SOUTHEASTERN EUROPE INNERMED NETWORKING MEETING

Joint meeting of the “InnerMeD-Information Network” project partners and collaborating partners and experts from the Southeastern Europe

Zagreb, Croatia

Hotel „Palace“

May 11th – 13th, 2015

Program & Abstracts
Dear colleagues, collaborators, co-workers, guests and friends,

It is our honour and great pleasure that you are taking part in the “Southeastern Europe InNerMeD Networking Meeting”, thus confirming belonging to the idea of joined efforts as the best way to succeed in coping with the burden of inherited metabolic disease affecting the nervous system. The meeting is a unique opportunity to strengthen and enlarge the “InNerMeD”-network and to intensify our collaboration in the field of inherited neurometabolic disorders with the final aim to help patients and their families suffering from the growing number of inherited neurometabolic diseases.

As you know, “InNerMeD” is an European project and it has been funded by the Executive Agency for Health & Consumers (DG-SANCO) under the Second Programme of Community action in the field of Health, 2008-2013 to be the first European Network on neurometabolic diseases. Since its beginning, it has had the focus to create a network of information targeted on diagnosis and treatment of iNMDs based on the collection and exchange of validated information among scientific community, health professionals, patients, patient associations and all interested stakeholders. In this sense, our joint meeting between project partners and colleagues from Southeastern European countries is a perfect tool to spread idea of the project in this part of Europe.

We are confident that both scientific content and the atmosphere of the meeting will guarantee that all participants will take home pleasant memories and strong motivation to continue working on the “InNerMeD”-project goals in their countries by further spreading of the networking and strengthening of our future collaboration.

We wish you a fruitful participation in the “Southeastern Europe InNerMeD Networking Meeting” and pleasant stay in Zagreb.

On behalf of the InNerMeD project:

Professor Ivo Barić, MD, PhD
(president of the organizing committee)

Professor Maurizio Scarpa, MD, PhD
(project coordinator)
ORGANIZERS

ORGANIZER: “Inherited NeuroMetabolic Diseases Information Network”, an European project funded by the Executive Agency for Health & Consumers (DG-SANCO) under the Second Programme of Community action in the field of Health, 2008-2013
CO-ORGANIZER: Section for Metabolic Diseases, Croatian Pediatric Society, Croatian Medical Association

ORGANIZING COMMITTEE

Ivo Barić (president), Maurizio Scarpa (project coordinator), Ljerka Cvitanović-Šojat & Danijela Petković Ramadža (secretaries), Nina Barišić, Ksenija Fumić, Ivan Lehman, Smiljka Vikić-Topić, Lucija Debeljak, Angeles Garcia Corzola, Christine Dali, Franco Bartoloni, Fedele Bonifazi

GENERAL INFORMATION

TIME - May 11th to 13th, 2015


THE OFFICIAL LANGUAGE OF THE MEETING: English

CONTACTS FOR INFORMATION:
Prof Ivo Barić, tel. +385-1-2388121; fax. +385-1-2376023; e-mail: ibaric@kbc-zagreb.hr
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SPEAKER’S INFORMATION - Presentations are expected to be made in Power Point program and given to the projection staff at least 15 minutes before the session to which presentation is scheduled. If other form of presentation is needed, please, contact the organizers as soon as possible. Speakers are kindly asked to keep strictly with time schedule.

PARTICIPATION VALIDATION: Participation will be credited according to the Croatian Medical Chamber rules on permanent education.

NAME BADGES - Only name badges holders can participate in scientific and social programme.

REGISTRATION AND REGISTRATION FEE - The registration desk for all participants is in the hotel lobby. It will be open on Monday, May 11th from 7 to 8 p.m., on Tuesday, May 12th, from 7.30 a.m. to 6 p.m., and on Wednesday, May 13th, from 8 a.m. to 2 p.m.
There is no registration fee, but registration is necessary for participation. The registration covers the participation in the scientific program, get together dinner, drinks and meals during the meeting, dinner on Tuesday, as well as all printed material.

LOGISTICS

PerfectMeetings.hr
Dražena Erceg, Executive Manager, Draskoviceva 66, HR-10000 Zagreb, Croatia; T: +385-1-4827-280; F: +385-1-4832-330, drazena.erceg@perfectmeetings.hr, www.perfectmeetings.hr
SOUTHEASTERN EUROPE INNERMEDI NETWORKING MEETING

Joint meeting of the “Innermed network” partners and collaborating partners and experts from the Southeastern Europe

PROGRAM

**Monday, May 11th, 2015**

13.30 - 19.00 InNerMeD - core network partners closed meeting - part I (visit to the Croatian Brain Research Institute)

20.00 - _get together dinner in Hotel Palace_

**Tuesday, May 12th, 2015.**

8.30 - 10.00 Hall „Lenuci“

InNerMeD- core network partners closed meeting - part II

Hall „Strossmayer“

Pre-symposium session - (contributed by the Section for Metabolic Disorders of the Croatian Pediatric Society)

Chairs: Ksenija Fumić, Danijela Petković Ramadža

8.30 _Fran Borovečki_ (Zagreb, Croatia): “Next generation sequencing in diagnosis of neurometabolic disorders”

9.00 _Danijela Petković Ramadža, Ivo Barić_ (Zagreb, Croatia): „Pompe disease-Diagnostic and treatment challenges“

9.30 _Ivan Pećin, Diana Muačević-Katanec, Iveta Merčep, Željko Reiner_ (Zagreb, Croatia): „Is Gaucher disease type 1 really a non-neuropathic disease?“

- coffee break -
Hall „Strossmayer“

10.30 **Welcome and opening:** Ivo Barić (Zagreb, Croatia), Vlasta Zmazek (Zagreb, Croatian Alliance for Rare Diseases, Eurordis), Maurizio Scarpa (Wiesbaden/Padova)

10.45-12.45 **INNERMED PARTNERS PRESENTATIONS**

Chairs: Maurizio Scarpa, Ivo Barić

10.45 **Maurizio Scarpa (Wiesbaden, Germany / Padova, Italy):** „The Inherited Neurometabolic Diseases Information Network (InNerMeD-I-Network) - a project to be joined“

11.15 **Christine Dali, Alan Lund (Copenhagen, Denmark):** „Lysosome disorders with CNS manifestations“

11.45 **Ksenija Fumić (Zagreb, Croatia):** „Laboratory diagnosis and biochemical monitoring of lysosomal disorders“

12.15 **Maurizio Scarpa (Wiesbaden, Germany / Padova, Italy):** „New therapies for neurometabolic disorders“

- **lunch** -

13.45 -15.25 **SOUTHEASTERN EUROPE INNERMED NETWORKING MEETING - presentation of countries - part I** (Greece, Slovenia, Albania, Bosnia & Herzegovina, Bulgaria)

Chairs: Angeles Garcia Corzola, David Neubauer

13.45 **Dimitrios Zafeiriou (Thessaloniki, Greece):** „Diagnostic role of MRI in neurometabolic diseases“

14.05 **David Neubauer, Barbara Gnidovec Stražišar, Mojca Tanšek Žerjav, Urh Grošelj, Barbka Repič Lampret (Ljubljana, Slovenia):** „Some rare diseases in child neurology“

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[Logo of Health Programme of the European Union]
14.25 Artan Haruni, Valentina Tashko (Tirana, Albania): “Challenges of diagnosing and managing patients with inherited metabolic disorders in Albania”, "Ketogenic diet in neurometabolic disorders"

14.45 Smail Zubčević (Sarajevo, Bosnia and Herzegovina): "Neuronal ceroid lipofuscinosis - challenges in diagnostics of neurometabolic diseases in countries with limited resources"

15.05 Ivanka Sinigerska, Maria Ivanova, Dobri Dimitrov, Alexej Savov, Ivo Kremensky (Sofia, Bulgaria): “The laboratory diagnosis of inherited neurometabolic disorders in Bulgaria”

15.25 - 15.55 INNERMED WORKSHOP

Angeles Garcia Corzola (Barcelona, Spain): "Neurometabolic diseases mimicking psychiatric disorders"

- coffee break -

16.15 - 17.35 SOUTHEASTERN EUROPE INNERMED NETWORKING MEETING - presentation of countries-part II (Croatia, Cyprus, Czech Republik, Hungary)

Chairs: Jolanta Sykut-Cegielska, Nina Barišić

16.15 Ivo Barić (Zagreb, Croatia): „Inherited disorders of methylation affecting the brain“

16.35 Theodoros Georgiou, Paola Nicolaidou, George Tanteles, Anastasia Hadjichristou, Maria Dionysiou, Eli Siama, Georgia Chappa, Violetta Anastasiadou, Anthi Drousiotou (Nicosia, Cyprus): „Glutaric aciduria type I in the Cypriot population“

16.55 Martin Magner, Jiri Zeman (Prague, Czech Republic): „Mitochondrial diseases in children“

17.15 Eszter Karg, Akos Barath, Peter Monostori, Gabor Racz and Ferenc Papp (Szeged, Hungary): „Newborn screening for neurometabolic disorders“

18.00 Zagreb - guided tour
Wednesday - May 13th, 2015

8.40 -10.20 SOUTHEASTERN EUROPE INNERMED NETWORKING MEETING - presentation of countries - part III. (Macedonia, Moldova, Montenegro, Poland, Romania)

Chairs: Beril Talim, Ljerka Cvitanović-Šojat

8.40 Filip Duma, Vesna Sabolić, Natalija Angelkova, Katerina Vasileva Dimovska, Elena Kochova (Skopje, Macedonia): "Neurometabolic diseases in Macedonia - Current diagnostic possibilities"
Natalija Angelkova, Vesna Sabolić, Elena Sukarova Angelovska, Tanja Zorčec, Elena Kochova, Filip Duma (Skopje, Macedonia): "Late diagnosis of phenylketonuria - Time for changes"

9.00 Natalia Usurelu, Victoria Sacara, Stefan Gatcan (Chisinau, Moldova): "The development of neurometabolic field in Moldova"
Victoria Sacara, Natalia Usurelu, Stanislav Groppa, Maria Duca (Chisinau, Moldova): “Interaction among folate/homocysteine metabolism genes and endothelial nitric oxide synthase gene polymorphisms in case of Duchenne muscular dystrophy”


9.40 Joanna Taybert, Jolanta Sykut-Cegielska (Warsaw, Poland): "Diagnostics of neurometabolic diseases in Poland - overview"
Jolanta Sykut-Cegielska, Krystyna Szymanska, Katarzyna Kusmierska, Krzysztof Bankiewicz (Warsaw, Poland): “AADC deficiency - from diagnosis to therapy"

10.00 Catrinel Iliescu (Bucharest, Romania): "Neurometabolic disorders and diagnosis in Romania"
- coffee break -
10.40 - 11.40 SOUTHEASTERN EUROPE INNERMED NETWORKING MEETING-presentation of countries - part IV (Serbia, Slovakia, Turkey)

Chairs: Eszter Karg, Smail Zubčević

10.40 Božica Kecman, Maja Đorđević, Sanja Grković, Adrijan Sarajlija (Belgrade, Serbia): “Neurometabolic disorders in Serbia”
Sanja Grković, Maja Đorđević, Božica Kecman, Adrijan Sarajlija (Belgrade, Serbia): “Laboratory for inherited metabolic disorders and diagnostic abilities of intermediary metabolism in Serbia”

11.00 Vladimir Bzduch, Katarina Brennerova, Miriam Kolnikova, Darina Behulova (Bratislava, Slovakia): “Our experience with the treatment of nonketotic hyperglycinemia”
Katarina Brennerova, Miriam Kolníková, Vladimír Bzdúch (Bratislava, Slovakia): “Synaptic congenital myasthenic syndrome – the cause of recurrent respiratory failure”

11.20 Beril Talim (Ankara, Turkey): “Congenital muscular dystrophies with brain involvement”

11.40 Viewpoint from patients & families

Vlasta Zmazek (Croatian Alliance for Rare Diseases, Eurordis): “Innermed and Eurordis”

12.00 -13.00 Unsolved neurometabolic case

Chairs: Christine Dali, Mojca Žerjav-Tanšek

Closing remarks

- lunch and farewell -
**POSTERS** exhibition: during the whole meeting in the “Academia” hall.

Poster presentations: during coffees brakes.

P.1

Vesna Sabolić (Skopje, Macedonia): “Niemann - Pick type C - case report”

P.2

Ljerka Cvitanović-Šojat, Maša Malenica, Monika Kukuruzović, Tamara Žigman, Kristina Kužnik, Ana Bielen (Zagreb, Croatia): „Mutations of NPC1 gene and the disease course”

P.3

Ivana Kavečan, Jadranka Jovanović Privrodski, Milan Obrenović, Tatjana Redžek Mudrinić, Marija Knežević Pogančev, Aleksandra Stojadinović, Dušan Vuković (Novi Sad, Serbia): „Inherited neurometabolic diseases in the autonomous province of Vojvodina, Republic of Serbia“

P.4

Sabina Salkanović Delibegović, Nevzeta Mustafić, Amelia Numanović (Tuzla, Bosnia and Herzegovina): Brothers with Menkes disease - clinical presentation and EEG changes - case report
ABSTRACTS

OF ORAL

PRESENTATIONS
NEXT GENERATION SEQUENCING IN DIAGNOSIS OF NEUROMETABOLIC DISORDERS

Fran Borovečki

Department of Neurology, University Hospital Center Zagreb, Kišpatićeva 12, Zagreb

Department for Functional Genomics, Center for Translational and Clinical Research, University Hospital Center Zagreb and University of Zagreb School of Medicine, Šalata 2, Zagreb

Neurological diseases entail a whole spectrum of central and peripheral nervous system disorders the etiopathological mechanisms of which are often not fully elucidated. Basic and clinical research of neurological diseases is especially hindered by an inability to analyze the central seat of pathological processes in live patients. Genomic approaches, such as gene chips or next generation sequencing have enabled considerable progress in our understanding of genetic processes involved in neurological diseases. The aforementioned methods have facilitated research of disease mechanisms and development of novel biomarkers through a systems medicine approach. Novel approaches based on next-generation sequencing, such as exome sequencing, panel sequencing or RNA sequencing, will enable a more detailed insight into rare genetic variants responsible for disease development. New studies are aimed primarily towards identification of risk genes, selection of promising therapeutic targets and development of novel peripheral biomarkers. Application of novel discoveries will enable better patients care, but will also bring forth a whole new set of challenges in everyday clinical application.
POMPE DISEASE - DIAGNOSTIC AND TREATMENT CHALLENGES

Danijela Petković Ramadža¹, Ivo Barić¹²

¹University Hospital Centre Zagreb, Department of Pediatrics, Zagreb, Croatia, ²University of Zagreb, School of Medicine, Zagreb, Croatia

Pompe disease is an autosomal recessive inherited glycogen storage and lysosomal storage disorder. Due to mutations in the GAA gene, deficiency of acid a-glucosidase (GAA) results in massive glycogen accumulation and cellular dysfunction, with prominent involvement of cardiac, smooth and skeletal muscles. Incidence is approximately 1 in 40,000. Clinical spectrum ranges from infantile-onset to late-onset disease. Complete deficiency of the GAA enzyme (activity <1% of normal controls) causes infantile-onset disease. Newborns typically present within the first months of life with hypotonia, feeding difficulties, cardiomyopathy and respiratory insufficiency. Without treatment, cardio-respiratory failure leads to death within the first two years of life. Later onset disease (LOPD) is clinically heterogeneous and associated with some residual enzyme activity (2% to 40% of normal controls). Main features are proximal myopathy, with predilection of lower limbs, paraspinal muscle involvement and respiratory weakness. Respiratory involvement doesn't necessarily correlate with skeletal muscle involvement. Other problems are feeding difficulties, malnutrition and osteopenia. Patients usually have elevated serum CK. Muscle histology shows glycogen accumulation and vacuolar myopathy, but up to 20% of LOPD patients may have normal muscle glycogen. Urinary tetrasaccharides may be used as a biomarker, although excretion may be normal in LOPD patients.

Assays of GAA enzyme activity in whole blood or dried blood spots are reliable for diagnostics, but the diagnosis must be confirmed by gene sequencing or enzyme assays in different tissue (fibroblasts of muscle). Enzyme replacement therapy (ERT), available since 2006, has markedly extended ventilator-free and general survival and improved cardiac function in infants with Pompe disease. Approximately 20% of infantile Pompe patients produce no endogenous GAA enzyme (CRIM-) and develop high levels of IgG antibodies to ERT. High sustained antibody titers (HSAT) have been correlated with poor response to ERT and clinical decline. Different immunomodulation protocols were designed to prevent or eliminate immune responses to ERT. Immunomodulation in CRIM negative patients is of major importance for improving outcome. In patients with LOPD ERT improves motor function and stabilizes or slightly improves respiratory function. ERT is well tolerated and most adverse events are mild or moderate, although development of HSAT in a subset of patients with LOPD may as well have potentially negative impact on clinical response to ERT. Therapies under investigation (ERT with improved uptake by muscle cells, small molecule therapy and gene therapy) could overcome disadvantages of current ERT. One of the most important aspects of management is a close monitoring for respiratory failure with timely interventions that include aggressive treatment of infections, immunizations, respiratory therapy, non-invasive or invasive ventilatory support. Other interventions include regular physiotherapy, maintaining weight with balanced diet, tube and/or enteral feeding, etc. As patients with infantile-onset disease survive longer, a new phenotype is emerging. It includes dysphagia, hypernasal speech, osteopenia, perceptive hearing loss, arrhythmias, anterior horn cell involvement, etc. In both in infantile and LOPD patients small- and medium-vessel arteriopathy and small-fiber neuropathy are occasionally reported. Considering rarereness and complexity of the disease it is understandable why these patients should be followed in specialized metabolic centers by multidisciplinary team.
IS GAUCHER DISEASE TYPE 1 REALLY A NON-NEUROPATHIC DISEASE?

Ivan Pećin, Diana Muačević-Katanec, Iveta Merčep, Željko Reiner

Zagreb School of Medicine; University Hospital Center Zagreb; Department of Internal Medicine; Division of Metabolic Diseases, Zagreb, Croatia

Gaucher disease is the most common lysosome storage disease. Gaucher disease results from an autosomal recessive deficiency of the lysosome enzyme acid beta-glucosidase (glucocerebrosidase), which is responsible for hydrolysis of glucocerebroside (glucosylceramide [GLC]) into glucose and ceramide. Absent or reduced enzymatic activity leads to multisystem accumulation of GLC in various tissues, principally in lysosomes of macrophages, consequently compromising the spleen, liver, bone marrow, bone mineral, and, less often, the lungs, skin, conjunctiva, kidneys, and heart. Upon clinical presentation we can distinguish three types (phenotypic forms) of the disease. Neuronal involvement was characteristic of type 2 and 3 Gaucher disease (which are distinguished in between on the age of onset and the course of disease progression). In the last decade several authors have reported that neuronal involvement isn’t just exclusive to type 2 and 3 but some of the neurological symptoms can be observed in Gaucher disease type 1 patients. Those symptoms differ significantly from GD 2 and 3 form of disease and mostly include mono- and poly-neuropathy and higher incidence of Parkinson disease. This lecture will bring to the front issue about neurological involvement in patients with Gaucher disease type 1.
THE INHERITED NEUROMETABOLIC DISEASE NETWORK (InNerMeD-I-Network) - a project to be joined

M. Scarpa¹, I. Baric², F. Bartoloni³, C. Bellettato¹, F. Bonifazi³, D. Bonifazi³, A. Ceci³, F. D’Avanzo¹, V. Giannuzzi³, C. Dali⁴, C. Lampe⁴, A. Lund⁴, M. Lupo⁵, D. Negri⁵, L. Cvitanovic-Sojat², E. Cortes-Saladelafont⁶, R. Tomanin¹

¹Brains for Brain Foundation, Padova, Italy; ²University of Zagreb, School of Medicine, Zagreb, Croatia; ³Fondazione G. Benzi, Bari, Italy; ⁴Region Hovenstadet, Copenhagen, Denmark; ⁵Weber Shandwick, Bruxelles, Belgium; ⁶Hospital San Juan de Deo, Barcelona Spain.

Inherited NeuroMetabolic Diseases (iNMDs) are genetic rare diseases constituted by metabolic disorders that impact on the brain from birth.

Drugs replacing the missing enzyme or detoxifying cell metabolism can, if promptly used in newborns or young children, slow the neurodegeneration and increase life expectancies. In addition, pre-symptomatic, prenatal diagnosis and newborn screening are already available for about 70 diseases and will be soon routine tools of prevention in newborns.

But data on iNMDs are few, often bad disseminated, and experts are poorly connected. Then a challenge is to increase the general and medical awareness on iNMDs by disseminating proper information.

Inherited NeuroMetabolic Disease-Information-network (InNerMeD-I-network) is aimed to develop a network of information targeted on diagnosis and treatment of inherited iNMDs based on the exchange of information among experts, collect standardised data and disseminate validated data among patients and all the interested parties.

The general objective of the proposed Network is to create a critical mass of knowledge encompassing multi-specialist competences to be disseminated in order to:

• increase awareness on iNMDs among experts and general public;
• straighten research capacities and foster innovation in iNMD field;
• provide practical support for sharing experiences and results;
• circulate knowledge on clinical and experimental approaches for diagnosis and treatment of iNMDs and skills in view of empowering patients and families as actors of the disease management;
• expand network activities with networks of excellence focused on rare, paediatric and CNS diseases.
The major tool with which we aimed to reach our goals is the creation of an online repository. The repository will contain data spanning research, clinical features, results from clinical trials, data from available therapies and off-label application, biomarkers and genetic data which allow all the stakeholders to get all information necessary to have a clear picture of disease of interest using a single source.

The Network also aims to apply for the criteria of European Reference Network (ERN) and European Network of Paediatric Research at the European Medicines Agency (Enpr-EMA).

The Network is open for collaboration and aims to expand its activity with no territorial limit. For information please contact Maurizio.scarpa@brains4brain.eu or iberic@kbc-zagreb.hr.
LYSOSOME DISORDERS WITH CNS MANIFESTATIONS

Christine i Dali, Alan Lund

Clinical Genetic Department, Rigshospitalet, Copenhagen, Denmark

Lysosomal storage disorders (LSD) are a heterogeneous group of approximately 50 monogenically inherited orphan conditions. A defect leads to the storage of complex molecules in the lysosome, and patients develop a complex multisystemic phenotype of high morbidity often associated with premature death.

The biochemical identification of this storage material led to the traditional classification of LSDs into lipidoses (including sphingolipidoses), mucopolysaccharidoses (MPSs), glycogenosis, cystinosis, mucolipidoses, oligosaccharidoses, and neuronal ceroid lipofuscinoses. Each of these disorders is distinct with its own pathophysiology and clinical presentation. LSDs are in general progressive disorders that can manifest within a heterogeneous somatic and neurological spectrum such as hydrops fetalis, dysmorphism, dysostosis multiplex, hepatosplenomegaly, central nervous system disease, ophthalmologic, cardiovascular, renal, or cutaneous disease features.

Between 1983 and 2013, fourteen drugs for seven conditions received FDA approval. Primarily neurological LSD diseases have been neglected. Focus of this presentation are lysosomal storage disorders with CNS manifestations.


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LABORATORY DIAGNOSIS AND BIOCHEMICAL MONITORING OF
LYSOSOMAL DISORDERS

Ksenija Fumić

Department of Laboratory Diagnostics, University Hospital Center Zagreb, Zagreb, Croatia

Lysosomal storage disorders (LSD) are a clinically heterogeneous group of approximately 60 inherited disorders. They are caused by deficiency of a specific lysosomal protein and characterized by progressive intracellular storage. The importance of lysosomal pathways including autophagy is emphasized by recent findings that reveal new roles for lysosomal proteins in cellular physiology and in an increasing number of diseases that are characterized by defects in lysosome biology.

Over recent decades, considerable progress has been made in the treatment of LSDs and in patient outcome. The efficacy of many current and proposed therapies relies heavily on early detection and treatment prior to onset of irreversible pathology. Newborn screening holds the promise of early detection. However, presymptomatic diagnosis raises a number of issues related to patient management and treatment. LSD diagnostic strategy still mostly relies on initial clinical suspicion of individual symptoms that may occur from early infancy period to adulthood. Examination is usually followed by adequate specialist and laboratory management.

Laboratory diagnosis of lysosomal storage disease should be performed in several steps, depending on initial clinical symptoms. It most often begins with determination of specific metabolites in urine and/or serum and, depending on results, is followed by measurement of residual activity of lysosomal enzymes. Dried blood spot methods are currently available for identification of a range of LSDs. Molecular genetic testing is necessary to confirm the diagnosis of most LSDs and for diagnostics of X-linked diseases and pseudodeficiencies. Whenever genotype/phenotype correlations are available, they can be helpful in prognosis and in making decisions about therapy.

We are, actually, still searching for "ideal" biomarkers for all LSDs. Ideally, a good biomarker is the one that gives low false positive rates, is clearly elevated in patients with disease and ameliorates in proportion to the level of clinical correction following treatment. Preferably, it has direct link to disease pathology and needs to be cheap and technically easy to apply. Clinical applications of current biomarkers (primary and secondary accumulating metabolites or proteins specifically secreted by storage cells) involve aiding diagnosis, monitoring disease progression, and assessing therapeutic efficacy. Further research to identify novel predictive biomarkers in LSDs is essential.

Due to clinical variety LSDs still represent a challenge in modern medicine so that it is of particular significance to use recommended integrative algorithms for diagnostics of treatable LSDs.
NEW THERAPIES FOR NEUROMETABOLIC DISORDERS

M. Scarpa\textsuperscript{1,2,3}, R. Tomanin\textsuperscript{1,2}, A. Zanetti\textsuperscript{1,2}, F. D’Avanzo\textsuperscript{1,2}, L. Rigon\textsuperscript{1,2}, M. Salvalaio\textsuperscript{1,2}, A. Rampazzo \textsuperscript{2}, C. Bellettato\textsuperscript{1}, C. Lampe\textsuperscript{1,3}

1: Brains For Brain Foundation, Padova Italy; 2: Dept. Of Pediatrics University of Padova, Italy; Center for Rare Diseases, Horst Schmidt Klinik, Wiesbaden Germany.

To date 5,000 to 8,000 distinct rare diseases have been identified, affecting between 5% and 8% of the population in total. Among these, inherited NeuroMetabolic Diseases (iNMDs) represent a major public health concern in European societies, as they are mostly associated with high morbidity and mortality rates. The majority of iNMDs appear at early age or young adulthood, are chronic and invalidating, and share common problems of invisibility to the health care systems, lack of experts and of proper treatments, and social exclusion for the patients and their families. Many of the available therapies can reverse the natural history of the disease in peripheral organs (i.e. Enzyme Replacement Therapy, ERT) but unfortunately, are still not able to effectively reach the central nervous system (CNS) because they cannot cross the blood-brain barrier (BBB) that surrounds and protects the brain. Research efforts are therefore focused on the development of new alternative strategies to enhance drug delivery across the BBB. Significant advancements have already been made and different approaches including ERT. Nanoparticles have been designed in order to deliver the therapeutic agent across the BBB via specific linkers able to recognize receptors of the surface of the BBB, monoclonal antibodies specifically recognizing BBB receptors have been designed and will be challenged in phase I/II clinical trials, gene therapy approaches are at the moment under testing and direct injection of recombinant proteins have shown to be able to deliver active enzyme in CSF with therapeutic effects.

All these approaches, although still requiring a full clinical evaluation, give huge hope to patients affected by neurometabolic diseases, and their family, that in the next few years methods to modify favorably the neurologic impairment and halt the progression of the disease will be available.
DIAGNOSTIC ROLE OF MRI IN NEUROMETABOLIC DISEASES

Dimitrios Zafeiriou
Aristotle University, Thessaloniki, Greece

MRI of the brain sets new perspectives into diagnosis of neurometabolic diseases.

The first important decision whether the disease involves primarily the gray matter, the white matter, or both the gray and the white matter.

The second important decision is whether the symptomatology includes:

- Seizures, dementia (cortical gray matter disorders)
- Chorea, dystonia, athetosis (deep grey matter disorders)
- Spasticity, ataxia (white matter disorders)
- Mixed symptoms (both grey & white matter disorders)

Once a disorder is identified as being primarily of gray matter, the next step is to determine whether the cerebral cortex or the deep gray matter nuclei are primarily involved.

If only deep gray matter is involved, the identification of the specific structures that are affected and the signal intensity of the affected structures is crucial [involvement of the striatum (caudate and putamen and/or globus pallidus).

Primarily cortical involvement (cortical thinning with enlarged cortical sulci) is usually seen in neuronal ceroid lipofuscinoses, mucolipidoses, glycogen storage diseases and GM1 gangliosidosis.

In primary white matter disorders there are four important discriminators:

1. Deficient myelination vs. other pathology
2. Confluent vs. focal or multifocal abnormalities
3. Major preferential localizations (frontal, parieto-occipital, periventricular, subcortical, diffuse cerebral, posterior fossa, brainstem)
4. Special MRI characteristics (i.e. cystic white matter degeneration, anterior temporal cysts, megalencephaly, enlarged perivascular spaces or small cysts, additional gray matter lesions like cortical dysplasia, cortical lesions, basal ganglia lesions, contrast enhancement, calcium deposits, microbleeds, spinal cord involvement, evolution over time)

Furthermore, magnetic resonance spectroscopy (MRS) can further help in confirming diagnosis, instituting therapy or assessing response to therapy in specific neurometabolic disorders (i.e. maple syrup urine disease, Canavan disease, nonketotic hyperglycinemia, creatine deficiency syndromes, PKU, X-linked ALD, vanishing white matter, complex II deficiency, Sjögren-Larsson syndrome, leukoencephalopathy with a disturbance in the metabolism of polyols etc.).
SOME RARE DISEASES IN CHILD NEUROLOGY

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Objectives: Rare diseases are serious chronic diseases which can often be life-threatening, and affect only small number of children. For European population a disease is considered rare when it affects 1 person per 2000 population. It is also known that some diseases can be considered rare in certain areas in the world but being quite often in some other countries. There are around 6000 rare diseases, and each week 5 new are encountered. Most genetic diseases are rare, but rare diseases are not always caused by genetic defects, and can be of various aetiology: infectious, autoimmune, neuromuscular, neurodegenerative, but in most cases they are of unknown origin. Signs and symptoms are extremely diverse and can be found in newborn or during early infancy, however the majority of signs and symptoms will emerge during adolescence and early adulthood. Clinically and scientifically have been overlooked for a long time and are therefore regarded as orphan diseases. However there is another reason for such a description – there is usually no effective treatment. Good and above-standard care and some palliative treatments, usually the use of off-label drugs and good and continuous rehabilitation, can quite considerably improve quality of life in such persons. During recent years many programmes and projects have been developed, especially designed for people (and particularly for children) with rare diseases (such projects are also included within 7th framework programme under translational research studies – rare diseases and children and also as a single-stage project for next year, partly funded by EMEA for adapting off-patent medicines to the specific needs of paediatric populations. Very successful non-governmental organisations have also been established within Europe (Orphanet and Eurordis) for support of patients and their families but also supporting and financing research, especially in the field of development of new drugs and new strategies (like enzyme-replacement therapy, chaperon treatment, some techniques for improvement of bone marrow transplants). Similar organisations can be also found in USA, where the most known one is National Organisation of Rare Diseases (NORD) which includes 200 similar small strong affiliated organizations and strongly supports the research in the field.

Results: Some rare diseases are presented from groups of patients having infectious and parainfectious diseases, inborn error of metabolism, neuromuscular diseases, peroxysomal diseases, some syndromes and glycogen storage diseases, as well as lysosomal and leucodystrophies and diagnostic and therapeutic issues briefly discussed.

Conclusion: For the time being and with the support of the above mentioned institutions as well as research projects’ funds we can expect in some future years that rare diseases will be much more appreciated in the medical world and that with good and extensive collaboration in this field we will be able to register them correctly through European networking. On the other hand there is some hope for future effective development of different treatment approaches as well as for some techniques which have already given some positive results in the management of rare diseases today.
CHALLENGES OF DIAGNOSING AND MANAGING PATIENTS WITH INHERITED METABOLIC DISORDERS IN ALBANIA

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The diagnosis and management of patients with inherited metabolic disorders in Albania is still a challenge for clinician that face in everyday work with that kind of problems.

Actually there is inadequate number of clinicians and scientists with experience inherited metabolic disorders. The lack of funding, lack of well organized plan and not well equipped laboratory facilities are some of the reasons.

Actually neonatal screening for metabolic disorders is not performed in public hospitals in Albania. Recently children born in private hospitals are lucky to perform this test. But these is just a small number of population in comparison with children born in public hospitals.

KETOGENIC DIET IN NEUROMETABOLIC DISORDERS

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Ketogenic diet (KD) therapy is mainly used for intractable childhood epilepsy; neurometabolic diseases are rare indications for the diet.

In two diseases of brain energy metabolism, glucose transporter type 1 (GLUT1) deficiency and pyruvate dehydrogenase deficiency (PDHD), the diet is the treatment of choice.

It provides ketones as an alternative fuel to the brain. In addition, the diet has been used in several other neurometabolic conditions, but the available data are insufficient to recommend the diet as an established treatment.

Applying the diet in neurometabolic conditions is generally very similar to the KD used in intractable childhood epilepsy. The main differences are the duration and thus potential long-term consequences of the diet in these disorders.
NEURONAL CEROID LIPOFUSCINOSIS - CHALLENGES IN DIAGNOSTICS OF NEUROMETABOLIC DISEASES IN COUNTRIES WITH LIMITED RESOURCES

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Neuronal ceroid lipofuscinoses (NCL) are heterogeneous group of neurodegenerative disorders, regarding clinical and genetical aspects. They are characterized by the intracellular accumulation of autofluorescent lipopigment storage material in different patterns ultrastructurally. There are at least eight neurodegenerative disorders that result from accumulation of lipopigments in tissues. Newer classifications divide it by the associated gene: PPT1, TPP1, CLN3, CLN5, CLN6, CLN8, DNAJC5, MFSD8, CTSD.

We are presenting patient born after normal pregnancy and caesarean section delivery due to imminent asphyxia, birth weight 3250g, birth length 50cm. Early psychomotor development normal, started walking at the age of 13 months. Substantial delay in speech development, first words at the age of 30 months. Diagnosed at regional hospital as delayed psychomotor development and observed as possible muscular dystrophy since two father’s siblings suffered from congenital muscular dystrophy (non-specified). At the age of 4 years he developed myoclonic seizures that were medically intractable. Behavioural and cognitive regression became more prominent at that time and patient was referred to Paediatric Hospital in Sarajevo where brain MRI was done that was described as showing zone of changed signal intensity in parietal, paraventricular regions, with deep sulci, remanding changes in leukodystrophy. Since further diagnostic of hereditary neurodegenerative disorders could not be done in Bosnia and Herzegovina patient was referred to Metabolic Unit, University Hospital Centre Zagreb, Croatia, where new brain MRI was interpreted as diffuse reduction of white matter with hyperintensity of deep white matter, more pronounced in periventricular region, and cerebellar atrophy. Vision impairment was suspected, but this couldn't be thoroughly investigated due to patient's non-compliance. Fundoscopy showed pigmentary retinopathy. Electron microscopy of skin found fingerprint and curvilinear inclusions. PPT1 and TPP1 analysis were done in Hamburg, Germany, both enzymes were within the reference range. As clinical course was consistent with NCL, samples were sent to Naples, Italy, and the analysis showed the homozygous pathogenic variant c.754+2T>A in the major facilitator superfamily domain containing 8 (MFSD8). The report is consistent with the diagnosis of ceroid lipofuscinosis neuronal type 7 (CLN7).

Presenting this case report we wanted to emphasize fact that good networking in diagnostics of heredodegenerative neurometabolic diseases can enable patients from countries with limited resources to get final diagnosis of their disease.
THE LABORATORY DIAGNOSIS OF INHERITED NEUROMETABOLIC DISORDERS IN BULGARIA

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The National Genetic Laboratory (NGL) is the only one in Bulgaria (population of about 7.2 millions) offering genetic services to patients from specialized clinics and genetic consultants in 5 medical universities, about 70 pediatric and 119 neonatal units. NGL provides wide spectrum of modern tests for screening, diagnosis, prevention and follow-up of treatment for a variety of inherited metabolic disorders (IMD). Mass neonatal screening for phenylketonuria (PKU), analyte and enzyme selective screening for more than 70 IMD, enzyme and molecular confirmatory diagnosis (postnatal and prenatal) of more than 30 diseases are carried out.

Since 1978 about 2.5 millions newborns have been screened for PKU and 88 patients with the classical PKU and 92 with hyperphenylalaninemia have been detected. The experience from newborn screening is a good base for the introduction of expanded metabolic screening program by MS/MS technology – the best tool for detecting of more than 30 IMD in neonates.

The comprehensive selective screening program has been developed for diagnosis of IMD in patients with clinical signs of neurometabolic disorder. Each sample sent for testing is accompanied by an application form with detailed clinical information for the patient and his family. Our analytical approach includes: metabolite analyses in urine, plasma or dried blood spots by various spectrophotometric, fluorometric and chromatography techniques (TLC, HPLC GS/MS and MS/MS), in some cases followed by enzyme and molecular confirmation of diagnosis. All laboratory procedures are under strict internal and external laboratory control. The laboratory participates in the European external quality control scheme (ERNDIM Diagnostic Proficiency Testing).

For a period of more than 35 years over 10 000 patients with clinical and laboratory data for IMD have been included in the comprehensive selective screening program. The definitive diagnosis, set in the total of 669 (about 6.7 %) cases is as follows: 175 (26.2 % of the diagnosed) patients with amino acid disorders, 154 (23 %) patients with organic acidurias, 31 (4.6 %) patients with congenital lactic acidosis, 23 (3.4 %) patients with urea cycle defects, 15 (2.2 %) patients with fatty acid oxidation disorders, 15 (2.2 %) patients with peroxisomal disorders, 4 (0.6%) patients with glycoprotein degradation disorders, 76 (11.4%) patients with mucopolysaccharidosis and mucosulphatidosis, 10 (1.5%) patients with mucolipidosis II/III, 33 (4.9%) patients with carbohydrate disorders, 159 (23.8%) patients with sphingolipidosis and one (0.1%) patient with Wolman disease (LAL deficiency). Molecular genetic testing for
Wilson disease has been performed in 126 cases. Prenatal diagnosis has been carried out in 92 pregnancies at risk for 15 IMD.

The confirmed diagnoses do not fully correspond to the real prevalence of IMD in Bulgarian population, as most probably considerable number of patients remain un- or misdiagnosed, due to the fact that the majority of these disorders are quite rare, clinically heterogeneous and unrecognizable by many physicians.

To rule out some less common disorders, requiring more sophisticated testing, the patients with clinical findings, suggesting neurometabolic disorder, but with “normal” results from the selective screening, are considered for additional studies in more specialized laboratories abroad.
PSYCHIATRIC MANIFESTATIONS OF NEUROMETABOLIC DISEASES

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Neurometabolic diseases can present with psychiatric and behavioural symptoms as the initial sign of the disease or as prominent manifestations at any moment of the disorder outcome. In general they appear in combination with other clinical manifestations and although they can mimic well-defined psychotic disorders (DSM-IV), most often they appear as atypical clinical presentations. They can present as acute psychotic states and in these cases they deserve an emergency treatment. Acute manifestations include delirium, hallucinations, psychosis, and catatonia and can be present in urea cycle disorders, homocystinurias, Wilson disease, cerebrotendinous xanthomatis and some lysosomal disorders such as Niemann-Pick type C, gangliosidosis and leukodystrophies. Chronic manifestations include autism spectrum disorders, aggressiveness, ADHD, anxiety-depression, obsessive compulsive disorder, bipolar disease and dementia, and are present in a wide variety of neurometabolic diseases. In this workshop we will consider different clinical cases to exemplify the main neurometabolic diseases that can present acute and chronic psychiatric manifestations. Main messages will address the necessity of considering an urgent metabolic work-up in the acute cases and thinking first about treatable disorders in any kind of presentation.
INHERITED DISORDERS OF METHYLATION AFFECTING THE BRAIN

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Inherited methylation disorders are considered those, which affect transfer of methyl groups from methionine via adenosylmethionine (AdoMet), which is major methyl group donor in humans, by numerous methyltransferases to a large number of acceptors, including important molecules like DNA, RNA, lipids, proteins, amino acids, neurotransmitters, etc. The common product of all reactions mediated by methyltransferases is S-adenosylhomocysteine (AdoHcy), which is a potent inhibitor of these reactions, regulated actually by the AdoMet, AdoHcy and their ratio. Therefore, the pathogenecity of these disorders is very complex. This pathway is part of methionine cycle and it occurs in all mammalian cells reflecting its significance. Four inherited disorders affecting this pathway have been described. These are methionine adenosyltransferase (MAT) deficiency and three disorders described in this century - the glycine-N-methyltransferase (GNMT) deficiency, S-adenosylhomocysteine hydrolase (SAHH) deficiency and adenosine kinase (AK) deficiency.

All inherited methylation disorders either affect the brain or have the potential to affect it. Clinically, MAT deficiency presents only in some patients, with variable neurological symptoms. GNMT deficiency seems relatively benign and can be characterized by hepatomegaly, failure to thrive and elevated aminotransferases. However, plasma methionine concentration can be high enough to be itself risky for the brain. SAHH deficiency is characterized by congenital myopathy with high creatine kinase, delayed psychomotor development, frequently with attention deficit-hyperactivity disorder and delayed myelination. Severe cases died in early infancy and had brain anomalies. Patients with AK deficiency had severe developmental delay, macrocephaly, frontal bossing, early onset epilepsy and liver disease characterized mostly by cholestasis.

Hypermethinoninemia is the most frequent, relatively specific, biochemical marker of inherited methylation disorders. However, it is not always present in all patients, in particular in newborns and young infants, making the expanded newborn screening unreliable for this group of disorders. Although clinical signs and symptoms can be clues for diagnosis, measurements of AdoMet and AdoHcy are frequently a necessary diagnostic step. Confirmatory tests are enzyme assays and/or gene analysis, depending on the disease.

Specific treatment options include low methionine diet and supplementation of AdoMet in some MAT-deficient patients and low methionine diet, choline and cysteine being partly
useful in some SAHH deficient patients. GNMT deficiency possibly does not need treatment and AK deficiency in most patients does not seem amenable to treatment.

Since decreased ratio of AdoMet to AdoHcy is supposed to be at least one of the pathogenetic mechanisms in numerous inherited and acquired pathological conditions associated with hyperhomocysteinemia the research of this metabolic pathway and its inherited disorders has special significance. It has further potential because it can reveal important mechanisms of damage of organs affected in mentioned disorders (brain, muscle, liver), regulatory factors in transsulfuration processes and cancer development (also associated with disturbed AdoMet/AdoHcy ratio).

We stay at disposition for related diagnostic assistance and research (ibaric@kbc-zagreb.hr).
Glutaric aciduria type I (GAI) is an autosomal recessive metabolic disorder caused by a deficiency of glutaryl-CoA dehydrogenase, an enzyme involved in the catabolic pathway of lysine, hydroxylysine and tryptophan. More than 100 disease causing mutations have been identified so far and certain mutations show predominance in specific populations. Ten Cypriot children have been diagnosed with GAI in the last twenty years. Diagnosis was based on their urinary organic acid and plasma acylcarnitine profile. Molecular analysis of the \textit{GCDH} gene was performed by direct sequencing of the patients’ genomic DNA. \textit{In silico} tools were applied to predict the effect of the novel variants on the structure and function of the protein. All disease alleles were characterized (mutation detection rate 100%). Five missense mutations were identified: c.192G>T (p.Glu64Asp) and c.803G>T (p.Gly268Val), which are novel, and three previously described mutations, c.1123T>C (p.Cys375Arg), c.1204C>T (p.Arg402Trp) and c.1286C>T (p.Thr429Met). The novel mutations, p.Glu64Asp and p.Gly268Val, account for the majority of the disease alleles (76.5%) in the Cypriot population and are postulated to have arisen through founder effects. Identification of the GAI mutations in the Cypriot population has facilitated carrier detection as well as post- and prenatal diagnosis.
MITOCHONDRIAL DISEASES IN CHILDREN

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Mitochondrial disorders represent a clinically, biochemically and genetically heterogeneous group of more than 260 diseases associated with dysfunction of the oxidative phosphorylation system (OXPHOS) and pyruvate dehydrogenase complex with estimated incidence of 1 : 5000. In these disorders, organs with the highest energy demand (brain, heart and skeletal muscles) are predominantly affected. Mitochondrial diseases may manifest at any age since birth until late-adulthood with acute manifestation or as a chronic progressive disease. The clinical presentation and course of patients with mitochondrial syndromes are extremely diverse, even among patients or relatives with identical enzymatic or genetic defects. For diagnostics of mitochondrial disorders, it is necessary to take into account the particular family and personal history, the course of the disease, the comprehensive clinical examination, results of specialized examinations (especially cardiology, visual fundus examination, brain imaging, EMG), laboratory analyses in body fluids (e.g. increased lactate, alanine), and examination of biopptic samples of muscle, skin, and liver, eventually. The definitive diagnosis is confirmed by the molecular-genetic examination. Therapy of mitochondrial disease is largely supportive, although some therapeutic modalities may significantly influence the course of the disease (e.g. arginine supplementation in MELAS syndrome, ketogenic diet in PDH deficiency). In the area of central Europe, TMEM70 deficiency is one of the most common diagnosed mitochondrial disease. This was originally described as a disease with early neonatal onset and poor prognosis linked to the Roma population, due to a homozygous mutation c.317A>G in the TMEM70 gene, but recent reports from different regions demonstrated a much broader spectrum of clinical symptoms including patients with later disease onset and/or attenuated course. The most discriminating and nearly constant clinical symptoms in children with TMEM70 deficiency are the early onset of hypotonia, failure to thrive, short stature, microcephaly, developmental delay, non-progressive hypertrophic cardiomyopathy and attacks of metabolic crises. The most common results of metabolic analyses are hyperlactataemia, hyperammonaemia and 3-methylglutaconic aciduria. TMEM70 deficiency is a relatively common panethnic, multisystemic disease with variable outcomes depending mainly on adequate management of the hyperammonaemic crises after birth or in early childhood.
NEWBORN SCREENING FOR NEUROMETABOLIC DISORDERS

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The genetic diseases that belong to the group of inherited metabolic disorders (IMD) or neurometabolic disorders (NMD) are not strictly defined. Yet, newborn screening (NBS) for IMDs inevitably encompasses NBS for NMDs. NBS is justified if early diagnosis, possibly before symptoms emerge, is to advantage of the newborn as defined by the Jungner Wilson criteria and its updates. NMDs are represented in each of the three main groups of IMDs, i.e. they may induce 1. acute toxicity, 2. energy deficiency or 3. cellular damage due to problems in the synthesis or degradation of macromolecules. Efficient therapy is available for several of the amino acid disorders and sugar intolerance of the first group, though the benefits of early treatment of organic acidurias or neurotransmitter diseases are less pronounced. Significant benefit of NBS is proven for medium-chain acyl-CoA dehydrogenase deficiency belonging the second group of disorders. In recent years, the introduction of enzyme replacement therapy provided the rationale for screening of lysosomal storage disorders. Screening panels differ greatly between countries, at present 26 IMDs of the 1. and 2. groups are screened for in Hungary. Since the introduction of expanded newborn screening, patients with amino acid (phenylketonuria, citrullinemia I and II, tyrosinemia I) and neurotransmitter (tetrahydrobiopterin deficiency) disorders, organic acidurias (propionic aciduria, glutaric aciduria, isovaleric aciduria, methylcrotonylglycinuria), biopterin deficiency, galactosemia, and lipid oxidation disorders (middle chain-, long chain 3-hydroxyacyl- and very long chain-CoA dehydrogenase deficiencies, as well as multiple acyl-CoA dehydrogenase deficiency) have been detected.
NEUROMETABOLIC DISEASES IN MACEDONIA - CURRENT DIAGNOSTIC POSSIBILITIES

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Growing possibilities for treatment of inborn errors in metabolism (IEM) require establishing the diagnosis as soon as possible. There are two approaches that should be implemented within the medical care system. First aim is to improve basic knowledge of primary care physicians and pediatricians about the first clinical signs of these diseases. Constant improving of the laboratory possibilities is also goal that should be reached.

There is an improved medical educational system along the three levels of medical care in Macedonia.

Diagnostic guidelines were prepared for more frequent IEM in order the first signs of the disease to be noticed by the physicians, i.e lethargy, vomiting, convulsions, etc. Regular courses dedicated to IEM were held organized on different levels.

The utilization of tandem mass spectrophotometry is announced and should provide the possibility to establish the diagnosis of 15 IEM, including the most frequent amino and fatty acid metabolism disorders. The pilot project was offered on a selected population of newborns and young infants where clinical suspicion or basic biochemical disorder for IEM appear. Comprehensive strategies organized by medical authorities can lead to timely recognition of common IEM in Macedonia.
LATE DIAGNOSIS OF PHENYLKETONURIA - TIME FOR CHANGES

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Hyperphenylalaninemia has been detected in four children in first 20 months of age during evaluation of psychomotor delay. The standard amino acid analysis done by liquid chromatography detected higher levels of PHA from just above the upper limit 460 μmol/L till 1200 μmol/L considering 120-360 μmol/L as normal ranges.

Three boys and a girl had been followed because of delayed fine motor skills, lack of attention, poor visual contact. There were episodes of anger and excitation including selfmutlation .There was no initiation of speech at all four of them. The severe deficits were in cognitive function and communication skills.

EEG recorded epileptiform activity in one case where tonic seizures occurred. The other 3 EEG findings were in normal ranges.

Areas of delayed myelination were detected on MRI as well as cortical thickness and atrophy in frontal and temporal regions

Phenylalanine free diet started, with difficulties to maintain PHA close to normal levels. The early outcome considered improvement in growth and nutrition. In 2 cases with good compliance there is improvement of communication but with poor speech which started at age 4 and 5. Hyperactivity was reduced, they started special education. Two severe cases, with poor diet are highly disabled, hyperactive and aggressive, they need permanent nursing and care.

Neonatal screening should obtain early diagnosis and treatment which is prerequisite of better developmental outcome and quality of life.
INTERACTION AMONG FOLAT/HOMOCYSTEINE METABOLISM GENES AND ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE POLYMORPHISMS IN CASE OF DUCHENNE MUSCULAR DYSTROPHY

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Introduction. Have been modeled gene-gene interaction between 5 mutations in 4 genes, involved in folat/homocysteine metabolism (FHMG) and eNOS which play a pivotal role in vascular homeostasis and endothelial function, that may be alter the severity (in our case on the age at wheelchair dependency- 9 or 12 years) of DMD.

Methods: A retrospective single institution long-term follow-up study was carried out in 148 corticosteroids-free DMD patients. The genotyping of dystrophin gene were performed by the MPCR to detect the deletion in DMD gene and the PCR-RFLP to identify the MTHFR C677T and A1298C, MTRA2756G, MTRRA66G, eNOS polymorphisms. For statistical analysis we used the program SPSS (version 20). Gene-gene interactions were analyzed using entropy-based multifactor dimensionality reduction (MDR).

Results: We evaluated single-site allelic and genotypic associations, genotype equilibrium and multilocus genotype associations, using MDR, which failed to show a genetic model of severity of myopathic process. Multinomial logistic regression method helped us to choose the right model intensity due to genetic indicators and other clinical data at the speed of progression of the pathological process. Positive assessment of MTHFR C677T heterozygous and homozygous MTHFR C1298C mutations mean that the corresponding categories act as the highest category of the dependent variable (p=0.004 and 0.039, respectively). Mathematical value of the parameter estimates of the regression showed a statistically significant (p<0,05) value for compound heterozygotes MTHFR677, MTHFR1298 and MTR (β = 33,7) and MTHFR 677 , MTRR, MTR (β = 34,7). The genetic basis of susceptibility to the progression of myopathic process and early disability (up to 9 years old) show a strong synergism interaction between genes of folate cycle, methionine ( FMC ) and endothelial NO synthase gene. Have been adopted the MDR method to explore the synergistic effects of the studied polymorphisms on modifying to myopathic process. We selected the best model, which included the. MTHFR677T and A1298C, MTRA2756G, eNOS polymorphisms, cross-validation consistency is 9/10 (χ² =54.22, p<0.0001) for case of wheelchair up to 12yrs). Since the selected polymorphisms were not associated with DMD there is evidence for the existence of epistasis between the two polymorphisms MTHFR677T and eNOS (CVC10/10, χ²=5.3, p=0.02) in case of wheelchair up to 9yrs.

Conclusion: Our results indicate that the MTHFR, MTR and eNOS genes are modifying loci and presence of the high-risk alleles may associate with an increase in the severity of DMD.
THE DEVELOPMENT OF NEUROMETABOLIC FIELD IN MOLDOVA

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The IEM require very expensive equipment and a very good trained multidisciplinary team of professionals to be developed. In Moldova this domain is not evolved according to many standards due to very limited financial possibilities, but the diagnosis of IEM, beside PKU where there is the neonatal screening, is successfully initiated through a very fruitful collaboration with the foreign partners, supporting in such a way the creation of the National Register of Rare Diseases in Moldova.

Methods: Thus, the biochemistry as the ammonia, lactate, glucose, pH and amino acids of body fluids by liquid chromatography (LC) were performed in Moldova; the $^1$H-NMR spectroscopy for organic acids of urine/CSF/amniotic fluid – in Romania; tandem MS with acylcarnitine profile – in Hungary and Romania; neurotransmitters in CSF – in Germany and IEF of transferrin – in USA.

Results: For the first, the National Register includes 102 PKU patients, the majority of them being diagnosed by neonatal screening existing about 25 years in Moldova which rate is 88-97% during last years. PKU is registered as 1:7325 newborns and each year about 3-4 PKU newborn are found. The diet and management of PKU patients are individualized according to the results of amino acids in blood and urine using LC. The genotype analysis is routinely performed in PKU patients only on 12 common mutations found in ~75% of them, the BH$_4$ loading test is not applied. We report on 3 cases of maternal PKU with a better outcome in a case when the strong diet was kept during all the pregnancy, but the child died from a non-maternal-PKU problem. The new starting screening for CH is in progress now.

As about other IEM, we could report on many cases diagnosed during the last years using different methods through our collaboration. Thus, we have been found 5 children with methylmalonic aciduria and 1 child with glutaric aciduria type 1 positively diagnosed by both $^1$H-NMR spectroscopy of urine and by Tandem MS. Four children were diagnosed with galactosemia using neonatal screening and $^1$H-NMR spectroscopy of urine and confirmed by enzyme assay for GALT deficiency. Five children were discovered having high urinary 2-oxoglutarate and lactate by $^1$H-NMR spectroscopy; 3 of them were clinically confirmed with glycogen storage disorder and other two were suspected for possible mitochondrial disorder with PDHC or KDHC deficiency due to additional high blood Ala and lactate. More than 10 cases were suspected for mitochondrial disorders according to their clinical manifestations having high blood/urinary lactate, Ala (>450 mkmol/l) and Ala/Lys ratio (>3) appreciated by LC, no other investigations to confirm it are not performed in Moldova. In a case with dark urine of a suspected patient high level of homogentisic acid in urine by $^1$H-NMR spectroscopy...
was appreciated and alkaptonuria was confirmed. One newborn with a deep coma in the 3rd day of life after a short period (2 days) of wellness was appreciated with urea cycle disorder. In order to exclude a congenital disorder of glycosylation we send the samples for the IEF of transferrin, no CDG patients were found yet. Two cases of GM1-Gangliosidosis were confirmed in EU.

In about 10 children the neurotransmitters disorders were suspected due to their neonatal onset of convulsions and resistance to the anticonvulsive therapy. The amino acids of blood, urine and CSF have been analysed and the clinical trials with pyridoxine, folic Acid and pyridoxal-5-phosphate have been performed under EEG control. Only one child was investigated for neurotransmitters metabolites and pterins in CSF. Two children were suspected for hyperekplexia due to their clinical presentations.

**Conclusions:** In the absence of a system for IEM performing in Moldova the collaboration with other institutions is very important. The evaluation of clinical manifestations remains the most important to detect an IEM patient. Among most accessible specific investigations for Moldova the $^1$H-NMR spectroscopy of urine performed in Romania (the nearest lab) seems to provide high analytic information improving the orientation in diagnosis of IEM in unclear patients, but all methods are very necessary and should be developed for the better IEM diagnosis. We are waiting for a new amino acids analyzer and other equipment (genetic analyzer, qPCR). Early diagnosis of IEM is very important for the specific therapy initiation and to prevent some of them by prenatal tests. Our further developmental strategy is to improve the field of IEM in Moldova through the common European projects.

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DIAGNOSTIC AND TREATMENT OF INHERITED NEUROMETABOLIC DISEASES IN MONTENEGRO: POSSIBILITIES AND LIMITATIONS

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Neuro-metabolic diseases (InNerMeD) are defined as inborn errors of metabolism affecting the central nervous system, causing progressive destruction of mental and motor functions, usually starting early in childhood, often very unspecific, affecting multiple organ systems, varying from those of acute life-threatening disease to subacute progressive degenerative disorder associated with earlier death often before adulthood.

InNerMeD present a significant group of inherited diseases, but with very low individual prevalence in general population (less than 1 in 10,000 births), difficult to diagnose and with no treatment available or rarely expensive to treat and with uncertain outcome. Following these facts InNerMeD are classified as rare diseases (RD), and are recognized as one of health priorities in Europe.

InNerMeD represent a significant challenge in small countries with limited professional and financial capacities. According to characteristics of population in Montenegro (approx. 670,000 citizens, life expectancy 73/76 yr man/women respectively, life birds approx. 8000/yr, infant mortality rate < 6/1000), access to InNerMeD is organized as unique centralized multidisciplinary service at tertiary health care level (pediatric neurologist, clinical genetics, medical biochemist, prenatal and postnatal genetic diagnostics and genetic counseling). Burdened with lack of professional experience, obscure clinical recognition and difficult and late diagnose, overall treatment of InNerMeD is predominantly focused on early clinical recognition and regional and cross Europe collaboration regarding diagnostic and treatment.

Facing with a lack of appropriate and unified registration of RD, including InNerMeD, at national level, but also with insufficiently raised awareness on RD among medical professionals and general population, Government of Montenegro adopted National Strategy for Rare Disease in Montenegro, with National Action Plan 2013–2020, in January 2013. The main goals of Strategy are to set the comprehensive institutional framework and mechanisms for better health care of patients with RD and to improve the response on RD at national level. Three main priorities are: holistic approach in recognition, diagnostic, treatment and social integration of patients with RD, training and building the professional capacities and giving a reference point for doctors to recognize rare diseases, and integrative data collecting and analyzing. The second, but also crucial priority is to improve research in the field of RD with establishment of cooperation at regional and EU level.
DIAGNOSTICS OF NEUROMETABOLIC DISORDERS IN POLAND - OVERVIEW

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Diagnostics of neurometabolic disorders in Poland is based on newborn screening and selective screening. In symptomatic patients the initial metabolic work-up starts mainly in local hospitals (in neurological, genetic and paediatric departments), depending on leading signs and symptoms.

Diagnostic procedures are usually consulted and coordinated by limited number of metabolic centres. There are focused on and dedicated to lysosomal storage disorders, mitochondrial diseases, neurotransmitter defects, congenital disorders of glycosylation and other small molecules diseases (as aminoacidopathies and organic acidurias), with long-term experience. Newborn screening by tandem MS allows early diagnosis and outcomes improvement in some aminoacidopathies and organic acidurias. Other neurometabolic disorders are merely frequently detected by molecular analyses, though biochemical and enzymatic assays are still of benefit.
Aromatic amino acid decarboxylase (AADC) deficiency (MIM 608643) is a rare recessive neurodevelopmental disorder that affects the synthesis of both groups of biogenic amines and results in combined catecholamines and serotonin deficiency. AADC deficiency may be a challenge for clinicians, regarding early diagnosis and effective treatment. The right diagnosis is frequently delayed, mainly due to insufficient awareness among physicians about this rare disease, though the clinical picture is rather typical. Just after establishing diagnosis of AADC, proper treatment should be recommended. Multiple pharmacotherapy is recommended, but response is variable and usually insufficient. Moreover some medications are not authorized for children and/or for this condition, therefore they may be not easily available. Currently the treatment should be individually adjusted to the patient and monitored by the experienced specialists in the field. Recently the new therapeutical method has appeared – gene therapy based on viral vector (AAV2), encoding the human AADC gene (hAADC), which has been developed for the treatment of Parkinson’s disease. Early studies have demonstrated that the gene can be safely delivered to the striatum in human subjects via targeted infusion.

One out of two cases of Polish patients with AADC diagnosis is discussed in the presentation.
NEUROMETABOLIC DISORDERS AND DIAGNOSIS IN ROMANIA

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Diagnosis of neurometabolic disorders is still challenging in Romania. The neonatal standard screening includes congenital hypothyroidism and phenylketonuria, and most of metabolic investigations needed for diagnosis are (still) not reimbursed nor performed by standard laboratories available in the hospitals, with very few exceptions.

We intend to present a case of a child with a neurometabolic disorder quite easy to diagnose usually, in whom an extremely high suspicion of the disorder existed for the pediatric neurologist in charge, but inclusion of the child in the national programme of rare disorders was not possible until definite diagnosis was made, and this was not possible in Romania, due to some particularities of the case.

Our conclusion would be that availability of a network of specialists in an area closer to the original country would increase the possibilities of diagnosis and decrease the costs, the time until a definite diagnosis is reached and the most appropriate case management is made.
NEUROMETABOLIC DISORDERS IN SERBIA

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Republic of Serbia is situated at the crossroads between Central and Southeast Europe with a total population of 7.2 million. Birth rate in Serbia is 9.1 births/1000 inhabitants which means that 65,000 live births occur in country per year. Main center for metabolic disease and site for National neonatal screening programme is Mother and Child Health Care Institute. Minority of metabolic patients are treated in other university pediatric hospitals in Serbia. Neurometabolic disorders are quite rare in general population but collectively they represent a significant part of pediatric morbidity. The presenting manifestations of neurometabolic disorders usually include progressive deterioration of mental and motor functions. The symptoms are often unspecific: delayed development, letargy, seizures, poor feeding and failure to thrive.

Newborn screening for phenylketonuria (PKU) is implemented in Serbia since 1982. Currently, 50 patients are treated for PKU with special diet. National Health Insurance Fund provides low phenylalanine food products.

Patients with intoxication type neurometabolic disorders form the largest group of our patients (maple syrup urine disease – 4 pts, methylmalonic aciduria – 5 pts, propionic aciduria – 1 pt, glutaric aciduria type 1 – 2 pts, L-2-hydroxyglutaric aciduria – 3 pts, urea cycle disorders - 6 pts, neurotransmitter metabolism – 1 pt).

Neurometabolic disorders involving energy metabolism comprise second largest group (oxydative phosphorylation defects – 15 pts , PDH – 4 pts, fatty acid oxidation defects – 5 pts, infantile Pompe disease – 1 pt).

Third largest group of our patients have some of disorders involving complex molecules: Gaucher disease chronic neuropathic form – 3 pts, Krabbe disease – 1 pt, Niemann Pick disease type C – 3 pts, metahromatic leukodystrophy – 3 pts, MPS type II – 7 pts, neuronal ceroid lipofuscinoses – 5 pts, X-linked adrenoleukodystrophy – 1 pt).

Our center provides important part of biochemical metabolic diagnostics but for definitive enzymatic and genetic analysis we usually rely on foreign laboratories, located mostly in countries of European Union. Enzyme replacement treatment is currently used in our Institute for 5 patients with Gaucher disease, 6 patients with Hunter syndrome and one patient with Pompe disease.

Majority of families of our patients participate regularly in activities of National Organization for Rare Disease. Physicians, biochemists and geneticists from our and other institutions also play significant educational role in meetings organized by parental support groups.
The laboratory for inherited metabolic disorders (IEMs) at Mother and Child Health Care Institute of Serbia is unique laboratory in the country with a mission to provide biochemical testing and result interpretation for multitude of disorders. Laboratory provides consultation with physicians for diagnosis and follow-up of patients mainly with intermediary metabolism disorders. IEMs are present in every population but are perceived to be very rare due to low recognition rate and scarce reporting. Thus, prevalence of IEMs in various population is mostly underestimated, except in cases of large population screenings. Currently, the only neonatal screening test for IEMs in Serbia is for phenylketonuria (PKU) and it is performed in our laboratory. Recent advances in tandem spectrometry (MS/MS) technology has facilitated the detection of multiple IEMs simultaneously on a single specimen and has been attracting increasing interest. Working group for IEM in Serbia in collaboration with the Ministry of Health plans effective integration of MS/MS technology into newborn screening programme. However, decision and implementation will take time due to the complexity of introducing novel technique with limited resources. Since the biochemical basis of IEMs is wide, our approach is to maximise the cost effectiveness of the allocated funds and broaden the diagnostic potential with the range of useful tests. We concluded that the use of an amino acids analyser, GC/MS instrument for the quantitative assay of organic acids, specific assays for carbohydrates, neurotransmitters, neopterin, biogenic amines and their metabolites enables our Institute to successfully diagnose a wide variety of disorders of intermediary metabolism i.e. amino acids and organic acid disorders, urea cycle defects, galactosemia, hereditary fructose intolerance and disorders of neurotransmitter metabolism. The tests targeted for disorders like cooper metabolism (Wilson and Menkes disease) and cholesterol metabolism (Smith-Lemil Opitz syndrome) require use of a wider range of instruments and methods such as enzyme analyses and DNA mutation studies. Proper interpretation of metabolic biochemical results and successful diagnosis of IEMs requires clinical information and usualy results of other laboratory and radiological findings. Therefore, good and continuous communication between clinical and laboratory staff is essential for correct diagnosis.
OUR EXPERIENCE WITH THE TREATMENT OF NONKETOTIC HYPERGLYCINEMIA

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Objective: Nonketotic hyperglycinemia (NKH) is a devastating neurometabolic disorder, leading to early death or severe disability. Classical treatment strategies for NKH included a reduction of glycine by sodium benzoate, and a blockade of the N-methyl-D-aspartate (NMDA) receptor by the NMDA receptor antagonist dextromethorphan.

Methods: Four patients (three with typical neonatal form and one with atypical late-onset form) are presented. Diagnosis was based on the finding of hyperglycinemia and hyperglycinuria in the absence of an organic acid disorder and on calculation the cerebrospinal fluid/plasma glycine concentration ratio greater than 0.08 (normal values below 0.04). All patients were treated with sodium benzoate 500mg/kg/day, dextromethorphan 5mg/kg/day and L-carnitine 50mg/kg/day. In one infant with neonatal form of NKH we started treatment with ketogenic diet at the age of 18 months after informed consent of parents.

Results: After classical treatment of NKH, glycine concentration in plasma decreased and condition of our 3 infants only temporarily improved. One died at the age of 34 days, other have suffered from severe brain damage. Only patient with atypical late-onset form have only moderate brain damage. In one male patient with neonatal form of NKH despite classical treatment extremely hypotonia and severe clinical state persisted, so we used a new treatment with ketogenic diet. Shortly after its institution, clinical state promptly improved and after one year of ketogenic diet, cerebrospinal fluid glycine markedly decreased from 124umol/L to 52.2umol/L (normal < 10,1), hypotonia and muscle strength improved, alertness increased and seizures ceased.

Conclusions: Our experience showed, that classical treatment of NKH favourably modified only early neonatal course, but does not prevent poor long-term prognosis of patients. In one patient with severe neonatal form of NKH a new treatment with ketogenic diet improved his clinical state and decreased cerebrospinal fluid glycine. Experience of other patients with NKH are required to confirm this beneficial effect of ketogenic diet.
SYNAPTIC CONGENITAL MYASTHENIC SYNDROME – THE CAUSE OF RECURRENT RESPIRATORY FAILURE

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The most common cause of congenital myastenic syndrome (CMS) in Slovakia is mutation of CHRNE (ε1267delG) that causes anomalies of acetylcholine receptor. This disease belongs to postsynaptic group of CMS and has a high incidence in the Roma ethnic group. The prognosis is good and the disease is non-progressive and benign in the most cases.

In our case report we describe the first case of synaptic CMS in Slovakia: a girl with recurrent respiratory failure from neonatal age. A few days before respiratory failure the child refused a food. After 2-3 days of ventilatory support she started to breath spontaneously, then about 10 days was sufficient food intake. At the age of 8 months during severe sepsis extubation after respiratory failure was not possible.

Ptosis on the left eye appeared at the age of 6 months, later bilateral ptosis was present. There were slow photoreactions. At the age of 16 months a EMG decrement - more than 20% - was described. Syntostigmin clinical condition deteriorated, 3.4 DAP had no therapeutic effect. After consultation with foreign specialists we have started treatment by ephedrine. On this treatment continuous ventilation was gradually reduced only to night mode ventilation through a tracheostomy.

The child is 9.5 years old now, after repeated scoliosis surgery, separately pass a couple of meters, eats mixed food. Ephedrine dosage is 5 mg / kg / day divided into 5 doses. She attends the primary school, learning problems attributed to repeated hypoxic conditions in respiratory failure. Hypomimia, ptosis and significant muscle weakness persists.

Molecular genetic analysis showed heterozygous mutation p.Q211X in the COLQ gene, but the second mutation is missing. The next genetic tests are not possible without a muscle biopsy, but the parents refused biopsy.

Conclusion: Each unclear respiratory failure in children requires EMG test to exclude myasthenic syndrome.
CONGENITAL MUSCULAR DYSTROPHIES WITH BRAIN INVOLVEMENT

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Congenital muscular dystrophies (CMDs) are characterized by muscle weakness presenting at birth or during the first few months of life and delayed developmental milestones. Apart from muscular symptoms and contractures, brain and eye involvement are present in some forms. Brain involvement can be in the form of variable degrees of mental retardation and structural malformations.

In the earliest identified genetic form, merosin deficient CMD caused by mutations of the LAMA2 gene encoding laminin alpha 2 of the extracellular matrix, patients have normal intelligence. However, brain imaging shows white matter changes in the periventricular area, due to disturbance of the blood-brain barrier.

Muscle-eye-brain disease, Walker Warburg Syndrome and Fukuyama type CMD are the classical severe phenotypes, presenting with variable degree of central nervous system malformations (ex. cobblestone lissencephaly) and ocular findings. A major breakthrough in the understanding of CMD with structural brain involvement has been the identification of deficiency of alpha dystroglycan (aDG) glycosylation. Alpha dystroglycan is a heavily glycosylated structural component of the sarcolemma, associated with extracellular matrix by an extensive network. This interaction is hampered due to hypoglycosylation of aDG and results in a wide spectrum of diseases from isolated muscular dystrophy (congenital or limb-girdle muscular dystrophies) to muscular dystrophy associated with brain and eye malformations. To date 17 genes responsible from defects in glycosylation of aDG have been identified. However, some patients with aDG deficiency still lack molecular diagnosis and more genes are to be discovered with the use of modern molecular techniques.

Another form of CMD with brain involvement is caused by mutations of the CHKB gene encoding choline kinase beta, the first enzyme in the phosphotidyl choline synthesis, which is a major component of the membrane phospholipids. This disease, also named as megaconial CMD, is characterised by muscle weakness (usually onset in the first few months but may present later in some cases), delay in developmental milestones, mental retardation, high creatine kinase and characteristic findings in muscle biopsy (enlarged mitochondria usually accumulated in the periphery of the fiber, leaving the center devoid of staining in oxidative enzyme stains). Autistic features and ichthyosiform skin changes are also common. Some patients develop dilated cardiomyopathy, which is the cause of early mortality.

The spectrum and etiology of brain involvement in CMD is variable. Careful clinical examination, brain imaging and muscle biopsy are crucial for correct diagnosis and they guide molecular investigations.
InNerMeD and EURORDIS

Vlasta Zmazek, EURORDIS Board of Directors, Croatian Alliance for Rare Diseases, Debra Croatia

What is connecting EURORDIS and InNerMed project?
EURORDIS is a non-governmental patient-driven alliance of organisations and individuals active in the field of rare diseases in Europe.
EURORDIS represents 678 rare disease patient organisations in 63 countries, covering more than 4000 rare diseases.

Eurordis Mission:
• To build a strong pan-European community of patient organisations and people living with rare diseases.
• To be their voice at the European level and to fight against the impact of rare diseases on their lives.

Rare Diseases: Public Health Priority – Achievements of EURORDIS from 2008-2015
• Contribution to the adoption of the EU Regulation on Orphan Medicinal Products in 1999
• Contribution to the adoption of the EU Regulation on Paediatric Drugs in 2006
• Contribution to the adoption of the EU Regulation on Advanced Therapy Medicinal Products in 2007
• Contribution to the adoption of the EU Commission Communication on Rare Diseases in 2008
• Contribution to the adoption of the EU Council Recommendation on European Action for Rare Diseases in 2009
• Contribution to the adoption of the EU Directive on Patients’ Right to Cross-Border Healthcare in 2011
• Contribution to the promotion and maintenance of rare diseases as:
  – EU Public Health Policy priority
  – EU Research Framework Programme priority
• Promotion of National Plans and Strategies on Rare Diseases in all 27 EU Member States and other European countries
• Contribution to the designation of over 1100 orphan drugs
• Initiation and organisation of the biennial European Conferences on Rare Diseases (ECRD)
• Initiation and annual coordination of the International Rare Disease Day

Common issue with InNerMed project: Reducing the gap in the field of Rare Diseases by networking, collection and exchanging of information.

European Reference Networks – role of Eurordis

Key words: Rare Diseases, information, exchange, networking
ABSTRACTS
OF POSTER
PRESENTATIONS
NEIMANN-PICK TYPE C - CASE REPORT

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Niemann Pick type C (NPC) is an autosomal recessive lysosomal storage disorder characterized by accumulation of cholesterol and gangliosides. NPC has wide clinical spectrum and variable age of onset. Patients demonstrate neurological dysfunction with cerebellar ataxia, dysarthria, seizures, vertical gaze palsy, motor impairment, dysphagia, psychotic episodes and progressive dementia.

Treatment with miglustat can stabilise neurological progresson of disorder

Our patient was 12 years old girl, first child of healthy and nonconsanguineous parents. Her early development was normal until 9 years of age when neurological symptoms started with learning difficulties, dysarthria, ataxia . At 12 years she admitted hospital after first seizures. Hepatosplenomegaly, severe cerebellar ataxia, dystmetria, dysarthria, supranuclear paralysis of vertical gaze was found on examination. Bone marrow puncture showed blue histiocytes and foam cells. Enzyme analysis for lysosomal disorders was chitotriosidase positive. Fibroblasts cultured from skin were tested with filipine staining. More of 90% of fibroblast cells stored cholesterol. This test was consistent with the diagnosis of Nimann Pick Type C. DNA analysis confirmed the diagnosis.
Niemann-Pick disease type C is a rare autosomal recessive disorder, caused in 95% of the cases by mutations in NPC1 gene. Consequently, unesterified cholesterol accumulates in late endosomes/lysosomes causing extremely various neurovisceral symptoms. For many countries including Croatia, there are no reported NP-C cases to date, mainly because the accurate diagnosis of NP-C requires not easily accessible biochemical and molecular-genetic laboratory tests. Therefore, with the aim of improving the clinical praxis and understanding of NP-C in the region, we present the first siblings with NP-C recorded in Croatia. The diagnosis was based on histological, biochemical and genetic tests. Namely, filipin staining showed accumulation of unesterified cholesterol and cultured skin fibroblasts were deficient in esterification of exogenously administered cholesterol. Electron microscopy of skin biopsy revealed the presence of sequestrated lipids in lysosomes. Molecular analyses showed that both siblings are compound heterozygotes for two disease-causing mutations of NPC1 protein, N1156S and Q922X. Based on the comparison with previously reported N1156S homozygotes, we propose that Q922X mutation, causing the formation of a truncated NPC1, had more severe impact on the clinical outcome. Further, we observed pronounced differences in the disease course in the siblings; i.e. in the boy we observed an earlier onset and a much faster neurological deterioration (late infantile onset), suggesting other genetic and/or environmental factors influencing the course of the disease. In contrast, girl exhibited juvenile type of NP-C. In conclusion, when progressive neurological symptoms develop in late childhood and with previous history of neonatal cholestasis, a classical late infantile or juvenile type of NP-C must be suspected.
BROTHERS WITH MENKES DISEASE - CLINICAL PRESENTATION AND EEG CHANGES - CASE REPORT

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Menkes disease is a lethal multisystemic disorder of copper metabolism characterized by connective tissue abnormalities, progressive neurodegeneration and peculiar "kinky hair." This is X linked recessive neurodegenerative disorder, results from a mutation in the gene coding for the copper transporting ATPase (ATP7A). Epilepsy is one of the main clinical features of this disease. Patient's history, clinical exam, and laboratory findings of low serum copper and ceruloplasmin could lead us to diagnosis. In this case report we described the clinical presentation, evolution of epilepsy for three brothers who had Menkes disease. Same parents have one healthy daughter. The elder brother has died at age of 6,5, the other one at the age of 1,2 and the third one was 8 months old. Younger children had an earlier manifestation of disease whith more progressive neurodegeneration and more intensive drug resistant epilepsy. Despite the genetical counseling after the first child was diagnosed, parents decided to have more children. They avoided prenatal diagnosis, hiding information from ginecologist and pediatrician, because of bad social enviroment. Now, when we have possibility for prenatal diagnosis, this is rarely seen presentation of the genetics disorder in same family.
INHERITED NEUROMETABOLIC DISEASES IN THE AUTONOMOUS PROVINCE OF VOJVODINA, REPUBLIC OF SERBIA

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Autonomous Province of Vojvodina is part of Republic of Serbia, located in the northern part of the country. Novi Sad is the largest city and administrative center of Vojvodina and the second largest city in Serbia. Vojvodina has a population over two and half million, comprising a multi-ethnic and multicultural identity with 6 official languages and 23 nationalities.

The University of Novi Sad (http://www.uns.ac.rs), Medical Faculty (www.medical.uns.ac.rs/en) is established in 1960 and is the educational institution for medical training and research. The University of Novi Sad is the largest in Vojvodina and the second in Serbia.

Institute for Child and Youth Health Care of Vojvodina (http://izzzdiovns.rs) in Novi Sad, Vojvodina, Republic of Serbia is a health and scientific-educational institution that provides health care for newborns, children and adolescents, aged 0-18 years. The Institute is a representative institution that provides the most complex medical procedures, Institute services cover the province of Vojvodina.

In previous period we identified patients who suffer from inherited neurometabolic diseases such as: Wilson disease, PKU, MPS, Neuronal ceroid lipofuscinoses, Metachromatic leucodystrophy, Galactosemia, Hereditary fructose intolerance, Niemann-Pick disease, Glycogen storage diseases, Leigh disease, Lesch-Nyhan disease, MCAD and others. In most cases clinical diagnosis, general laboratory investigations and neuroimaging were made in our Institute and genetic analyses were done in cooperation with colleagues and laboratories from different countries.

We suppose that there are some cases of inherited neurometabolic diseases that are not diagnosed yet, so we expect that including our Centre to Inherited Neurometabolic Diseases Information Network will help us to solve unsolved cases, do earlier diagnosis and earlier treatment for treatable neurometabolic diseases, make prenatal prevention when there is a need and connection with colleagues in other Centres who have experience with inherited neurometabolic diseases, so there is a need for the network and many benefits of the developing as well.