UNIVERSITATEA DE MEDICINĂ ŞI FARMACIE „IULIU HAŢIEGANU” CLUJ-NAPOCA

ŞCOALA DOCTORALĂ

HABILITATION THESIS
Domain: Medicine

Genes and metabolism: from molecular mechanisms to implications in biomedical sciences

Conf. Dr. Romana Vulturar

CLUJ-NAPOCA 2020

ABSTRACT

This habilitation thesis presents, in synthesis, my professional, scientific and didactic achievements in the domain of medical genetics (biochemical, molecular and clinical fields) accomplished after defending my PhD thesis; in the second part, the thesis presents the plans for the development of the future professional, research and didactic activity.

After a short introduction, the first chapter of the thesis entitled "*Genes and metabolism: from molecular mechanisms to implications in biomedical sciences*", summarizes the most relevant professional results, classified in four subchapters, corresponding to the scientific domains with important results that can be described as follows:

(1) Contributions in the field of inborn errors of metabolism; (2) Studies related to neurosciences – candidate gene approaches related to serotonin metabolism and other molecules involved in signaling or neuronal development [like Brain Derived Neurotrophic Factor (BDNF), Catechol-O-methyltransferase (COMT), the Corticotropin-releasing factor type one receptor (CRFR1), glucocorticoid receptor (GR)). Besides, our studies were addressed to the *hypothalamic-pituitary-adrenal gland* (HPA) axis, that is involved in stress reactivity and we have studied in adults and newborns. (3) Contributions in the field of normal and pathological metabolism, applications of molecular nutrition, and (4) Studies of genetic features in clinical adult pathology.

After defending my PhD thesis, I have continued to be involved in the diagnostic and follow-up of several inborn errors of metabolism, partially as an integrated physician in the Ist Laboratory of Genetic Explorations of Cluj County Emergency Hospital. Moreover, I've introduced a metabolic evaluation on a research basis with chemists from the Group of Biospectroscopy from the "Petru Poni" Institute of Molecular Chemistry Iași, and Institute of Biochemistry – Romanian Academy Bucharest. Urinary NMR spectroscopy is a relatively new analytical complementary method that may be applied to body fluids and help in the diagnosis of numerous congenital metabolic diseases. Being part of the team, we used this highly performant method in biomedical investigations for several hundreds of samples from patients suspected for inborn errors of metabolism, in

parallel with chromatographic evaluations in the laboratory integrated into the Discipline of Cell and Molecular Biology, UMF Cluj-Napoca. The NMR spectroscopy shows the majority of proton-containing compounds, and therefore, provides an overall view of metabolism; giving characteristic 'fingerprint' of almost all hydrogen nuclei in a metabolite, we have helped clinical services for the diagnostic of several genetic rare metabolic disorders. Most of these investigations were referred from pediatrics, child neurology and neonatology clinical services. Regarding the molecular genetic tests, I was participating in several molecular diagnostic steps in cystic fibrosis, and starting with 2005-2006 I have introduced several molecular analyses for the most frequent mutations in phenylketonuric (PKU) Romanian patients; molecular analyses in these patients are important nowadays for the new therapeutical approach. Regarding the advanced techniques (Next Generation Sequencing - NGS) applied worldwide for a complete genetic evaluation, the study has continued within an international team (coordinated from Zürich), and we have established the molecular profile in 80 PKU patients, and identified three new types of defects in Romanian populations, with results published in 2016. We published and communicated in international journals many of our results, together with specialists from clinical departments in journals like *Gene, Medicina, Revista de Chimie, Journal of Inherited Metabolic Disease, European Journal of Human Genetics*.

Starting with 2007, I became member in the Cognitive Neuroscience Laboratory (CNL) in Cluj-Napoca, coordinated by Prof. Andrei C. Miu, and I contributed to several studies that are important for understanding the risk for psychopathology. We have integrated methods from molecular genetics and cognitive science to investigate genetic influences on psychological and biological aspects of emotion and cognition, for understanding individual differences in emotion regulation, emotion-cognition interactions, and emotional vulnerability. Using gene candidate studies, we were focused on functional genetic polymorphisms that may contribute to individual differences in emotion regulation or moderate its effects on emotional symptoms. The molecular genetic studies were integrated in a wide array of methods, including neuroendocrine assays to autonomic psychophysiology and optical neuroimaging. Several studies aimed to identify genetic influences on emotional development during adolescence and early adulthood, genetic moderators of the relations between emotion and cognition, neural endophenotypes of emotional vulnerability. Another direction of studies coordinated in CNL is related to the interest in the development of the hypothalamic-pituitary-adrenal (HPA) axis and its relation to behavior; our non-

invasive evaluation in newborns, as an approach of stress reactivity and correlations with the type of delivery, showed that mode of delivery influences neonatal stress reactivity. Other studies of our team were focused on gene-environment interaction in one type of emotion regulation strategy (reappraisal) that has been linked with the psychopathology (depression, anxiety disorders); we found that a genetic variant (Met) in the BDNF (*brain-derived neurotrophic factor*) gene was a significant moderator in the relation between childhood trauma and reappraisal. There was a negative relation between the number of childhood traumatic events and reappraisal ability in BDNF Met carriers, but not Val homozygotes. This finding suggests that BDNF Val66Met contributes to susceptibility to childhood stress, with long term impact on emotion regulation. The results of these studies are published in important international journals like *Psychoneuroendocrinology*, *Genes-Brain-and-Behavior*, *PlosOne*, *Emotion*, *Psychophysiology*, *Neuroscience Letters*, and others.

(3) The contributions in the field of nutrition (involving amino acids metabolism, micronutrients, etc) are related to several monogenic disorders for which the diet is important to modulate the physiopathology of the disease. These have major implications for the management of several abnormalities in which the interactions "*nature versus nurture*" are extremely important (i.e. phenylketonuria, galactosemia, methylmalonic aciduria, lactose intolerance, and others). Recent approaches regarding the normal metabolism and the benefits of nutritional interventions have helped us to form a large collaboration platform with researchers from clinical departments (internal medicine, nutrition and metabolic disorders, cardiology), informatics, and the team from Molecular Nutrition Laboratory - Department of Food Science that belong to University of Agricultural Sciences and Veterinary Medicine (USAMV) Cluj-Napoca. Besides, our recent studies have been applied for *in vitro* methods evaluating, through multiplex RT-PCR, the genes expressions that are modulated after the treatment with nano-compounds influencing signaling pathways that, together with identification of new biomarkers, are important in oncogenesis and oncosupression. Results were published and communicated in *Foods, Nutrients*, *Medicina*, *Medicine and Pharmacy Reports*, *Romanian Journal of Rare Diseases*, and others.

(4) Studies of genetic features in clinical adult pathology included the contributions regarding rare adult disorders with relevance in rheumatology speciality; besides, several genetic variants were evaluated for their contributions in cardiovascular drug therapy (pharmacogenetics). Regarding the gastro-enterology, we have studied several Interleukines gene variants related to

gastro-esophageal reflux disease (GERD). The articles in this field are published in *Diagnostics, Medicina, Experimental and Therapeutic Medicine*, and others.

Regarding the didactic activities, after defending my PhD thesis, I have been involved in teaching lectures and/or practical works in Cell and Molecular Biology for the Medicine and Dentistry 1st year students for all the three lines in our university: Romanian, French and English, holding the permanent foreign language certificates (Delf for the French language, and Cambridge for English). Besides, for several years (till 2017) I have taught courses and worked in the laboratory with Romanian resident physicians in Laboratory Medicine, Medical Genetics and Neonatology. During the past years, I have co-organized workshops (hands-on) for students and coordinated students for participating with oral and poster scientific presentations to the international and national congresses/conferences; one oral presentation was awarded - IInd prize at the International Student Medical Congress in Bucharest, December 2019.

The scientific and publishing research activity, after defending the PhD thesis, can be quantified as follows: 2 chapters in international publishing houses, 4 chapters and 3 scientific books in national publishing houses; 12 ISI articles published as one of the main authors and 14 ISI articles being co-author (totally 26 ISI articles of which 13 are in quartiles Q1 and Q2) and 6 International databases (IDB) articles and more than 70 abstracts published in ISI and IDB/ISBN journals as participation at national and international conferences. These are reflected on the ISI Thomson platform (accessed on 15.09.2020) by the following features: H-index = 7 (200 citations, with an average of 5.56/item). I also was member in 13 research projects, and have coordinated 5 other grants (one of this these projects have allowed self-improvement by participating in the "*Hands-on Clinical Molecular Biology Course*" organized in Medical Faculty Milano, Italy). Other courses "*Focused courses on Mitochondrial Medicine*" in the Centre for Mitochondrial Disorders, Radboud University Nijmegen Medical Centre in the Netherlands, or Course "*Academy on metabolic disorders"*, London, UK, were dedicated for both clinical and laboratory approaches. I was rewarded with 18 prizes (for research and didactic activities). I am reviewer for several ISI journals, and I am member in national and international scientific societies as Romanian Society of Medical Genetics, European Society of Human Genetics (ESHG), Society for Study Inborn Errors of Metabolism (SSIEM), Romanian Society of Bioinformatics, and others.

The second part of the thesis presents the perspectives for professional and academic development, having as a first objective the increase of the scientific quality, the visibility at the national and international recognition of our

researches. I hope that my future research activity, as part of collaborative teams, will be focused on main following domains:
(1) Studies of genetic and biochemical bio-markers (*genes and metabolism*) with implications in more rare monogenic disorders (inborn errors of metabolism and others), and multifactorial diseases, giving benefits for the patients' evaluation and treatment. These studies are possible through collaborations with specialists in analytical chemistry, pediatrics, cardiology, rheumatology, pharmacy, internal medicine, neurology, laboratory medicine etc, involved either in the management of monogenic rare disease, or in the investigation of complex cases with genetically conditioned pathology.
(2) Fundamental studies, as part of the team in Cognitive Neuroscience Laboratory, for candidates genes and biochemical markers targeting the interactions of environmental and genetic factors involved in psychological and biological aspects of emotion and cognition, in traits giving vulnerability for psychopathology.
(3) Research projects on dietary interventions (molecular nutrition), including gene expression studies, with teams from USAMV, that will focus on therapy and prophylaxis of metabolic disorders, or tumours, with genetic or mixed substrates.
For all these desideratum, you will also benefit from the international collaborations already established with specialists from the Netherlands, Canada, the United Kingdom and France. I hope that these studies, involving students, MA students and PhD candidates, will contribute to modern approach for the management of patients with genetic causes of diseases in our country, and, in plus, will contribute to the research domain entitled "*from genetics to disease biology*".

In the same time, the didactic activities will be continuously improved by adding new scientific aspects and/or new subjects of interest accordingly to the latest scientific developments. Regarding the teaching activities, I will continue to sustain the modern methods of teaching, extremely useful for students in biomedical fields, modern genetics offering so many opportunities in the development of practical and analytical skills.
In this manner, I hope that in my future activity, these fields (the educational and research activities) will be continuously correlated with requirements in the health system.
The last part of this thesis includes the bibliographic references used in writing the thesis.