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GENOTYPE-PHENOTYPE CORRELATIONS IN CATECHOLAMINE SECRETING TUMORS

ABSTRACT OF THE Ph.D. THESIS

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I. Introduction

Catecholamine secreting tumors, represented by pheochromocytomas and paragangliomas (PPGLs) are rare tumors of the chromaffin tissue (i.e. neural crest-derived tissues) that store and secrete catecholamines in excess. [1]

Pheochromocytomas (PHEOs) arise in the adrenal gland while paragangliomas (PGLs) in sympathetic and parasympathetic ganglia along the nerves from the abdomen, thorax, pelvis or head and neck area. Approximately 80% of catecholamine-secreting tumors are PHEOs, the rest of them being represented by PGLs. In the literature, only one report in which PGL were documented in a higher percentage (50.9%) than PHEOs. [2], [3], [4] About 30-50% of sympathetic PGLs arise in the abdominal ganglia, 5% in the pelvis, 5 % mediastinal and approximately 50-70 % of PGL occur in the head and neck area - the head and neck PGLs (HNPGL). Most of them arise in the parasympathetic paraganglia and are non-functional, but they can also arise in the sympathetic head and neck paraganglia and secrete catecholamines. [4], [5]

Approximately 30-40% of patients with PPGLs have a germline mutation, the rest of them (60-70%) are sporadic. Among sporadic PPGLs, ~ 30 % harbor a somatic mutation [6] and about 30% of patients with apparently sporadic disease or patients with clinical phenotype of heritability do not carry mutations in any known gene. Therefore, PPGLs are the solid tumors with the highest genetic variability (nowadays, more than 21 genes are known to be involved in these pathologies). PGLs are more often caused by a germline mutation than PHEOs. [7] This elevated percentage of tumors with molecular pathogenesis indicates genetic testing is a mandatory step in the management of patients with PPGLs.

Currently, more than 21 genes are known to predispose to hereditary pheochromocytomas and paragangliomas. Based on their transcriptional signature, these genes have been grouped into three clusters: *pseudohypoxia cluster 1* (1A and 1B), *kinase-signalling cluster 2*, and *Wnt signalling cluster 3.* [8] Each cluster have a specific clinical behaviour, biochemical phenotype, aggressivity and prognosis. This cluster classification is based almost exclusively on Caucasian population studies.

II. Work Hypothesis

Genotype-phenotype correlations in catecholamine secreting tumors started up at the same time with genetic testing era. In the beginning, due to cost-efficiency reasons, the European Society Guidelines developed algorithms for genetic tests, based on clinical phenotype specific to each cluster. [9] In the present, the standard of care in PPGLs management is genetic testing for all patients with PPGLs, by Next Generation Sequencing (NGS) based technique, independently to their clinical presentation. [10] In case of a patient with germline PV the next step is screening of first-degree relatives.

In Romania, NGS for all patients with PPGLs still remains an aim towards we are moving with small but secure steps.

Starting from this necessity, the main aim of this work was to make genotype-phenotype correlations in Romanian patients with PPGLs and to emphasize their particularities. This work included not only genetically testing and analysing Romanian patients, but also retrospectively analysing a Flemish cohort of patients with PPGLs which will be presented and compared with our cohort in the Special part of this paper.

The following assumptions stand out at the foundation of this work:

1. The continuing growing spectrum of genetic mutation and the unique mechanisms of tumorigenesis in these tumors, along with the clinical variability specific for each cluster, lead to the idea of making a characterisation of Romanian patients with PPGLs.
2. The geographical variability of genetics in these tumors, referred as the 'founder effect' (*SDHD* for Italian and Dutch ancestry, *SDHB* for Spanish population, etc.). [11], [12] and the fact that we did not find any report about genetic landscape of PPGLs patients from Romania or Eastern Europe, raised our scientific consciousness about patients with PPGLs from Romania.
3. The extended scientific context developed in Western Countries had also triggered the necessity to extend the knowledge in this fields with our reports and to be a part of this scientific community.

This paper has the following content:

The first part - **General part** is dedicated for understanding the physio-pathological and molecular mechanisms of these tumors in the context of actual filed of knowledge along with historical and geographical distribution of their genotype.

The second part - **Special part,** contains three original studies:

The first study, entitled '**Particularities of genotype-phenotype correlations in a Romanian cohort of patients with PPGLs'** is a retrospective and prospective study, in which we genetically characterized patients with PPGLs and made genotype-phenotype correlations, emphasizing their particularities. **This is the first report about genetic landscape of patients with PPGLs from Romania.** Reported to rarity of these tumors, we had a large cohort of patients. This study is a retrospective and prospective research. Clinical, biochemical, imaging, histological and genetic data of patients diagnosed with PPGLs between-1976-2024 in 'CI Parhon' National Institute of Endocrinology were retrospectively retrieved. For newly diagnosed patients DNA samples were extracted from the peripheral blood cells and submitted to genomic study by NGS or by Direct sequencing.

The second study entitled '**Comparison between Romanian versus Flemish cohort of patients with PPGLs'** is a retrospective study, in which we emphasized genetic, clinical, biochemical, imaging and outcome differences between two cohorts of patients with PPGLs from two different geographical regions.

The third, and the last study entitled '**Metastatic PPGLs-genotypic and phenotypic particularities-descriptive study',** is a retrospective and prospective descriptive study. The main aim of this study was to identify genotype-phenotype corelations in Romanian patients diagnosed with PPGLs and to emphasize the particularities in presentation of PPGLs from our cohort.

In extensions, I will present the outcomes of this project:

A. Periodically follow-up with clinical, biochemical, and imaging screening for patients with personal history of PPGLs, according to international guidelines.
B. Genetic screening for early detecting (since childhood) of mutant genes in first-degree relatives of patients carrying a germline mutation related to PPGLs.
C. Romanian patients with PPGLs registered in the International ENSAT (European Network for the Study of Adrenal Tumors) database, which will allow us to be a part of various research projects. Participation to Working groups of ENSAT Network.
D. Finally, the last and the most productive objective of this project is to implement the NGS test for all the patients diagnosed with PPGLs, in Romania.

III. Study I - Particularities of genotype-phenotype correlation in a Romanian cohort of patients with PPGLs

III.1. Introduction

Working in the most representative centre of Endocrinology – 'C.I. Parhon' National Institute of Endocrinology from Bucharest, where, despite the rarity of these diseases, we had a large and complex cohort of patients with PPGLs, lead to the idea of the first study entitled **Particularities of genotype-phenotype correlations in a Romanian cohort with PPGLs.**

III.2. Material and methods

This is an analytical study and has a retrospective and prospective character. Retrospective data of patients consecutively diagnosed with PPGLs between 1976-2020 in Department IV of 'CI Parhon' National Institute of Endocrinology were retrieved from electronic records of the patients in our local database. These data included: anthropometrics, clinical, biochemical, histological phenotype, imaging data, therapeutic approach, genetics and outcome. New patients (diagnosed with PPGLs between 2020-february 2024) were prospectively evaluated starting from clinical to genetic approach.

Newly diagnosed patients were recruited from Department IV of 'CI Parhon' National Institute of Endocrinology. Patients' clinical, biochemical and imaging exams were performed in the same institution. Surgery was performed after an adequate pre-surgical alfa-blockade.

In total, 140 patients were included in this study.

Patients' **clinical data** included age at diagnosis, gender, previous history and family history of PPGLs, presence of other syndrome components, signs and symptoms reported at diagnosis. For the new patients, complete clinical exam, including ambulatory 24-h blood pressure monitoring was performed (ABPM) while being admitted in the hospital. The devise we used - TONOPORT™ V from GE Healthcare® with the CardioSoft™ Diagnostic System Ambulatory Blood Pressure (ABP) application. [13]

Signs and symptoms were grouped as following:

-symptoms related to catecholamine excess: secondary arterial hypertension, hypertension paroxysms, classic triad (headache, palpitations, sweating), trembling, nausea, weight loss, anxiety;

-local complaints caused by tumor compression;

-life-threatening events (myocardial infarction, Tako-tsubo cardiomyopathy, cardiac arrest, pulmonary oedema, epileptic crises, feto-placental apoplexy, pancreatitis).

Life-threatening events were considered those situations in which patients needed intensive care unit admission.

Method of discovery was categorized as:

-incidentally discovered

-genetic screening

-symptoms based diagnosis

Biochemical data included measurements of plasma and urinary fractioned normetanephrine, metanephrine and chromogranin A for the prospective evaluations and retrospectively retrieve MN values from the patient electronical records. Sampling plasma catecholamine metabolites was conditioned by fasting state and supine position for 30 minutes. Measurements were made using liquid chromatography separation followed by tandem mass spectrometry. The cutoffs for PPGLs diagnosis was set, as International Guidelines suggest, to > 2-3 folds upper limit of normal (ULN). [9] Noradrenergic secretion pattern was considered when normetanephrine levels were > 2-3-fold ULN, accompanied by metanephrine increase < 1.5 fold ULN, whereas adrenergic/mixt secretion pattern was considered when metanephrine levels were increased > 2-3 fold ULN.

Tumor localisation and dimensions were confirmed in most of the cases by contrast CT scan, and in some of them by MRI scan.

Diagnosis of PHEO/PGL was confirmed in all patients by histological diagnosis, but immunohistochemistry was available only in few cases.

Genetical analysis

Patients with classic MEN2A/MEN2B phenotypes underwent direct analysis of *RET* proto-oncogene, in the Genetic Laboratory of National Institute of Endocrinology, Bucharest,

Romania and the remained samples were submitted to National Institute of Oncology from Budapest Hungary to complete NGS, with the curtesy of Prof. Dr. Attila Patocs.

Most of the DNA samples from peripheral blood cells were stocked in our biobank before the study begun. In some patients, the DNA was extracted after the informed consent was signed. In total we had the DNA from 88 patients. The rest of them were either lost to follow-up or diagnosed before the era of genetic testing begun. At the time of enrolment, some patients were already tested for *RET* mutation. DNA was extracted from peripheral blood cells following a standard manually method. [14]

The screening for *RET* mutation was done by direct Sequencing (on Beckman CEQ-8000-Beckman Coulter) for exons: 5,7,8,10,11,13,14,15,16. For each exon, the whole coding sequence and minimum ten flanking base were examined. PCRs were performed using 100 or 200 ng of genomic DNA as input, in a final volume of 25 µl [20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM deoxynucleotide triphosphate, 1 U Taq polymerase, 1 mM specific primers].

The NGS sequencing was done using a commercial cancer panel -Trusight Hereditary Cancer Panel - targeting 113 genes, from Illumina®. In this panel, genes related to PPGLs (*VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2, NF1, MAX, TMEM127, HIF1A, FH, CDKN1B, MET, PPKAR1A*) and other non-related to PPGLs genes were covered (Image 5.1.). DNA input from patients' samples was prepared using Nextera Flex for Enrichment and sequenced on the MiSeq System; data was evaluated using the Base-Space Enrichment App version 3.1.0. The analysis included identification of germline pathogenic genetic variants, copy number variations (CNV). Somatic DNA was extracted only in 4 patients and the NGS genetic test was performed in the National Centre of Oncological Studies, Madrid, Spain coordinated by Dr. Mercedes Robledo.

Related to results from the genetic tests, PPGLs were classified as **hereditary, variant of undetermined significance (VUS) and sporadic**, in accordance to the American College of Medical Genetics (ACMG). [15], [16] Benign findings were not reported.

The study conforms to the Declaration of Helsinki and Good Clinical Practice guidelines. All patients were recruited under study protocols approved by the appropriate local institutional review boards or ethics committees of the participating centres. Informed consent for genetic analyses and use of existing clinical data was obtained from all patients.

III.3. Results

III.3.1. General characteristics of the cohort

In the study were included 140 patients with PPGLs, with a mean age at diagnosis of 47.9 ± 15.6 years. Ninety-seven (69.2%) were women, and 43 (30.7%) were men; 130 (92.8%) presented with PHEOs and 10 (7.2%) with PGLs.

In PHEO group, 71 (54.6%) were right sided while 44 (33.8%) were left sided and 15 (11.5%) were bilateral [9 (60%) synchronous and 6 (40%) metachronous].

In PGL group, 4 (40%) patients presented HNPGL, 4 (40%) retroperitoneal PGLs, 1 (10%) Zuckerkandl PGL, 1 (10%) mediastinal PGL. Four of them were non-functional. Eleven patients (8.4%) presented metastatic disease.
Mean tumor diameter at diagnosis was 51 ± 22 mm. Median plasmatic MN levels were 239 pg/ml (10-3365), 2.6-fold ULN, whereas median NMN levels were 1018 pg/ml (30-9808), 3 fold ULN. Median Chr. A levels were 176 ng/l (0-4630), 5 fold ULN. We found a direct correlation between NMN levels and tumor diameter ($p<0.001$).

III.3.2. Genotype-phenotype correlations in the Romanian cohort

From these 140 patients, 88 (62.8 %) were submitted to the genomic study. The genetic landscape of those 88 PPGL patients submitted to the genomic study showed the following:

Among them, germinal mutations were identified in 36.3 % (n=32) (22 *RET*, 3 *VHL*, 1 *SDHB*, 2 *NF1*, 2 *SDHD,* 1 *FANCA*, 1 *CASR*), VUS were described in 22.7 % (n=20). Patients from sporadic group represented 41 % (n=36) out of tested patients. In 3 cases, the interpretation of VUS was either likely pathogenic in ClinVar and Franklin [17], [18] Patients from hereditary group presented with bilateral tumors (n=12; 37%), right PHEO (n=10; 31%) and left PHEO (n=9; 28%), and HNPGL (1; 4%), while those with VUS presented right PHEO in a proportion of 75% (n=15). Mean age at diagnosis in patients with germinal mutations was significantly lower compared to those with VUS (37±15 vs. 53±12 years old, $p<0.001$) and to sporadic group (37±15 vs. 49.9±12.2 $p=0.001$). Patients from sporadic group presented higher NMN levels compared to hereditary cases ($p=0.003$)

From 36 patients of sporadic group, 26 (72%) were women and 10 (28%) men. Mean age of diagnosis for sporadic group patients was 49.9±12.2 years old. In this group, 32 had PHEOs

(17 right, 15 left) and 4 patients presented with PGLs (1 glomus caroticum, 1 pulmonary, 1 retroperitoneal, 1 Zuckerkandl). Two of them had metastatic disease and some of them had apparently hereditary treats.

Thirty-seven percent patients (n=52) were not tested due to lack of DNA samples. Their mean age at diagnosis was 52±16 years old, 37 F, 15 M. Twentynine (55.8%) of them presented right PHEO, 15 (28.8%) left PHEO, 3 bilateral PHEO (5.8%), 3 (5.8 %) abdominal PGL, 2 HNPGL (3.8%). Seven of them developed metastatic disease.

I. Hereditary group

Patients with *RET* mutation

Patients with *RET* pathogenic variant represented the majority (68.7 %) of patients with germinal mutation and 25 % of the tested patients.

Twenty-two patients from fifteen families were analysed. All of them presented MEN2A syndrome. Fourteen patients (63.6%) were women and 8 (36.4%) were men. Mean age for diagnosis was 36.6±2.8 years. Nine patients (40.9%) developed bilateral tumors (4 synchronous or 5 metachronous). Maximum tumour diameter was 46.4±3.5 mm. Five patients were diagnosed due to genetic screening. In 10 patients, PHEO was the first manifestation of the disease. One of them presented with metastatic disease, case that will be presented in the Study no.3. Median follow-up duration was 8 (IQR 11.5) years.

These patients, had the following genetic profile: eighteen patients (from 12 families) had a mutation of exon 11 [*p.Cys634Trp* in (55.5%), *p.Cys634Arg/Tyr* 22.2%/16.6%; *p.Asp631Tyr* (5.5%)]; 4 (from 3 families) had a mutation of exon 10 [*p.Cys618Arg* (75%), and *p.Cys618Tyr* (25%)].

Three (3.75%) out of 88 patients and 9.3% of patients from hereditary group had *VHL* mutation. Two patients presented with *VHL type* 2A syndrome (mother and daughter) - *c.245G>T; p.Arg82Leu*, and 1 with *VHL type* 2C (*c.482G>A; p.Arg161Glu*). Patients from the same family presented with PHEO and hemangioblastomas, while the third patient presented with isolated PHEO and with a family history of hypophyseal adenoma (her mother). All patients developed PHEO in childhood (mean age of diagnosis 12.3±2.3 years), all of them were women. All three patients presented with synchronous bilateral tumours. None was metastatic. Median follow-up duration for these patients was 5 years (range 3-38).

One patient with VHL2A, died due to VHL syndrome related complications (hemangioblastomas, back spine tumors). The third patient with VHL2C was disease free at 10 years of follow-up.

NF1 *(c.5513_5514del; p.Ser1838Tyrfs*23);(c.1308G>A; p.Ser436=):* Male patient with clinical signs of neurofibromatosis was diagnosed at 39 years old with a left PHEO with noradrenergic secretion pattern. He did not develop tumor recurrence at 10 years follow-up. Woman diagnosed with PHEO at 52 years old, with family history of colonic cancer but with no other tumors or clinical signs of *NF1*.

SDHD *(c.341A>G; p.Tyr114Cys);* large deletion*:* One patient, woman, was diagnosed at 56 years old with left PHEO, with noradrenergic secretion pattern. She also had renal and splenic cysts. The second patient presented with HNPGL located latero-cervical on the left side at 67 years old. He also developed a pulmonary tumor with adrenal metastases.

SDHB *(c.725G>A; p.Arg242His);**MLH1** (c.902A>G; p.Gln301Arg) (VUS*): This patient was diagnosed with right PHEO at 36 years old at diagnosis and no recurrence at 7 years from the diagnosis. He had no family history of PHEOs, but his siblings were not tested yet for SDHB mutation. This patient associated a VUS of *MLH1* gene.

CASR *(c.1856_1857delTT)*: This patient was diagnosed at 57 years, with left PHEO and noradrenergic pattern. She had no parathormone or calcium abnormalities but presented hepatic and renal cysts. She had no family history of tumors.

FANCA Patient aged 53 years old, was diagnosed with right PHEO with noradrenergic secretion pattern. He had no other diseases related to *FANCA* mutation, but had a daughter diagnosed with hepatocarcinoma at 37 years old. He was disease free at 9 years of follow-up.

II. Patients with VUS

This group represented 22% (n=20) of tested patients. Mean age at diagnosis was 51.9±12 years, four of them had less than 40 years at diagnosis. Women were predominant n=14 (70%) compared to men n=6 (30%). All of them presented with PHEO, 13 (65%) on the right side and 7 (35%) on the left. Some of them presented clinical phenotype of hereditary PHEO.

One patient (*MSH 6 c.1474A>G*) associated PHEO with pulmonary adenocarcinoma; another patient (*BRIP 1 c.728T>C*) had an aggressive pattern of malignant PHEO and family history of colonic cancer.

One patient had clinical phenotype of neurofibromatosis. She was diagnosed with PHEO at 35 years and her son has also clinical signs of neurofibromatosis. In her case, we identified somatic mutation of NF1 (*c.7966del*), and a VUS in germline (*MSH2+ATM c.1134_1136delAGA+ c.1444A>C)*.

In two patients we identified calcium metabolism abnormalities: one patient (*MEN1 c.526G>T*) associated PHEO and familial hypocalciuric hypercalcemia, another one had PHEO and idiopathic hypercalcemia (*RB2 c.644C>T*) respectively primary hyperparathyroidism (*ATM c.5639C>T*). Another patient with a VUS in *BARD1* (*c.1333G>A*) presented PHEO, papillary thyroid carcinoma and colonic polyps and had a family history of leukaemia and sudden death.

III.3.3. PPGLs associated with life threatening events and asymptomatic PPGLs

Seventeen (12 %) patients needed intensive care unit admission due to life threatening events. Most of them suffered for cardiovascular complications. Two of them developed complications during surgery for PPGL. Median age of diagnosis for these patients was 43.5 years (9-72), most of them were women (n=14, 82 %). PHEOs were the cause of these events in 14 (82 %) patients while in two patients aggressive mediastinal PGL respectively juxta renal PGL were the cause of the life-threatening events. Most of them had benign disease, but 2 (11.7%) presented metastatic disease. Median tumor diameter was 48 mm (20-140).

The most reported event was Tako-Tsubo cardiomyopathy (n=5, 29 %), followed by myocardial infarction (n=3, 17.6 %). These events happened more frequently in age interval of 40-65 years. In younger patients (18-40 years), obstetrical complications were more often reported.

Regarding genetic status of these patients, 2 had hereditary disease (*RET* and *VHL* mutation), 5 were sporadic, and 3 of them had a VUS. The rest of them were not tested.

All patients with complications survived. Patients with Tako-Tsubo cardiomyopathy had normal cardiac function few days after the event. One patient with acute coronary syndrome and pulmonary edema but a background of other cardiac comorbidities had the ejection fraction of 40% after this event. A woman with feto-placental apoplexy had a negative outcome: her baby died in uterus and she underwent hysterectomy at 34 years old.

Comparing patients with life-threatening events with those without life-threatening events we found that there is any significant difference in tumor diameter, plasma MN levels between both groups, but patients with complications were younger (44±15.6 years) than those without complications (48±15.6 years), respectively, had urinary MN levels significantly higher compared to those without complications (urinary MN: 4395 vs.1035, p=0.002).

Twenty (14.3%) patients were incidentally discovered. Among them, fifteen (10.7%) were asymptomatic at diagnosis (i.e. free of catecholamine excess symptoms or local complaints for cervical PGLs). Nine patients were discovered due to genetic screening (6.4%).

At a deeply anamnesis, the incidentally discovered patients presented either anaphylactic shock and cardiac arrest during delivery, tremor, anxiety, lumbar pain, sweating, headache or hypertension. Mean age at diagnosis in asymptomatic patients was 53.6±15.9 years, 10 women and 4 men. Four of them had cervical, non-functional PGLs and 11 presented with PHEOs. Two patients with PHEO were diagnosed after imaging investigations for abdominal pain, the rest of them were discovered either after a routine screening.

Between asymptomatic patients and incidentally/symptom based discovered patients, we identified a significant difference in age of diagnosis (55.5 ± 15.6 versus 47 ± 15.4 years, p= 0.047), but any differences in MN or NMN levels or in tumor diameter (45.2 ± 52.7 mm). When comparing age in asymptomatic patients versus in those with life-threatening events we did not find significant differences between MN levels, tumor diameter or age at diagnosis.

III.3.4. Bilateral PHEO

Fifteen patients (10.7%) presented bilateral PHEOs, 9 (60%) synchronous and 6 (40%) metachronous. Median age of diagnosis for these patients was 32 (9-76) years (3 had paediatric age at diagnosis). Most of them were women 10 (66.7%). Median follow-up duration was 5 years (1-41). Two out of 15 patients presented metastatic disease at diagnosis, while another patient developed metastases after 11 years of follow-up. Maximum tumor diameter at the first diagnosis was 49 mm (15-70). A noradrenergic secretion pattern was reported in 7 patients, whereas adrenergic secretion pattern was reported in 6 patients and in 2 patients we did not have the information about secretion pattern.

Regarding genetic status, 12 (80 %) of them had hereditary disease (9 MEN2A syndrome and 3 VHL syndrome). In patients with MEN2A, the most frequent mutation was in 634 (*p.Cys634Arg*) codon (8 out of 9 patients), while 1 patient had 618 (*p.Cys618Arg),* codon mutation. Two of the patients with *VHL* mutations were part of a family (mother and daughter),

expressing *VHL* 2A syndrome and one patient, without family history of PPGLs had *VHL* type 2 C syndrome.

Six patients underwent adrenal sparing surgery:

-5 bilateral; 1 cortical sparing on one side and total adrenalectomy on the other side. Three of them developed adrenal insufficiency. One patient with *VHL* syndrome and benign bilateral PHEOs developed recurrence at 7 years (bilateral recurrence after CSS) and 24 years after the first surgery (unilateral recurrence after total adrenalectomy), respectively.

In 7 patients total adrenalectomy was performed. All of them developed adrenal insufficiency. Recurrence was detected after 11 years from unilateral adrenalectomy in 1 patient with MEN2A syndrome He had a calcified contralateral tumor that was inoperable because of the calcification. This patient developed metastatic disease.

III.4. Conclusions

Our study is the first report that summarised genetic landscape of patients with PPGLs from Romania. PHEOs/PGLs ratio is higher in our cohort compared to the percentage already known. Women develop PPGLs more frequently than men.

PPGLs were related to a germinal mutation in approximately 36% of cases. Patients with hereditary PPGLs develop the disease in younger age compared to sporadic or VUS cases. Patients from hereditary group had lower NMN levels compared to sporadic group.

In patients with hereditary disease, *RET pCys634TRp* associated to MEN2A Syndrome was the most prevalent mutation in our cohort, as a founder effect for Romanian population of PPGLs. Patients with mutations in *RET* 634 codon, develop the tumor in younger age and have a more aggressive pattern compared to *RET* 618 codon.

The second mutation in order of frequency in our cohort, was *VHL* mutation.

Some of the patients with VUS associated tumors suggestive for a hereditary disease or a syndrome. In this cases, cumulative reports about this variants and functional testing of these genes should be applied, in order to establish the pathogeny of those variants.

Among sporadic group, there were patients with characteristics of hereditary disease. In their cases, other genes that were not included in the gene panel could be involved.

Patients with functional PPGLs can develop life-threatening complications. The most reported life-threating event in our cohort was Tako-Tsubo cardiomyopathy followed by myocardial infarction.

Patients with asymptomatic PPGLs have lower age at diagnosis and lower urinary MN levels compared to patients with life-threatening events.

Bilateral PHEOs are most frequently associated to *RET (p.Cys634Arg* being the most prevalent variant) or *VHL* mutation. Synchronous tumors were more frequently described.

In bilateral PHEOs the risk balance between adrenal insufficiency and recurrence should be carefully evaluated by a multidisciplinary team.

In case of a familial MEN2A syndrome, genetic counselling has an important role for establishing the perfect time for prophylactic thyroidectomy in mutation carriers.

IV. Study II - Comparison between Romanian versus Flemish cohort of patients with PPGLs

IV.1. Introduction

The aim of this study was to compare the genotype and phenotype characteristics from two cohorts of patients with PPGLs from two different geographical and historical regions of Europe. This is an analytical cohort study.

IV.2. Material and methods

For this study, retrospective data was collected from electronic medical records of 67 consecutively registered patients diagnosed with PPGL in Endocrinology, Pathology and Surgery Departments from Ghent University Hospital (Belgium) registry, between 2002-2020.

According to anatomic location, patients were divided into two groups PHEO vs. PGL; further, each group was divided according to genetic status in hereditary versus sporadic. Seven out of 67 patients, did not perform genetic test, therefore they were not included in any of the groups, but discussed separately.

Anthropometric data, age at diagnosis, gender, clinical phenotype (symptoms, localization, multiple tumors at diagnosis, metastatic behaviour, biochemical phenotype, tumor

diameter and *SDHB* immunostaining were compared between two groups hereditary versus sporadic PHEO/PGL.

The diagnosis cutoffs for MN levels were set, as International Guidelines suggest, to > 2-3 folds ULN. [9] Noradrenergic secretion pattern was considered when normetanephrine levels were > 2-3-fold ULN, accompanied by metanephrine increase < 1.5 fold ULN, whereas adrenergic secretion pattern was considered when both metabolites were increased > 2 fold ULN.

Signs and symptoms were grouped as PPGLs related symptoms: secondary arterial hypertension, hypertension paroxysms, classic triad (headache, palpitations, sweating), trembling, nausea, weight loss, anxiety, local complaints and life-threatening events (myocardial infarction, Tako-tsubo cardiomyopathy, pulmonary oedema etc.).

Method of discovery was categorized as:

-**incidentally discovered** for PPGLs discovered for symptoms non-related to PPGLs or for general screening;

-genetic screening for PPGLs diagnosed in patients with a family history of PPGLs or for patients with a confirmed germinal mutation;

-symptoms based diagnosis for patients diagnosed due to PPGLs-related symptoms.

Metastatic PPGLs was defined as tumor spread to non-chromaffin tissues. Multiple tumors were defined as presence of bilateral adrenal primary tumors or multiple PGL synchronous to diagnosis.

Recurrence was defined if a patient presented with a metachronous tumor in the same place of the primary tumor, while new disease was defined when a new tumor in other chromaffin tissue occurred.

Genetic test was performed using NGS or direct sequencing for patients with clinical features for a PPGLs related syndrome, at the Centre for Medical Genetics, Ghent University Hospital, Belgium. The technology used was KAPA HyperCap technology (Roche Sequencing) or other depending on diagnosis date. Gene panel covered most frequently genes involved in PPGLs pathogenesis: *RET*, *VHL*, *SDHA, SDHB, SDHC, SDHD, SDHAF2, NF1, MAX* and *TMEM 127*.

Material and methods used for the first study, which generated the results from Romanian cohort, were described in the first Chapter of the special part.

The first phase of this study was to analyse the above-mentioned characteristics of the Flemish cohort and to analyse and describe the differences between sporadic versus hereditary PHEO/PGL groups of the Flemish cohort.

The second phase of this study was to compare the general characteristics between the two groups: PHEO/PGL distribution, genetic profile, age at diagnosis, gender distribution, clinical presentation, presence of multiple tumours at diagnosis, metastatic rate, methods of discovery, tumour dimensions, biochemical phenotype, histological aspects and outcome.

IV.3. Results

IV.3.1. Phase I-Characteristics of the Flemish cohort and comparison between sporadic versus hereditary PHEO/PGL groups

In the study were included 67 patients, 38 (56.7%) women, 29 (43.3%) men, most of them presented with PHEO 42 (63%) and 25 (37%) with PGL (6 had multiple tumors at diagnosis). Mean age at diagnosis was 50 ± 19 years (range 13-85 years). From 60 genetically tested patients, 24 (40%) had germinal mutation and were included in the hereditary group, while 36 (60%) did not present any mutation in the tested genes. Among patients with hereditary disease, in order of frequency we found mutations in the following genes: *SDHD* (n=10; 41.7%), *SDHB* (n=8; 33.3%), *VHL* (n=2; 8.3%), *RET* (n=2; 8.3%), *MAX* (n=1; 4.2%) and *NF1* (n=1; 4.2%). PHEO cases were more frequently sporadic than hereditary, while PGL were in most of the cases hereditary (27 vs.8), respectively (9 vs.16). Patients with PHEO were diagnosed at older age than those with PGLs (55 ± 17 vs. 40 ± 18 p=0.001). Six patients presented metastatic disease [4 (9.5%) PHEO and 2 (8%) PGL] either synchronous (4) or metachronous (2).

Regarding methods of discovery, half of the patients were discovered due to PPGLs related symptoms [PHEO: 21 (50%); PGL: 13 (52%)], and the other half were incidentally or due to genetic screening discovered [PHEO: 21 (50 %); PGL: 12 (48 %)]. Patients with PGLs more often accused local complains than those with PHEOs: 1 (2.3%) vs. PGL: 7 (28%); p = 0.009.

Life-threating events were more frequently reported in patients with PHEO than in those with PGL [PHEO: 14 (33.3%); PGL: 3 (12%); p = 0.022].

We found a direct relationship between normetanephrine levels and tumor diameter and the occurrence of a life-threatening event. Urinary normetanephrine levels and tumor diameter were higher in patients with life-threatening events than in those without life-threatening events (5885 vs. 3089 µg/24h, p=0.001) respectively (51.4 vs. 28.6 mm, p=0.02). All PHEOs and about 2/3 of PGLs were functional.

Patients with PHEO were treated by surgical intervention, with one exception: the patient had large vessels transposition; therefore, the surgery was improbable. Four patients with metastatic disease, from PHEO group, underwent MIBG therapy (3) and radiotherapy (1), after surgery. From PGL group, 5 patients with HNPGL were treated only with radiotherapy, because of the surgically inaccessibility.

IV.3.2. Comparison between Romanian and Flemish cohort

The first major difference between Romanian and Flemish cohort is the large number of patients in Romanian cohort (140 vs. 67). Flemish patients presented with a significantly higher percentage of PGLs compared to Romanian population (37% vs. 7.2%, p=0.001). HNPGLs were most frequently described in the Flemish cohort (56%), while in Romanian cohort, abdominal and HNPGL had the same proportions (40%).

Patients from Romanian cohort presented more frequently with bilateral PHEOs, compared to Flemish cohort (15; 19.3% vs. 3; 7.1%; p=0.002). Most of them were hereditary (80%) in Romanian cohort and 100% in Flemish cohort, related to *RET* or *VHL* mutation in Romanian cohort and with *VHL* and *MAX* mutation in Flemish cohort.

Multiple PGLs were reported in Flemish cohort in a proportion of 24% while in Romanian cohort, none of the patients with PGLs presented multiple tumors.

No difference in age at diagnosis was observed between the two cohorts. In Romanian cohort, the percentage of woman, was higher, but without a significant difference between cohorts. Interestingly, in Flemish cohort, a higher number of patients were incidentally diagnosed compared to Romanian cohort.

Patients from Flemish cohort had a better access to genetic test, therefore, a higher percentage of hereditary cases were identified in Flemish cohort compared to Romanian cohort (40 vs. 36%). From hereditary group, Romanian patients presented most frequently with *RET* mutation (68.7%), while in Flemish cohort, the most prevalent mutation was *SDHD* (41.7%).

Patients with RET mutation from Romanian cohort, presented in 100% of cases with PHEOs, while in Flemish cohort, 90% of *SDHD* carriers developed HNPGL and the rest PHEO. Interestingly, 40 % of patients with *SDHD* mutation developed multiple tumors (i.e. other PGL associated to HNPGLs).

The second most reported mutation was *VHL* in Romanian cohort (9.2%), while in Flemish cohort, *SDHB* was described in 33% of cases.

IV.4. Conclusions

We can conclude that in Romanian cohort was observed a higher PHEO/PGL ratio compared to Flemish cohort. Romanian hereditary PPGLs are more frequently associated to *RET p.Cys634Trp* pathogenic variant, while in Flemish cohort, we found a *SDHx* related PPGLs predominance (*SDHD c.170-1 G>T* was most frequently described followed by *SDHB c.206 G>T p.Gly69Val).*

Based on this genetic background, Romanian patients presented more often with bilateral PHEOs, while Flemish patients presented more often with multiple PGLs. HNPGL had similar proportions among PGL group in both cohorts.

Patients from Belgium are more often incidentally diagnosed with PPGLs or due to genetic screening than Romanians, suggesting a higher addressability of them to medical services.

PGLs are more frequently caused by a germinal mutation than PHEOs, therefore, PGLs are discovered at a younger age. Patients from PHEO group presented more often cardiovascular complications, since most of them are functional, compared to PGLs. Somatic mutation can lead to aggressive forms of the disease, therefore, patients with apparently sporadic PPGL but with an aggressive behaviour should be tested for somatic mutations. In addition, genetic diagnostic laboratories should make a personalized gene panel in which they can include new discovered genes such as *FH, SLC25A11, GOT2* and others, at least until whole exome/genome sequencing is routinely available and try to make genotype-phenotype correlations for these new genes, to facilitate the diagnosis.

This clinical and genetic variability could lead to the conclusion that PPGLs have specific pattern of presentation, dependent to geographical area.

V. Study III - Metastatic PPGLs-genotypic and phenotypic particularities-descriptive study

V.1. Introduction

Since 2018, WHO classification of pheochromocytomas and paragangliomas referred to PPGLs as metastatic rather than malignant and non-metastatic, rather than benign. Metastatic PHEO/PGL are those tumors with secondary lesions in non-chromaffin tissues. [19], [20] Common metastasis sites are bone, lymph node, liver or lungs. [21]

About 10% of PHEOs and ~40% of sympathetic paragangliomas have metastatic behaviour. Most of HNPGLs are benign. [22], [23] From metastatic cases, more than 40% of cases are caused by a germline mutation (even in the context of a negative family history). Synchronous metastases are reported in 35–50% of cases, whereas metachronous metastases can develop within months or decades. [23] The median survival rate of patients with metastatic PPGL is ~ 6 years, but the progression rate in variable. [21], [24] Some metastases may occur even at 50 years from the first diagnosis. [23], [25]

V.2. Material and methods

This is a retrospective and prospective study. From the electronic records of patients diagnosed with PPGLs between 1986-2024, in the 'CI Parhon' National Institute of Endocrinology, we retrieved those patients with metastatic PPGLs, based on functional imaging confirmation of secondary lesions in non-chromaffin tissues. [19] Clinical, biochemical, imaging, histopathological and genetic data were retrieved from those patients. The same data was collected for the newly diagnosed patients.

Patients were tested for germinal mutations using NGS based technique.

Most of the DNA samples from peripheral blood cells were stocked in our biobank before the study begun. In some of the patients the DNA was extracted after the informed consent was signed. The rest of them were either lost to follow-up or diagnosed before the era of genetic testing begun.

Inclusion criteria: patients with imaging/histological confirmation of synchronous or metachronous metastases of PHEO/PGL.

V.3. Results

Eleven patients (7.9%) presented with metastatic PPGLs, 8 women (72.7%) and 3 men (27.3%), most of them presented with PHEOs 9/11 (81.8%) and two patients (18.1%) with PGLs (1 retroperitoneal and 1 mediastinal). Most of the PHEOs were right sided (8/9), and one patient presented with synchronous bilateral PHEO. Median age at diagnosis was 52 years (range 31-62). Age at diagnosis did not differ from non-metastatic cases. Median follow-up duration was 8.5 years (range 8-21). Patients with metastatic disease presented with larger tumors at diagnosis compared to non-metastatic patients (50±20 vs. 74±35, p value= 0.003). Seven of them presented with noradrenergic secretion pattern while 4 of them had mixt secretion (adrenergic and noradrenergic). Seven patients were tested for geminal mutations. Among them, 1 had pathogenic variants of *RET* gene (MEN2A syndrome) and one of them had a VUS of *BRIP 1* gene. Four patients presented with synchronous metastases, while 7 had metachronous metastases. Metastases occurred up to 10 years from the first diagnosis. Most of them were located to lungs, liver, and abdominal lymph nodes. Some of the patients developed metastases in unusual places such as: ocular or urinary bladder. Four patients underwent radionuclide therapy (3 MIBG and 1 MIBG+ PPRT). Their outcome was the following:

-1 had a good response on MN levels, symptomatology, and stationary lesions at 6 years of follow-up

-1 was completely non-responsive to MIBG therapy

-1 had a partial control: symptomatology control and stationary lesions but high metanephrine levels.

-1 was non responsive to MIBG but partially responsive to PRRT (symptomatology control, but high MN levels and progressive lesions. In the patient with PPRT, lesions were stable 2 years after PRRT, but metanephrine levels still increased. This case is also detailed in the following pages (Case no 1.)

Recurrence occurred in six patients (54.5%), before metastatic disease spread.

Interestingly, the patient with retroperitoneal PGL associated a gastric neuroendocrine tumor and a pulmonary hamartoma, suggestive for Carney Triad but any germinal mutation.

VI. Discussion and conclusions

Study no. 1

This study is the first report, including a large number of patients, which describes the genetic landscape of PPGLs from Romania. Furthermore, the first study of this work extensively described the genotype-phenotype correlations in patients with PPGLs from Romania.

PHEOs/PGLs ratio was higher in our cohort compared to the already known data from the literature. Women develop PPGLs more often than men.

Patients with PGLs presented most frequently with abdominal and HNPGLs.

Although we tested only 62% of our patients, compared with the percentage known from the literature, among them, we found a similar proportion of hereditary PPGLs. [6], [26] As expected, patients with hereditary PPGLs, developed the disease in younger age compared to sporadic or VUS group.

In the hereditary group, we identified a predominance of *RET* mutation associated to MEN2A, followed by *VHL.* We found a smaller proportion of *SDHx*-related PPGLs compared to other studies. [27], [28]

In all studies, MEN2A-related PHEO have a higher penetrance in patients with 634 codon mutation; usually, the expression of PHEO is 50% for codon 634 mutation carriers and 33% for codon 618. [29] The predominance of this codon was also described in a cohort from Brazil and in another small cohort of patients from Slovakia and Spain. [30], [31], [32], [33] A large study, including 812 patients with pheochromocytoma in MEN2A syndrome from 4 different regions of the globe (South America, South Europe, Western Europe and Central Europe), showed as well, that in all geographical regions 634 codon mutation was most frequently reported, followed by 618 codon. [34]

Moreover, codon 634 cysteine is a hot spot for MEN2A syndrome, *p.Cys634Arg* is the most representative pathogenic variant of codon 634, in Europe. [35],[36] On the other hand, in our *RET* group of patients, mutation of 634 (*p.Cys634Trp*) codon was the most frequently reported mutation.

The predominance of this variant (*p.Cys634Trp*) in our cohort could be considered as a founder effect, as this variant is specific for our country. Other studies from countries with Latin origins, reporting the genetic background of MEN2A related PHEO, found that the most prevalent variant was *p.Cys634Gly* (in a large cohort from Brazil); respectively *p.Cys634Tyr* in a cohort from Spain and Italy. [35] In the Spain cohort, *p.Cys634Trp* was associated to a lower PHEO penetrance. [30], [33] In Italy, Greece, Slovenia, Cyprus, Turkey, the most reported RET variant was *p.Cys634Arg*. [35]

Similar results as in our cohort, with a predominance of PHEO expression in *p.Cys634Trp* mutation (80% of patients with this variant developed PHEO) was described in a study from 2007 developed in USA, including a large cohort of patients with MEN2A. [29]

Among patients with VUS, there are some clinical associations suggestive for a syndrome, which were not described earlier. For this category of patients, further reports, even functional testing of the genes should be applied in order to categorise them as pathogenic. Among sporadic group, there were patients with characteristics of hereditary disease. In their cases, other genes that were not included in the gene panel could be involved.

Patients with functional PPGLs can develop life-threatening complications, even in an incidentally finding of PHEO/PGL. The most reported life-threating event in our cohort was Tako-Tsubo cardiomyopathy followed by myocardial infarction.

Patients with life-threatening events present higher levels of urinary MN compared to patients without these complications. Patients with life-threatening events are diagnosed at a younger age and have higher urinary MN levels compared to asymptomatic cases.

Bilateral PHEOs are in most of the cases hereditary, associated to *RET* (9/15) or *VHL* (3/15) mutation. Patients with bilateral PHEOs, presented a higher percentage of synchronous than metachronous tumors. In this situation, before surgery, the risk balance between adrenal insufficiency and recurrence should be carefully evaluated by a multidisciplinary team.

In summary, PPGLs have a high genetic determinism, and there is still a lot to discover about their genotype-phenotype association. A new entity of patients with PPGLs that have a VUS and clinical aspects of hereditary disease is up to be explored.

While every cohort report, brings new characteristics reported for these patients, the already known classification of PPGLs will stand for new changes. Although genetic diagnosis

is achieved in about 75–80% of these patients, genetic aetiology remains unexplained in a significant percentage of cases.

Study no.2:

In this study we emphasized the differences between the two cohorts of patients with PPGLs from 2 different geographical regions.

In both cohorts the percentage of hereditary cases were similar to those described in the literature. [37] Romanian patients presented more frequently with *RET p.Cys634Trp* mutation while Flemish patients with *SDHD c.170-1 G>T* pathogenic variant. The genetic background of Flemish cohort, it is an expected situation, as many studies report a predominance of *SDHD* related HNPGLs, followed by *SDHB* mutations in Netherlands. [12], [38], [39] They stipulate the presence of a founder effect of *SDHD*, c.274G>T*; p.Asp92Tyr* [38]. But, in our cohort, the most frequently *SDHD* mutation variant was *SDHD c.170-1 G>T,* a fact that may also be considered as a founder effect for Flemish population.

After a literature search, we did not find studies about mutational profile of patients with PPGLs from Eastern Europe. Only a small Slovak report showed a predominance of *RET* mutation in their cohort. [31] However, this result could be biased by the fact that in our Centre, *RET* mutational profile is the only genetic test that can be performed. Furthermore, being a Referral Centre of Endocrinology from Romanian, most of the patients diagnosed with thyroid nodule as possible MTC, are redirected to our Centre.

In both cohorts, PHEOs were more frequently reported than PGLs, but Romanian patients presented with a higher PHEO/PGL ratio than Flemish patients. This difference in presentation was also observed in a study describing a cohort from Slovakia, a country from Eastern Europe where patients presented with PGLs in a lower proportion than those described in the literature. [31] Another study, comparing two cohorts from Western Europe, respectively Asia, described a lower PHEO/PGL ratio compared to European cohort (75%/25% vs. 81/29%). [27], [31] A lower ratio of PHEO/PGL presentation was also described in other studies from Netherlands. [39]

In Romanian cohort, women had a greater ponder than men, while in Flemish cohort, no significant differences between gender distribution were seen. It is known that PHEOs are more frequently reported in women. This instance may be explained by the predominance of PHEOs

in Romanian cohort. Mean age at diagnosis was similar between two cohorts, and similar to already known data. [40], [41]

Bilateral PHEOs were identified in a higher proportion in Romanian cohort. On the other hand, multiple PGLs were more frequently reported in Flemish cohort. This situation may be generated by the genetic background of PHEOs in Romanian cohort, where most of them were associated to *RET* mutation, a mutation that predispose to bilateral tumors in approximately 50% of the cases [42], while the clinical expression of PGLs in Flemish cohort may be explained by the genetic background of the two cohorts - higher percentage of *SDHx* related PGLs compared to Romanian cohort.

The predominance of incidental finding diagnosis in Flemish cohort, compared to Romanian cohort it may be explained by a higher access to healthcare in developed countries. Moreover, patients' relatives in Belgium, had a higher addressability for genetic screening than in Romania.

Study no.3:

This study emphasized the heterogenous behaviour of patients with metastatic PPGLs. The percentage of patient with metastatic PPGLs is the same as in the literature. Even though PGLs are more often associated to metastatic disease, in our cohort, most of the patients had PHEO. We observed a predominance of women diagnosed with metastatic PPGLs. Mean age of diagnosis in metastatic PPGLs did not differ from the mean age for diagnosis of non-metastatic PPGLs.

Patients with metastatic PPGLs have a higher primary tumor dimension compared to non-metastatic cases. Metastases occurred even 10 years after the first diagnosis, therefore a close follow-up is recommended for patients at risk to develop a metastatic PPGLs: older age at diagnosis, male sex, synchronous metastases, high dopamine levels, tumor dimensions higher than 6 cm and/or PASS score >4.

Most common metastases sites are liver, lungs and bones, but they can also occur in non-ordinary places such as eyes, urinary bladder.

In our cohort, most of metastatic PPGLs were not hereditary cases. Moreover, we observed that *RET* mutations can be related to aggressive forms of PPGLs, un unexpected situation. There are no predictor factors for diagnosis of metastatic PPGLs.

Limitations

This project may also have some limitations. Due to the partially retrospective character of the studies and due to the subjectivity of the anamnestic data, some clinical details were missing or were incomplete, leading to inconsistent data. On the other hand, the large diagnosis and follow-up interval lead to the heterogeneity of paraclinical details, such as in some patients we only had urinary or plasma MN while in other had only adrenaline levels. Another important limitation, is that caused by the inaccessibility to NGS in our centre at the time of diagnosis, therefore we had a restricted number of patients addressed to NGS.

VII. Personal contributions and future directions

This work is the first report, (including a large number of patients) about the genetic landscape of patients with PPGLs from Romania.

Through this work we brough insight about the genotype-phenotype profile of patients with PPGLs from Romania, deeply describing their clinical behaviour and genetic profile. We identified a founder effect specific for our country, which was not identified in other countries from Europe. In Western Europe, patients with PPGLs usually harbour a mutation in *SDHx* complex.

The final outcomes of this work are the following:

A. Periodically follow-up with clinical, biochemical, and imaging screening for patients with personal history of PPGLs, according to the international guidelines.
B. Awareness about the importance of genetic screening for early detecting (since childhood) of mutant genes in first-degree relatives of patients carrying a germline mutation related to PPGLs.
C. Registration of Romanian patients with PPGLs in the International ENSAT (European Network for the Study of Adrenal Tumors) database, which will allow us to be a part of various research projects. Participation to Working groups of ENSAT Network.
D. Finally, the last and the most productive objective is the implementation of NGS test for all the patients diagnosed with PPGLs, in Romania. In the present the NGS test is implemented in 'CI Parhon' National Institute of Endocrinology, but only for selected cases.

Future directions:

Next step of this project is to make the genetic screening of all patients' first degree relatives. For mutation carriers, we will continue the clinical, biochemical and imaging screening for an earlier diagnosis of the disease. In this way we can prevent disease evolution to an advanced stage. In this phase, genetic counselling has a huge importance. PPGLs management needs a translational approach

A special attention in the future will be applied to NF1 patients. We are willing to specifically retrieve data about patients with NF1 and to screen them for PHEO.

Another research direction is to investigate clinical and immunohistochemical aspects of metastatic PPGLs to develop a better predictability score for metastatic potential and to further explore the therapeutic options for these patients.

I assume that this work achieved its objectives and brought a new perspective about the importance of genetic test in PPGLs patients from Romania, hoping that this work will be a foundation stone for further NGS testing as a routine practice in patients with rare diseases such as PPGLs.

Bibliography

[1] A. Cascón *et al.*, "Genetics of Pheochromocytoma and Paraganglioma in Spanish Patients," *J Clin Endocrinol Metab*, vol. 94, no. 5, pp. 1701–1705, May 2009, doi: 10.1210/JC.2008-2756.

[2] A. A. Leung *et al.*, "Epidemiology of pheochromocytoma and paraganglioma: population-based cohort study," *Eur J Endocrinol*, vol. 184, no. 1, pp. 19–28, Jan. 2021, doi: 10.1530/EJE-20-0628.

[3] J. Holland and V. Chandurkar, "A retrospective study of surgically excised phaeochromocytomas in Newfoundland, Canada," *Indian J Endocrinol Metab*, vol. 18, no. 4, pp. 542–545, Jul. 2014, doi: 10.4103/2230-8210.137514.

[4] A. Rahman, A. Subhi, V. Boyle, M. S. Elston, and M. Elston, "Systematic Review: Incidence of Pheochromocytoma and Paraganglioma Over 70 Years," *J Endocr Soc*, vol. 6, pp. 1–9, 2022, doi: 10.1210/jendso/bvac105.

[5] C. Offergeld *et al.*, "Head and neck paragangliomas: clinical and molecular genetic classification," *Clinics*, vol. 67, no. Suppl 1, p. 19, 2012, doi: 10.6061/CLINICS/2012(SUP01)05.

[6] A. Cascón *et al.*, "Genetic bases of pheochromocytoma and paraganglioma," *J Mol Endocrinol*, vol. 70, no. 3, Apr. 2023, doi: 10.1530/JME-22-0167.

[7] C. G. Turin, M. M. Crenshaw, and L. Fishbein, "Pheochromocytoma and paraganglioma: germline genetics and hereditary syndromes," *Endocrine Oncology*, vol. 2, no. 1, pp. R65–R77, Jun. 2022, doi: 10.1530/eo-22-0044.

[8] S. Jhawar *et al.*, "New Insights on the Genetics of Pheochromocytoma and Paraganglioma and Its Clinical Implications," *Cancers*, vol. 14, no. 3. 2022. doi: 10.3390/cancers14030594.

[9] J. W. M. Lenders *et al.*, "Pheochromocytoma and Paraganglioma: An Endocrine Society Clinical Practice Guideline," *J Clin Endocrinol Metab*, vol. 99, no. 6, pp. 1915–1942, Jun. 2014, doi: 10.1210/JC.2014-1498.

[10] R. A. Toledo *et al.*, "Consensus Statement on next-generation-sequencing-based diagnostic testing of hereditary phaeochromocytomas and paragangliomas," *Nature Reviews Endocrinology*, vol. 13, no. 4. Nature Publishing Group, pp. 233–247, Apr. 01, 2017. doi: 10.1038/nrendo.2016.185.

[11] A. Cascón *et al.*, "Genetics of Pheochromocytoma and Paraganglioma in Spanish Patients," *J Clin Endocrinol Metab*, vol. 94, no. 5, pp. 1701–1705, May 2009, doi: 10.1210/JC.2008-2756.

[12] E. F. Hensen *et al.*, "Mutations in SDHD are the major determinants of the clinical characteristics of Dutch head and neck paraganglioma patients," *Clin Endocrinol (Oxf)*, vol. 75, no. 5, pp. 650–655, Nov. 2011, doi: 10.1111/J.1365-2265.2011.04097.X.

[13] S. Lider and C. Badiu, "The role of ambulatory blood pressure monitoring in the diagnosis of pheochromocytoma," 2021. [Online]. Available: www.hypertens.org/jhr

[14] D. N. Lázaro-Silva, J. C. P. De Mattos, H. C. Castro, G. G. Alves, and L. M. F. Amorim, "The Use of DNA Extraction for Molecular Biology and Biotechnology Training: A Practical and Alternative Approach," *Molecular Biology and Biotechnology Training: A Practical and Alternative Approach. Creative Educa-tion*, vol. 6, pp. 762–772, 2015, doi: 10.4236/ce.2015.68079.

[15] “Home.” Accessed: Jan. 24, 2024. [Online]. Available: https://www.acmg.net/

[16] “Genetics101 for Endocrinologists.” Accessed: Jan. 24, 2024. [Online]. Available: https://www.acmgeducation.net/Public/Catalog/Details.aspx?id=9DPUYfsdAsvd%2fW0X1WCHtA%3d%3d&returnurl=%2fUsers%2fUserOnlineCourse.aspx%3fLearningActivityID%3d9DPUYfsdAsvd%252fW0X1WCHtA%253d%253d

[17] “Franklin.” Accessed: Mar. 30, 2024. [Online]. Available: https://franklin.genoox.com/clinical-db/home

[18] “ClinVar.” Accessed: Mar. 30, 2024. [Online]. Available: https://www.ncbi.nlm.nih.gov/clinvar/

[19] O. Mete, S. L. Asa, A. J. Gill, N. Kimura, R. R. de Krijger, and A. Tischler, “Overview of the 2022 WHO Classification of Paragangliomas and Pheochromocytomas,” *Endocrine Pathology 2022 33:1*, vol. 33, no. 1, pp. 90–114, Mar. 2022, doi: 10.1007/S12022-022-09704-6.

[20] A. K. yin Lam, “Update on Adrenal Tumours in 2017 World Health Organization (WHO) of Endocrine Tumours,” *Endocrine Pathology*, vol. 28, no. 3. Humana Press Inc., pp. 213–227, Sep. 01, 2017. doi: 10.1007/s12022-017-9484-5.

[21] D. Granberg, C. C. Juhlin, and H. Falhammar, “Metastatic Pheochromocytomas and Abdominal Paragangliomas,” *J Clin Endocrinol Metab*, vol. 106, no. 5, pp. e1937–e1952, Apr. 2021, doi: 10.1210/CLINEM/DGAA982.

[22] S. Lider and M. Gheorghiu, “Pheochromocytoma-paraganglioma: Translational approach from genetics to clinical neuroendocrinology,” *Acta Endocrinol (Copenh)*, vol. 14, no. 4, pp. 570–572, 2018, doi: 10.4183/AEB.2018.570.

[23] B. Sarkadi, E. Saskoi, H. Butz, and A. Patocs, “Genetics of Pheochromocytomas and Paragangliomas Determine the Therapeutical Approach,” *International Journal of Molecular Sciences 2022, Vol. 23, Page 1450*, vol. 23, no. 3, p. 1450, Jan. 2022, doi: 10.3390/IJMS23031450.

[24] G. De Filpo, · M Maggi, · M Mannelli, and · L Canu, “Management and outcome of metastatic pheochromocytomas/ paragangliomas: an overview,” *J Endocrinol Invest*, vol. 44, pp. 15–25, 2021, doi: 10.1007/s40618-020-01344-z.

[25] O. Hamidi *et al.*, “Malignant Pheochromocytoma and Paraganglioma: 272 Patients Over 55 Years,” *J Clin Endocrinol Metab*, vol. 102, no. 9, pp. 3296–3305, Sep. 2017, doi: 10.1210/JC.2017-00992.

[26] L. Fishbein and K. L. Nathanson, “Pheochromocytoma and paraganglioma: understanding the complexities of the genetic background,” *Cancer Genet*, vol. 205, no. 1–2, pp. 1–11, Jan. 2012, doi: 10.1016/J.CANCERGEN.2012.01.009.

[27] J. Jiang *et al.*, “Sino-European Differences in the Genetic Landscape and Clinical Presentation of Pheochromocytoma and Paraganglioma,” *J Clin Endocrinol Metab*, vol. 105, no. 10, pp. 3295–3307, Oct. 2020, doi: 10.1210/CLINEM/DGAA502.

[28] A. Cascón *et al.*, “Genetics of Pheochromocytoma and Paraganglioma in Spanish Patients,” *J Clin Endocrinol Metab*, vol. 94, no. 5, pp. 1701–1705, May 2009, doi: 10.1210/JC.2008-2756.

[29] F. J. Quayle, E. A. Fialkowski, R. Benveniste, and J. F. Moley, “Pheochromocytoma penetrance varies by RET mutation in MEN 2A,” *Surgery*, vol. 142, no. 6, pp. 800-805.e1, Dec. 2007, doi: 10.1016/J.SURG.2007.09.013.

[30] J. V Lima *et al.*, "Germline genetic variants in pheochromocytoma/paraganglioma: single-center experience," *Endocrine oncology (Bristol, England)*, vol. 3, no. 1, May 2023, doi: 10.1530/EO-22-0091.

[31] M. Felsoci, I. Lazurova, H. Wagnerova, and M. Svajdler, "Analysis of pheochromocytomas / paragangliomas from Eastern Slovakia," *452 Neoplasma*, vol. 60, 2013, doi: 10.4149/neo_2013_059.

[32] J. W. M. Lenders *et al.*, "Genetics, diagnosis, management and future directions of research of phaeochromocytoma and paraganglioma: A position statement and consensus of the Working Group on Endocrine Hypertension of the European Society of Hypertension," *J Hypertens*, vol. 38, no. 8, pp. 1443–1456, Aug. 2020, doi: 10.1097/HJH.0000000000002438.

[33] J. M. Rodriguez *et al.*, "Pheochromocytoma in MEN 2A Syndrome. Study of 54 Patients", doi: 10.1007/s00268-008-9734-2.

[34] F. Castinetti *et al.*, "The penetrance of MEN2 pheochromocytoma is not only determined by RET mutations," *Endocr Relat Cancer*, vol. 24, no. 8, pp. L63–L67, Aug. 2017, doi: 10.1530/ERC-17-0189.

[35] V. Neocleous, P. Fanis, S. Frangos, N. Skordis, and L. A. Phylactou, "RET Proto-Oncogene Variants in Patients with Medullary Thyroid Carcinoma from the Mediterranean Basin: A Brief Report," *Life 2023, Vol. 13, Page 1332*, vol. 13, no. 6, p. 1332, Jun. 2023, doi: 10.3390/LIFE13061332.

[36] S. Schirwani *et al.*, "Homozygosity for the pathogenic RET hotspot variant p.Cys634Trp: A consanguineous family with MEN2A," *Eur J Med Genet*, vol. 64, no. 2, p. 104141, Feb. 2021, doi: 10.1016/J.EJMG.2021.104141.

[37] J. Crona, A. Lamarca, S. Ghosal, S. Welin, B. Skogseid, and K. Pacak, "Genotype-phenotype correlations in pheochromocytoma and paraganglioma: A systematic review and individual patient meta-analysis," *Endocr Relat Cancer*, vol. 26, no. 5, pp. 539–550, 2019, doi: 10.1530/ERC-19-0024.

[38] van N. Duinen *et al.*, "High prevalence of founder mutations of the succinate dehydrogenase genes in the Netherlands The authors have no conflicts of interest," *Clin Genet*, vol. 81, pp. 284–288, 2012, doi: 10.1111/j.1399-0004.2011.01653.x.

[39] A. M. A. Berends *et al.*, "Incidence of pheochromocytoma and sympathetic paraganglioma in the Netherlands: A nationwide study and systematic review A R T I C L E I N F O," 2018, doi: 10.1016/j.ejim.2018.01.015.

[40] C. R. McHenry, "Pheochromocytoma: A Clinical Enigma," *AACE Clin Case Rep*, vol. 3, no. 2, pp. e180–e181, Mar. 2017, doi: 10.4158/EP161587.CO.

[41] P. T. K. Gunawardane and A. Grossman, "The clinical genetics of phaeochromocytoma and paraganglioma," *Arch Endocrinol Metab*, vol. 61, no. 5, pp. 490–500, Oct. 2017, doi: 10.1590/2359-3997000000299.

[42] F. Castinetti *et al.*, "Outcomes of adrenal-sparing surgery or total adrenalectomy in phaeochromocytoma associated with multiple endocrine neoplasia type 2: an international retrospective population-based study," *Lancet Oncol*, vol. 15, no. 6, pp. 648–655, 2014, doi: 10.1016/S1470-2045(14)70154-8.

Publications list

Articles

1. **Lider Burciulescu, S. M**., Gheorghiu, M. L., Muresan, A., Gherlan, I., Patocs, A., & Badiu, C. (2024). Bilateral pheochromocytomas: clinical presentation and morbidity rate related to surgery technique and genetic status. *Endocrine Connections*, *13*(4), 2024, e230466. Retrieved Mar 15, 2024, from *https://doi.org/10.1530/EC-23-0466.*
2. **Lider Burciulescu, S. M**., Randon, C., Duprez, F., Huvenne, W., Creytens, D., Claes, K. B. M., de Putter, R., T'Sjoen, G., Badiu, C., & Lapauw, B. Clinical presentation of sporadic and hereditary pheochromocytoma/paraganglioma. *Endocrine Oncology*, 2023, *3*(1), e220040. Retrieved Mar 15, 2024 *https://doi.org/10.1530/EO-22-0040*.
3. **Lider Burciulescu S.M.** Gheorghiu M, Milos I, Badiu C, Malignant Paraganglioma Non-Responsive to MIBG in MEN2A Syndrome *Acta Endo (Buc)*, 2022, 18:536-537 *doi: 10.4183/aeb.2022.536*.
4. **Lider Burciulescu S. M.**, Gheorghiu M, Pheochromocytoma-Paraganglioma: Translational Approach from Genetics to Clinical Neuroendocrinology *Acta Endo (Buc),* 2018, 14:570-572 *doi: 10.4183/aeb.2018.570.*
5. Mellid S, Gil E, Letón R, Caleiras E, Honrado E, Richter S, Palacios N, Lahera M, Galofré JC, López-Fernández A, Calatayud M, Herrera-Martínez AD, Galvez MA, Matias-Guiu X, Balbín M, Korpershoek E, Lim ES, Maletta F, **Lider S,** Fliedner SMJ, Bechmann N, Eisenhofer G, Canu L, Rapizzi E, Bancos I, Robledo M, Cascón A. Co-occurrence of mutations in NF1 and other susceptibility genes in pheochromocytoma and paraganglioma. *Front Endocrinol (Lausanne)*, 2023 Jan 25;13:1070074. *doi: 10.3389/fendo.2022.1070074.*
6. **Lider Sofia** and Corin Badiu. "The role of ambulatory blood pressure monitoring in the diagnosis of pheochromocytoma." *Journal of Hypertension Research*, vol. 7, no. 4, Oct.-Dec. 2020,131*GaleAcademicOneFile*,link.*gale.com/apps/doc/A691005224/AONE?u=anon~92bc6f4a&sid=googleScholar&xid=618cb17b.* Accessed 7 May 2024.

Book chapters

1. **Sofia-Maria Lider Burciulescu,** Monica-Livia Gheorghiu, Corin Badiu 'Evaluarea tumorilor secretante de catecolamine din perspectivă translaţională', Antropologia mileniului III Autori: Andrei Kozma,Cristina Glavce, Constantin Balaceanu Stolnici, Editura Academiei Romane, Bucuresti 2019, 210-226.

2. **Lider Burciulescu S.M.** & Gheorghiu, M. L. (2022). Advances in the Diagnosis and Treatment of Pheochromocytomas and Paragangliomas in the Era of Personalized Genetic Diagnostic. In (Ed.), Adrenal Glands - The Current Stage and New Perspectives of Diseases and Treatment, IntechOpen. https://doi.org/10.5772/intechopen.108298

Oral presentations:

1. **Sofia-Maria Lider-Burciulescu,** Monica Livia Gheorghiu , Andrei Muresan , Stanescu Laura-Semonia , Anda Dumitrascu, Corin Badiu Factors that influence pheochromocytoma penetrance in MEN2A Syndrome, *Endocrine Abstracts* (2024), European Congress of Endocrinology **99** RC3.7 | DOI: 10.1530/endoabs.99.RC3.7

2. **Sofia Lider,** Andrei Muresan, Attila Patocs, Corin Badiu, Differences in clinical presentation of PPGLs according to diagnosis age and genetic status, Al 7-lea Congres National de Psihoneuroendocrinologie, 2023, Timisoara, Romania

3. **Sofia Lider,** Monica Gheorghiu, Corin Badiu, Difficulties in the management of oligosymptomatic pheochromocytomas and their complications, Forumul tinerilor endocrinologi 2022, Bucuresti, Romania

4. **Sofia Maria Lider Burciulescu,** Monica Livia Gheorghiu, Tratamentul personalizat al acromegaliei – opțiuni și perspective, Ziua Acromegaliei, 2022, Bucuresti, Romania.

5. **Sofia Lider**, Monica Gheorghiu, Elena Emanuela Braha, Laura Semonia Stanescu, Corin Badiu, Particularitati fenotipice ale sindromului familial MEN2A, Conferinta zilele F. Rainer, 2022, Bucuresti, Romania.

6. **Sofia Lider,** Sorina Schipor, Monica Gheorghiu, Corin Badiu, Abordarea personalizata a pacientilor cu paraganglioame, Ziua bolilor rare 2022, on-line.

7. **Sofia Maria Lider Burciulescu,** Monica Livia Gheorghiu - Oral presentation 'Hipertiroidismul in primul trimestru de sarcina. Optiuni terapeutice'. Romanian National Congress of Endocrinology, 2022, Bucuresti, Romania.

8. **Sofia Lider,** Corin Badiu, Recurrence risk in bilateral pheochromocytomas reported to surgical technique and genetic status, Congresul National de Psihoneuroendocrinologie, 2022, Bucuresti, Romania.

9. **Sofia Maria Lider Burciulescu**, Monica Livia Gheorghiu, Corin Badiu'- Oral presentation: Managementul feocromocitoamelor si paraganglioamelor maligne' Forumul tinerilor endocrinologi 2021, on-line, Bucuresti, Romania.

10. **Sofia Lider**, Monica Gheorghiu, Corin Badiu- Oral presentation: 'Durata follow-up si riscul de recurenta in feocromocitoame si paraganglioame'- Forumul tinerilor endocrinologi 2020, on-line.
11. **Sofia Lider**, Monica Livia Gheorghiu, Corin Badiu-Oral presentation: `Frecventa insuficientei adrenale la pacientii cu feocromocitom dupa adrenalectomie-Congresul National de Psihoneuroendocrinologie, 2019, Timișoara, România.
12. **Sofia Maria Lider Burciulescu**, Monica Livia Gheorghiu, Anda Dumitrascu, - Oral presentation: **Isolated central cortico-adrenal insufficiency-Iatrogenic or organic? 2019** Postgraduate Clinical course in Endocrinology – Rotterdam, Netherland.
13. **Sofia-Maria Lider-Burciulescu**, Monica Livia Gheorghiu - Oral presentation: ¨Twin pregnancy after surgery for dopamine-agonist resistant macroprolactinoma with gonadotroph deficiency" 2019 Postgraduate course in endocrinology – Zagreb, Croatia.
14. **Sofia-Maria Lider Burciulescu,** Monica Livia Gheorghiu, Elena Emanuela Braha Andrei Muresan, Șerban Radian, Corin Badiu - Familial MEN 2A management and genetic counselling importance- Genetics National Congress 2018, Romanian Journal of Rare Disease, Supplement 1, 2018, 80.

Posters

1. **Sofia-Maria Lider-Burciulescu** , Cristina Stancu , Maria Anghel, Vlad Radulescu, Corin Badiu, A case of silent giant pheochromocytoma**,** *Endocrine Abstracts* (2024) **99** EP1059 | DOI: 10.1530/endoabs.99.EP1059

2. **Sofia Maria Lider Burciulescu**, Monica Livia Gheorghiu, Attila Patocz, Corin Badiu, ¨Genotype characterization of Romanian patients with PPGLs", 2023, ENSAT-COST Harmonisation Meeting, Dubrovnik P4.
3. **Sofia Maria Lider Burciulescu**, Monica Livia Gheorghiu, Attila Patocs, Corin Badiu, ¨New possible pathogenic variants involved in pheochromocytomas and paragangliomas", 2023, EYES Congress, Wurzburg, P6.
4. **Sofia Maria Lider Burciulescu**, Elena Emanuela Braha, Corin Badiu, "Strategies in the management of MEN2A syndrome as a potentially aggressive disease", 2022, ESE Summer School Innsbruck.
5. **Sofia Maria Lider Burciulescu**, Corin Badiu, ¨Clinical efficacy of PRRT treatment in a malignant paraganglioma related to RET mutation", 2022, 10th Baltic Congress.
6. **Sofia, M. L. B**., Costache, O. M., Bunea, I., & Livia, G. M. (2021, May). Transient thyrotoxicosis after laryngeal carcinoma surgery, 2021, European Congress of Endocrinology, In Endocrine Abstracts (Vol. 73). Bioscientifica, *Endocrine Abstracts* () **73** EP197.
7. Livia, G. M., Irina, A. B., **Sofia, M. L. B**., Anda, D., & Ramona, A. (2021, May). Rapid decrease of a pituitary mass with gonadotrophic and thyrotrophic insufficiency–the case for lymphocytic hypophysitis ? In Endocrine Abstracts (Vol. 73). Bioscientifica. DOI: 10.1530/endoabs.73, EP158

8. **Sofia Maria Lider Burciulescu**, Guy T'Sjoen, Corin Badiu, Lapauw Bruno, Characteristics of pheochromocytomas/paragangliomas in Flemish population, European Congress of Endocrinology, *Endocrine Abstracts* (2021) **73** AEP2 |DOI: 10.1530/endoabs.73.AEP2

9. **Lider Burciulescu Sofia,** et al. "Clinical presentation variations of pheochromocytomas." 22nd European Congress of Endocrinology. Vol. 70. BioScientifica,*EndocrineAbstracts* (2020) **70** AEP1010 | DOI: 10.1530/endoabs.70.AEP1010

10. **Sofia-Maria Lider Burciulescu,** Monica Livia Gheorghiu, Anda Dumitrascu, Dan Hortopan, Schipor Sorina, Corin Badiu 'Asymptomatic pheochromocytomas-an unelucidated physiopathology pattern." European Congress of Endocrinology, Endocrine Abstracts. Vol. 63. Bioscientifica, 2019. DOI: 10.1530/endoabs.63.P862

11. **Sofia Maria Lider Burciulescu**, Monica Livia Gheorghiu, Anda Dumitrascu, Dan Hortopan, Andra Caragheorgheopol, Carmen Iordachescu "Recurrent Cushing disease after bilateral adrenalectomy" National Congress of neuroendocrinology 2018- Vol XIV, Supplement 1, June 2018, 154-155.

12. Monica Livia Gheorghiu, **Burciulescu, S. M. L**, Dumitrascu, A., Hortopan, D., & Ionescu, V. (2019). Long-term follow-up in a series of pituitary stalk lesions. Endocrine Practice, 25, 246-246.

13. Gheorghiu, Monica-Livia, **Sofia-Maria Lider Burciulescu** and Luminita Nicoleta Ionescu. Atypical manifestation of pheochromocytoma. 7th ESE Young Endocrinologists and Scientists (EYES) Meeting. Vol. 67. BioScientifica, 2019. DOI: 10.1530/endoabs.67.GP22

14. **Sofia Maria Lider Burciulescu**, Anda Dumitrascu, Sorina Schipor, Monica Gheorghiu. Recurrent Thoracic paraganglioma- ENEA Cushing's Workshop 2021.

15. **Sofia Lider**, Cristina Stancu, Corin Badiu 'Aggressive Cushing's Disease in pandemic times' ENEA Cushing's Workshop 2021.

16. **Sofia-Maria Lider-Burciulescu**, Monica-Livia Gheorghiu, Andrei Muresan, Corin Badiu, Factors involved in response to antihypertensive drugs in Pheochromocytoma/Paraganglioma, ENEA 2020. E70

17. Monica-Livia Gheorghiu, **Sofia-Maria Lider Burciulescu**, Corin Badiu, Difficulties in diagnosing hypothalamus - pituitary germinoma, ENEA 2019 EP 63.

18. **Sofia-Maria Lider-Burciulescu,** Monica-Livia Gheorghiu, Corin Badiu "The response in patients with pheochromocytoma to the antihypertensive drugs correlated with the values of the catecholamines and with mutation"- Congresul National de Neuroendocrinologie, 2018, București, Romania

19. Gheorghiu, M. L., **Burciulescu, S. M. L**, Purice, M., & Alexiu, F. (2018, May). Conversion from thyroxine-treated autoimmune hypothyroidism to Graves. In 20th European Congress of Endocrinology, *Endocrine Abstracts* (2018) **56** P1102 | DOI: 10.1530/endoabs.56.P1102

20. Monica Livia Gheorghiu, **Sofia-Maria Lider-Burciulescu**, Andra Caragheorgheopol, Ionela Pascanu "Central corticosuprarenal deficiency in isolated inappropriate ACTH"

Congresul National de Endocrinologie-2018, Acta Endocrinologica, Vol XIV, Supplement 1, June 2018, 153-154.

Postgraduate courses

1. Postgraduate Clinical course in Endocrinology Rotterdam, **2019**
2. Postgraduate course in endocrinology-Zagreb, 2019
3. Neuroendocrine Tumors Masterclass-Budapest (27-28 November 2019).
4. *COST* Harmonisation Adrenal Tumor Master Class, 2022
5. ESE Summer School Innsbruck July 2022
6. Osteoporosis Essentials of Densitometry Diagnosis and Management Course, 2022

Awards

2020 - **BioscientificaTrust Grant**- Mobilities Grant Research stay at Ghent University Hospital, Belgium

2022 - '**Mihail Coculescu' Romanian PsychoNeuroEndocrine Society Award** for research in pheochromocytoma / paraganglioma field.

2023 - COST Harmonisation, **Short Term Scientific Missions (STSM)** – Observership at Instituto National de Investigaciones Oncologicas, Madrid, Spain.

2024 - **Agentia de Credite si Burse de studii - Bursa "H.G. nr. 118/2023", Ph.D.** Research stay at Universitary Hospital, Basel, Suisse.

Affiliations

Member of Romanian PsychoNeuroEndocrine Society

Member of European Society of Endocrinology

Member of ENSAT and COST Harmonization Group