 1-5 meetings and numerous Harmonis@tion Core Group meetings. From the perspective of education and training, two Adrenal Tumor Masterclasses were held and attended by more than 300 participants. Finally, the year ended with the 1st joint Harmonis@tion/ENS@T meeting held in Warsaw, Poland.

"I would like to announce that the 2nd joint Harmonis@tion/ENS@T meeting will be held from October 11 to 13, 2023, in Dubrovnik, Croatia"

The main tasks of the Action in the next year will refer to the analysis of existing practice in certain European countries related to the clinical management and research of adrenal tumors, as well as ethical and legal requirements and IT possibilities and limitations in the creation of a joint international adrenal tumor registry. Early-career Harmonis@tion members will continue to be one of the priorities, and, in addition to adrenal masterclasses and scientific conferences, they will be targeted also through short-term scientific missions whose main goals are to establish standard operating procedures for inter-institutional sharing of clinical dana and to train researchers in new technologies for adrenal tumor studies.

In the end, I would like to point out that in all its activities Harmonis@tion has felt great support from ENS@T, and, since this cooperation has proved to be very successful for both parties, it is planned to continue in the following years. In this regard, I would like to announce that the 2nd joint Harmonis@tion/ENS@T meeting will be held from October 11 to 13, 2022, in Dubrovnik, Croatia.

Darko Kaštelan
Harmonis@tion Chair
Department of Endocrinology, University Hospital Centre Zagreb
School of Medicine, University of Zagreb, Croatia

Working Group 3 Update

Since the start of the COST Action, the Working group 3 (WG3) has evolved to a well-rounded group of 44 members, with good gender balance, inclusion target country participation and a vibrant multidisciplinary landscape. The connection between data scientists, tech and artificial intelligence experts, researchers and clinicians has become integral to create new solutions and optimize the way we work in the medical field. Together with the Working group 4, the WG3 has taken several key steps which were established during regular meetings. The first was to create a detailed survey that would help us learn about the ethical and technical similarities and discrepancies between the way we collect, store, save, interpret, integrate and process our medical data on adrenal tumors all across Europe. The survey was sent out and the feedback was received from a total of 80 centers across Europe with very significant data. This data will now be processed and rounded up in a publication in a relevant journal in this field. Based on acquired knowledge, the second task is to commence a pilot data collection study on Adrenocortical carcinoma survival in Europe, with the aim to perform structured and pseudonymized data extraction from the electronic health records across Europe. This process will bring discussions and solutions between all working groups on obtaining ethical and regulatory approvals, creation of data collection tools and use of existing programs as well as their drawbacks, creation of local databases and secure data transfer solutions along with data valorization. The question on how ready are we to share data is ready to be answered in the field of adrenal endocrinology. With future interactions with other relevant European initiatives, a higher number of centers sharing data, inclusion of new types of data and having more complexed scientific questions, semi automation of regulatory clearance and automation of data collection, the aim is to reconfigure the scientific method, leading to better data, results and better management of various adrenal pathologies.

Antoan Stefan Šojat
WG-3 vice lead

The joint 21st ENS@T and 1st COST Harmonis@tion meeting

The goal of the joint 21st ENS@T and 1st COST Harmonis@tion meeting held in Warsaw (Poland), September 28-30, was to disseminate the results of the latest scientific research in the field of the adrenal diseases. The conference was attended on-site by 168 and on-line by 60 scientists from most countries in Europe, the United States and Mexico (including the scientific and organizing committees, a total of 240 participants). The scientific research results presented at the conference covered a wide range of genetics, molecular biology, biochemistry, biotechnology, medicine, pharmacy, and computer science at the highest, state-of-the-art level. The conference provided a unique opportunity to continue and establish multicenter, multinational research collaborations that will result in research published in top professional journals.

ENS@T - the European Network for the Study of Adrenal Tumors - is a scientific society established in 2002 to conduct basic and clinical research on adrenal diseases. The ENS@T Society now brings together the most important adrenal disease research groups in Europe, who have created a unique network of scientific collaboration. Publications based on multicenter projects carried out within ENS@T and through collaborations formed within ENS@T now account for the majority of adrenal disease publications from Europe.

This year, for the first time, the ENS@T meeting was held together with the COST action 20122 Harmonis@tion annual meeting.

Harmonis@tion's goal is to harmonize clinical care and research on adrenal tumours throughout Europe. This COST Action enabled researchers from most European countries to participate in the meeting and expand scientific collaborations. It is a unique in nature initiative based on the action of scientists to expand the network of scientific centers and support research in countries with less scientific involvement to date. The Harmonis@tion's Adrenal Master Class included 16 lectures which enabled young researchers to gain extensive knowledge from top European researchers in the field.

Conference in numbers:

· 240 participants

· 115 abstracts were submitted to the conference:

- Four oral sessions were held - 28 oral presentations were presented.
- The remaining abstracts - 87 - were presented as posters

· Four working group meetings were held

· One main session and general assembly of ENS@T were held

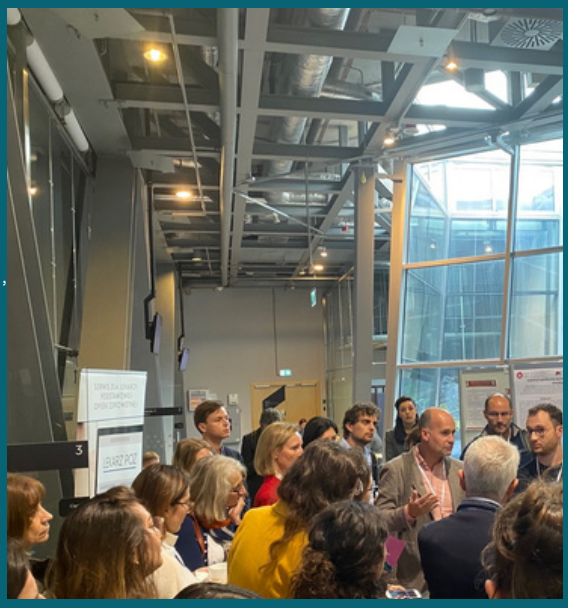
· 5 blocks of COST20122 meetings were held

Aleksander Prejbisz

LOC

National Institute of Cardiology, Warsaw, Poland

From the point of view of the molecular geneticist



The joint ENS@T and Harmonis@tion meeting held in Warsaw, Poland, delivered great insights on the diagnosis and treatment of adrenal tumors. The uniqueness of this event was the ”down to earth“ presentations during the masterclass session, where young physicians, together with recognized experts of adrenal tumors, presented their case reports.

All of the masterclass presentations ended up with a discussion and recommendations on how to deal with difficult, rare, or common disease cases.

These discussions gave confidence on the future decision making to the young physicians, and planted a seed of synchronizing the diagnostic and therapeutic practices of adrenal tumors in the whole Europe.

From the point of view of the molecular geneticist, this event showed what kind of tests physicians mostly rely on when making a decision on their patients' treatment. This information is crucial to researchers when making project proposals, since the desired result of the novel scientific findings is to implement them into clinical practice as soon as possible.

In addition, molecular experts, specializing in the genetics of adrenal tumors, presented what is already revealed in the genomic of adrenal tumors, and how to communicate more efficiently between molecular biologists and physicians.

Last, but certainly not least, an important topic during the ENS@T and Harmonis@tion meeting was the ethics of the research of adrenal tumors. During this session, listeners were able to understand the importance of informed consent, and what are the best practices to give one.

Best wishes from Kaunas, Lithuania

Rytis Stakaitis

Junior Researcher, Laboratory of Molecular Neurooncology, Neuroscience Institute

Lithuanian University of Health Sciences, Kaunas, Lithuania

INTERVIEW

Experiences of a nurse working with diseases of the adrenal glands

Tereza Jelača

1. What is the work of a nurse related to adrenal diseases? How much time do you spend with patients suffering from adrenal disease?

In the Department of Endocrinology and Diabetology at University Hospital Center Zagreb, I work only with adults. I deal with patients with adrenal diseases almost every day, probably because we are the reference center for adrenal diseases. My work starts with admitting the patient to the department and giving her/him basic instructions in simple vocabulary about the planned laboratory and radiology tests, further treatment procedures, options, proper therapy, reasons for taking medications and when and how to use them.

2. Can you briefly describe your responsibilities related to a specific adrenal pathology?

Adrenal incidentalomas = checking blood pressure and pulse, medical examination. We perform lab tests: plasma renin activity, aldosterone, cortisol in overnight dexamethasone suppression test (ODST), and metanephrine and normetanephrine in 24-hour urine.
Addison's disease = treatment consists of lifelong replacement therapy (hydrocortisone and fludrocortisone). It is important to educate patients on how to take the medications and when dose adjustments are needed.
Pheochromocytoma = detection of metanephrine and normetanephrine in 24-hour urine is critical for diagnosis. Treatment is surgical, but preoperative preparation is very important, starting 10 days before surgery with phenoxybenzamine and high-salt diet and infusions of saline and β-blockers a few days before surgery.
Cushing's syndrome = multidisciplinary approach and teamwork are required in recognition, diagnosis, and treatment. Clinical presentation of hypercortisolism: central type of obesity, moon face, neck hump, purple stretch marks, diabetes, hypertension, sexual dysfunction. Laboratory findings: increased amount of free cortisol in 24-hour urine, increased plasma cortisol concentration at midnight, lack of cortisol suppression in ODST, ACTH.
Adrenocortical carcinoma = the nurse's role is mainly to educate the patient about the use of the mitotane drug and to collect the blood sample for mitotane concentration.

3. Is it also part of the nurse's job to educate the patient about certain diseases and conditions?

A nurse's job is to accurately perform laboratory diagnoses, monitor 24-hour urine, and educate patients about the importance of prescribed therapy, why it is necessary, when to take it, and what to do if they become ill. A nurse's role is to assess health care, plan, implement interventions, and evaluate.

4. What do you think has changed in recent years?

In recent years, there have been more and more different disease patterns of the adrenal glands. Nurses are being trained more frequently, and therefore there is better collaboration. The nurse is one of the most important factors in a multidisciplinary team dealing with the treatment of patients with adrenal diseases.

5. Who is Tereza Jelača?

Tereza Jelača is a nurse and has been working in the Department of Endocrinology for 26 years. I have always loved helping people, I am very compassionate. Since I am interested in the human body, I chose to become a nurse. Detecting, diagnosing, and treating endocrine disorders requires a multidisciplinary approach and teamwork, and I enjoy that. The role of a well-trained nurse is very important, starting with recognizing symptoms and signs, performing endocrinology tests accurately, preparing for and participating in radiology exams, caring for patients before and after surgery, and providing good education. It is a great challenge, but it is often possible to achieve positive outcomes and happy life stories to our satisfaction.

Karin Zibar Tomšić

The 21st ENS@T Scientific Meeting Awards

During the joint 21st Ens@t Scientific Meeting and 1st Harmonis@tion meeting held in Warsaw at the end of September 2022, many interesting research studies were awarded also thanks to the contribution of the Harmonis@tion COST Action Program.

As in past editions, the Ens@t scientific prizes were awarded during the social dinner to the four ENS@T working groups: ACC, NAPACA, APA and PPGL.

The names of the winners are as follows:

For ACC, **Constanze Hantel** with the work "Innovative multidimensional models in a high-throughput format for different cell types of endocrine origin" and Andrea Abate with the article "Ribociclib Cytotoxicity Alone or Combined with Progesterone and/or Mitotane in in Vitro Adrenocortical Carcinoma Cells".

For APA, **Alaa Abdellatif** with the paper "Colocalization of Wnt/β-Catenin and ACTH Signaling Pathways and Paracrine Regulation in Aldosterone-Producing Adenoma" and Marta Araujo with the study "Predictive model of hypertension resolution after adrenalectomy in primary aldosteronism: the SPAIN-ALDO score".

For NAPACA, **Alessandro Prete** with the study "Cardiometabolic Disease Burden and Steroid Excretion in Benign Adrenal Tumors" and Roberta Armignacco with the paper "Identification of glucocorticoid-related molecular signature by whole blood methylome analysis".

For PPGL, **Svenja Nölting & Katharina Wang** with the work "Personalized drug testing in human pheochromocytoma/paraganglioma primary cultures" and Hanna Remde with the paper "Improved Diagnostic Accuracy of Clonidine Suppression Testing Using an Age-Related Cutoff for Plasma Normetanephrine".

But that's not all! This year there were more prizes awarded, for the best oral presentations and posters in the four categories. Awards for the Best poster went to Georgiana Constantinescu (APA), Hermine Mohr (PPGL) Louis Thomeret (NAPACA), Mariangela Tamburello (ACC) and Peter Turai (ACC). Here is a selection of the Oral communication winners!

ACC - OC: Juliane Lippert & Cristina Ronchi

Prognostic role of targeted methylation analysis in formalin-fixed paraffin-embedded samples of adrenocortical carcinoma

Adrenocortical carcinoma (ACC) is a rare aggressive disease with heterogeneous prognosis. Previous studies identified hypermethylation in the promoter region of specific genes to be associated with poor clinical outcome. Hereby, we provide a comparative analysis of the prognostic role of hypermethylated genes against established clinical prognostic tools. Tumor DNA was isolated from 237 formalin-fixed paraffin-embedded (FFPE) ACC samples. Targeted pyrosequencing was used to detect promoter region methylation in 5 preselected genes (PAX5, GSTP1, PYCARD, PAX6, G0S2). The prognostic role of hypermethylation pattern for progression-free (PFS) overall (OS) and disease-free (DFS) survival was compared to S-GRAS score. Hypermethylation in all individual genes, except GSTP1, was significantly associated with both PFS and OS (Hazard Ratios (HR) 1.4-2.3). However, only hypermethylation of PAX5 remained significantly associated with OS ($p=0.013$; HR=1.95, 95%CI 1.2-3.3) in multivariable analysis. A model for risk stratification was developed, combining PAX5 methylation status and S-GRAS groups, showing improved prognostic performance compared to S-GRAS alone (Harrell's C index: OS=0.751, PFS=0.711, DFS=0.688). In conclusion, hypermethylation in PAX5 is associated with worst clinical outcome in ACC, even after accounting for S-GRAS score. Assessing methylation in FFPE material is straightforward in the clinical setting and could be used to improve accuracy of prognostic classification, enabling the direction of personalized management.

PPGL - OC: Carlota Arenillas

Mechanistic insights on pheochromocytomas and paragangliomas from patients with cyanotic congenital heart disease

The hypoxia-inducible factor 2a (HIF2a) is a key driver of pheochromocytomas and paragangliomas (PPGL). We recently reported a high frequency of somatic gain-of-function mutations in the EPAS1 gene, encoding the HIF2a protein in PPGLs from patients with cyanotic congenital heart disease (CCHD) and life-long systemic hypoxia.

Through the creation of a CCHD-PPGL International Consortium, we gathered 34 CCHD-PPGL tumors and found that nearly 90% (24/27) of the sympathetic tumors carried EPAS1 gain-of-function mutations, which were not detected in parasympathetic PPGLs from CCHD (0/7, $p<0.001$) or non-CCHD patients (0/76, $p<0.001$), nor in thousands of non-PPGL tumors from publicly available genomic projects ($p<0.001$). This result pinpoints an extraordinary tissue and environmental specificity of EPAS1 mutations. Remarkably, some CCHD patients developed multiple PPGLs harboring different hotspot EPAS1 missense gain-of-function mutations. Whole-exome sequencing (WES) data uncovered that EPAS1 wild-type parasympathetic CCHD-PPGLs carried mutations in other genes of the hypoxia pathway. Mechanistically, we found decreased expression of DNA mismatch repair genes and proteins (MLH1, MSH2, MSH6, PMS2), increased expression of DNA damage marker (γH2AX), accompanied by a higher tumor mutation burden (TMB) in CCHD-PPGL systemic hypoxic tumors. Our findings provide a unique piece of evidence on systemic hypoxia can shape the genetic landscapes of PPGLs.

APA - OC: Sherazeed Boulkroun

Genome-wide association studies identify risk loci for primary aldosteronism and reveal new pathogenic mechanisms

Germline and somatic mutations have been identified in familial forms of primary aldosteronism (PA) and aldosterone producing adenoma (APA); however, the causes underlying a large proportion of cases of PA are still unknown. We hypothesised that subtle genetic variation may predispose to the development of PA. To identify genomic loci that may confer an increased susceptibility of developing PA, we conducted a genome-wide association study in a discovery cohort. We identified three loci on chromosomes 1, 13 and X at a genome-wide significance, and a fourth locus on chromosome 11 at suggestive significance; associations on chromosome 1, 11 and 13 were replicated in a second cohort and confirmed by a global meta-analysis. Candidate genes located within the two main loci (CASZ1 on chromosome 1, RXFP2 on chromosome 13) are expressed in human and mouse adrenals in different cell clusters and their overexpression in H295R-S2 cells modifies mineralocorticoid output under basal and stimulated conditions. Our study identifies the first risk loci for PA and highlights new mechanisms for the development of aldosterone excess. These loci are shared between APA and BAH, in accordance with accumulating evidence for a continuum between the two conditions.

Giulia Cantini

LATEST RESEARCH

The immune cell infiltrate in the tumour microenvironment of phaeochromocytomas and paragangliomas

The authors researched the tumor microenvironment (TME) in phaeochromocytomas and paragangliomas(PPGLs). Immunohistochemistry was compared between 65 PPGLs tumor samples and 20 normal adrenal medulla samples. In tumor tissue, a higher proportion of immune cells, mainly macrophages, was observed. A higher proportion of M2:M1 macrophages and T-helper lymphocytes existed in aggressive tumors compared to indolent ones. For SDHB-associated tumors, there was a higher proportion of M2 macrophage infiltration, with higher M2:M1 in aggressive SDHB PPGLs compared to indolent tumors. Authors concluded that PPGLs were immunologically active tumors and TME could be potential hallmarks to predict tumor behaviors.

Tufton, N. et al. (2022). The immune cell infiltrate in the tumour microenvironment of phaeochromocytomas and paragangliomas. Endocrine-related cancer, 29(11), 589-598.

First-in-human evaluation of [18F]CETO: a novel tracer for adrenocortical tumours

The authors performed a first evaluation of para-chloro-2-[18F]fluoroethyletomidate ([18F]CETO), for adrenocortical tumors. Fifteen adrenocortical tumor patients and five healthy volunteers were recruited. Research found that uptake of [18F]CETO was low in the liver and high in adrenals. Initial metabolization was rapid, followed by a plateau. Standardized uptake values (SUV) correlated well with the uptake rate constant K1. The authors concluded that [18F]CETO is a suitable tracer for adrenal imaging, and the low uptake in the liver would facilitate clinical assessment of right adrenocortical disease, especially in ruling out liver metastases.

Silins, I.et al. (2022). First-in-human evaluation of [18F]CETO: a novel tracer for adrenocortical tumours. European journal of nuclear medicine and molecular imagin.

Histopathology and Genetic Causes of Primary Aldosteronism in Young Adults

The authors aimed to characterize the histological and genetic landscape of lateralized primary aldosteronism in young adults (<35 years old). Immunohistochemical (IHC) analyzes for aldosterone synthase (CYP11B2) and CYP11B2 IHC-guided DNA sequencing were performed in 74 formalin-fixed, paraffin-embedded adrenal tissue sections from aldosterone-producing lesions. The most common histological feature was aldosterone producing adenoma (APA), whereas the second most common was aldosterone-producing nodule (APN). The most common somatic mutations for early onset of APA and APN were KCNJ5 and CACNA1D, respectively. No somatic mutations were identified for non-function adenomas (n=2).

Nanba et. al. (2022), Histopathology and Genetic Causes of Primary Aldosteronism in Young Adults, The Journal of Clinical Endocrinology & Metabolism, Volume 107, Issue 9, September 2022, Pages 2473–2482

Liang Zhang &
Kazhan Mollazadegan

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