MEETING REPORT

4th RARE DISEASE SOUTH EASTERN EUROPE (SEE) MEETING SKOPJE, MACEDONIA (November 14th, 2015)**Zoran Gucev¹, Velibor Tasic¹, Momir Polenakovic^{2*}**¹ University Children's Hospital, Medical Faculty Skopje, R. Macedonia² Macedonian Academy of Sciences and Arts, Skopje, R. MacedoniaCorresponding Author: Macedonian Academy of Sciences and Arts, Kej Dimitar Vlahov, Skopje,
Phone: + 389 70 27 97 42; E-mail: maknepo@t-home.mk**Abstract**

The 4th meeting on rare diseases in South Eastern Europe (SEE) was held in Skopje, at the Macedonian Academy of Sciences and Arts (MASA) on the 14th of November 2015. The focuses were metabolic, rare brain diseases as well as the rare dysmorphic syndrome. The authors of the report are particularly keen on stating that one of the main goals of the meeting, namely to help the treatment of patients with rare disease has begun to bear fruits. The talk on an iminosugar-based pharmacological chaperone compound as a drug candidate for the treatment of GM1-gangliosidosis and mucopolysaccharidosis IVB (Morquio disease type B) was enlightening. To date, there is no treatment available to be offered to patients, but chaperones lead mutated proteins to adopt a native-like conformation and to successfully traffic to their normal cellular destination. DORPHAN is developing an iminosugar-based pharmacological chaperone compound for the treatment of GM1-gangliosidosis and mucopolysaccharidosis IVB.

A talk on recent developments in the laboratory diagnosis of mucopolysaccharidoses (MPS) was particularly interesting, covering the laboratory diagnosis of the MPS diseases by a strategy of clinical examination, biochemical analysis of urine samples, enzyme tests and genetic characterization of underlying mutations. New techniques were developed, including analysis of urinary glycosaminoglycans with tandem mass spectrometry, miniaturized enzyme tests or novel synthetic substrates for enzyme assays using mass spectrometry detection of products using dried blood spots. Feasibility and cost-effectiveness of these methods in newborn screening programs have been demonstrated.

Neuromuscular RDs, and especially familial amyloid polyneuropathy (FAP) were a topic of the Bulgarian colleagues. Diagnosis, screening and the role of microglia were also topics of particular interest. In summary, this year RD meeting was exciting and productive on a wide range of diseases and on a novel insights on diagnosis and treatment. New methods are expanding our capabilities for a fast and precise diagnosis. Novel knowledge offers better distinction on whom to treat with which medications (e.g. steroid dependent nephrotic syndrome). Novel diseases or variants are published (segmental overgrowth). The authors of the report are particularly keen on stating that one of the main goals of the meeting, namely to help the treatment of patients with rare disease has begun to bear fruits. Namely, the Health Fund of Macedonia for the first time treats the patients with Gaucher's disease. We are hopeful that the number of patients treated for Gaucher's disease and the number of treated patients with other treatable RDs diseases will continue to grow.

Key words: Rare diseases, Macedonian Academy of Sciences and Arts, mucopolisaccharidoses, segmental overgrowth, steroid dependant nephrotic syndrome, neuromuscular RDs, familial amyloid polyneuropathy (FAP), phenulcetonuria, Pompe

The 4th meeting on rare diseases in the South Eastern Europe (SEE) was held in Skopje, at the Macedonian Academy of Sciences and Arts (MASA) on the 14th of November 2015. The focuses were metabolic, rare brain diseases, as well as the rare dysmorphic syndrome. The authors of the report are particularly keen on stating that one of the main goals of the meeting, namely to help the treatment of patients with rare disease has begun to bear fruits. Namely, the Health Fund of Macedonia for the first time treats the patients with Gaucher's disease. We are hopeful that the number of patients treated for Gaucher's disease and the number of patients treated for other treatable RDs diseases will continue to grow. The kind assistance of Mrs. Maja Ivanova, the first lady of Macedonia is particularly important.

The introductory lecture of Momir Polenakovic stressed the ongoing battle to curb the costs of diagnosis and treatment of RDs. Rare diseases are indeed rare, but cumulatively they represent a significant number of patients who require specialized treatment including many health professionals. To encounter only few renal diseases: steroid resistant nephrotic syndrome, Alport syndrome, Cystinosis, nephrophtisis, Fabry disease – they produce great costs for treatment of the ESRD with dialysis or transplantation. The early diagnosis and the appropriate treatment may retard the progression of the disease, to enable family screening and prenatal genetic diagnosis [1]. The first example how modern genetic techniques were applied in clinical medicine was the 5 month old Turkish baby who was mistakenly diagnosed and treated as Bartter Syndrome. The whole exome sequencing established mutation in *SLC26A3* gene and this lead to correct diagnosis of the congenital chloridorrhea [2]. The next examples are patients with steroid resistant nephrotic syndrome. The discovery of 39 dominant or recessive SRNS genes enabled a better understanding of the function of the glomerular podocytes and slit membrane [3, 4]. The detection of causative mutations is important because the patients do not have benefit from immunosupressants, there is no recurrence after transplantation, living related transplant donors are acceptable and there is a pos-

sibility for familial genetic counseling and prenatal diagnosis. With these examples the authors wanted to emphasize the importance of the modern genetic techniques for diagnosing and management of patients with rare diseases. The health authorities should understand the urgent need for clinical utility of the modern genetic techniques in our country!

Velibor Tasic, Skopje, Macedonia spoke about the steroid-resistant nephrotic syndrome (SRNS) [3, 4, 5]. SRNS usually progress to end-stage renal failure. According to the North American Pediatric Renal Trials and Collaborative Studies SRNS constitutes the second most frequent cause of ESRD in the first two decades of life. The majority of the SRNS patients have the histologic picture of focal segmental glomerulosclerosis. The recurrence of the disease in the kidney graft is significantly lower in patients who have genetic background than in those who do not have it. Hildebrandt's group used modern diagnostic techniques such as next generation sequencing and tested a large international cohort of SRNS patients (n = 1783 families). Single-gene cause was detected in 29.5% (526 of 1783) of families with SRNS that manifested before 25 years of age [3]. The identification of the causative single-gene mutations may have important therapeutic consequences in some cases, particularly in patients who carry mutations in a gene of coenzyme Q10 biosynthesis (*COQ2*, *COQ6*, *ADCK4*, or *PDSS2*). In these patients, the treatment with coenzyme Q10 may be indicated [6]. Also, patients with recessive mutations in *PLCE1* may respond fully to the treatment with steroids or cyclosporine A. Patients with *CUBN* may benefit the treatment with vitamin B12. Individuals with *ARHGDI1* may theoretically be responsive to the eplerenone treatment. The detection of causative mutations may be also very important for management of these patients (no benefit from immunosupressants), pre-transplant evaluation and acceptance of living donors, familial genetic counseling and for prenatal diagnosis.

The group of authors from Sofia, Bulgaria (Stayko Sarafov, Mariana Gospodinova, Velina Guerguelcheva, Andrei Kirov, Teodora Chamova, Tihomir Todorov, Alebena Todo-

rova, Ivailo Tournev) exposed the rare and fascinating epidemiology of familial amyloid polyneuropathy (FAP) in Bulgaria. Discovered in 2008 for the first time in Bulgaria, four mutations have been found in the country so far: Glu89Gln, Ser77Phe, Val30Met, Ser52Pro. The selective genetic screening program is performed in the affected families. A total of 261 individuals belonging to the affected families were examined. TTR-FAP mutations were found in 130 individuals. The authors concluded that the clinical phenotype and the place of origin are helpful for preliminary information for the expected mutation.

Hadil Kathom, Sofia, Bulgaria gave a lecture on phenylketonuria (PKU) in Bulgaria. Both the neonatal screening for the PKU, the epidemiology and the treatment availability with modified nutrition was exposed in detail. This presentation was followed by a lecture on molecular basis of the mental retardation in PKU and the specific role of microglia by Gudrun Schlegel et al. from Hamburg, Germany [7]. They used the Pahenu2 mouse, a mouse model of PKU with high levels of phenylalanine. Amdelay in synaptic pruning in hippocampus was observed. The activity of microglia was dramatically reduced in the hippocampus of the Pahenu2 mouse.

Ivailo Tournev, Sofia Bulgaria gave a fascinating talk on the selective screening, carriers testing and carriers follow-up program of the Bulgarian Neuromuscular Disease Society. The very existence of this selective screening, the expansion on carrier testing in a diverse group of neuromuscular RDs was exposed in detail [8].

Dr Vukasin Andric, Zagreb, Croatia (Genzyme a Sanofi Company) gave a talk on Pompe disease, its diagnosis and enzyme replacement therapy. This progressive, multisystemic, debilitating and potentially fatal neuromuscular disorder is linked to an inherited deficiency of the lysosomal enzyme, acid alpha-glucosidase (GAA). The result is intra-lysosomal accumulation of glycogen and is associated with the significant morbidity and/or premature mortality. The incidence of the Pompe disease is approximately 1 in 40,000 live births. The diagnosis from blood samples, including dried blood spots and confirmation by mutation analysis is the norm. Enzyme replace-

ment therapy (ERT), alglucosidase alfa is indicated in adults and paediatric patients of all ages.

As therapy for RDs is costly and often prohibitive in countries in development Dr. Günter Harms (Berlin, Germany; for the Shire Symposia) talked about the very imposing question: why are orphan drugs different? The main goal is to achieve sustainable access to the treatment for rare disease patients. As a company responsible for projects including market access, healthcare reform and cost containment issues his exposition was a remarkable value in Macedonia.

Dr. Stephane Demotz comes from a private pharmaceutical company. Having a professional development at the University of Lausanne, Switzerland and continuing to work as a scientist in a start-up company (Cytel, San Diego, CA) and 3 years at the Basel Institute for Immunology (Roche), Dr. Demotz has over 15 years of experience in drug development gained in pharmaceutical companies (Parke-Davis in Paris, then acquired by Pfizer, and Glenmark, Neuchâtel) and start-ups (Cytel, Dictagene and Apoxis, in Lausanne, then acquired by TopoTarget, Copenhagen). He also spent 4 years at Philip Morris International in Neuchâtel (2008–2012), and in 2008 Dr. Demotz co-founded Edimer Pharmaceuticals S.A., which had as goal the development of a drug for the treatment of the rare and orphan genetic disease X-linked hypohidrotic ectodermal dysplasia (XLHED). US\$ 50 million have been thus far invested in Edimer Pharmaceuticals and the drug candidate is currently undergoing phase 2 clinical trials in the US and Europe. In 2012, he co-founded DORPHAN S.A., whose aim is the development of drugs for orphan and rare genetic diseases, with a strong focus on mucopolysaccharidoses and related lysosomal storage diseases. His talk was on an iminosugar-based pharmacological chaperone compound as a drug candidate for the treatment of GM1-gangliosidosis and mucopolysaccharidosis IVB (Morquio disease type B). To date, there is no treatment available to be offered to patients, in most cases children, afflicted by one of these severe and often rapidly fatal diseases. In those diseases newly synthesized mutated proteins are believed to be improperly folded in the endoplasmic reticulum and are subsequently tagged as faulty by a cell quality sys-

tem, giving a signal leading to their premature degradation. Inhibitors of enzymes lead mutated proteins to adopt a native-like conformation and to successfully traffic to their normal cellular destination. DORPHAN is developing an iminosugar-based pharmacological chaperone compound for the treatment of GM1-gangliosidosis and mucopolysaccharidosis IVB. This compound is undergoing preclinical development, with the objective to commence its clinical evaluation in the coming 18 months. The talk was a rare and fascinating view on the complex processes underlying the creation of new medicines for RDs.

Eduard Paschke, Graz, Austria gave a talk on the recent developments in the laboratory diagnosis of mucopolysaccharidoses. Mucopolysaccharidoses are a group of lysosomal storage disorders (LSDs) caused by deficiencies of 11 lysosomal enzymes and result in multisystem disorders, mainly affecting the skeletal system, the central nervous system and the visceral organs. The laboratory diagnosis of the MPS diseases is done by a strategy of clinical examination, biochemical analysis of urine samples, enzyme tests and genetic characterization of underlying mutations.

The advent of causal therapies caused an urgent need for presymptomatic. New advanced techniques were developed, including analysis of urinary glycosaminoglycans with tandem mass spectrometry, miniaturized enzyme tests or novel synthetic substrates for enzyme assays using mass spectrometry detection of products using dried blood spots. The feasibility and cost-effectiveness of these methods in newborn screening programs have been demonstrated.

Zoran Gucev, Velibor Tasic, University Children's Hospital, Skopje Macedonia gave a history on the proteus spectrum [9, 10, 11]. This disease group has a common characteristic of overgrowth of part(s) of the body, from monstrous proportions of several body parts to a relatively modest overgrowth. They exposed a complex discovery route from a CLOVES syndrome to a new variant, or a novel entity of segmental overgrowth. This voyage also included the discovery of a somatic mutation of PIK3CA gene as a causative factor in segmental overgrowth syndrome [11].

Velibor Tasic, University Children's Hospital, Medical School, Skopje, Macedonia gave an insight in the idiopathic infantile hypercalcemia (IIH). Infants with IIH fail to thrive, had polyuria, dehydration crisis, vomiting, seizures, lethargy and in most severe cases coma and death. Laboratory investigations revealed hypercalcemia, hypercalciuria, elevated levels of 1.25 (OH)D3 and suppressed parathormon. The etiology of the idiopathic infantile hypercalcemia (IIH) was unknown until 2011 when Schlingmann et al reported in the New England Journal of Medicine that the mutations in *CYP24A1* gene were responsible for the disease in majority of the patients [12]. In Macedonia they have already diagnosed 6 infants with idiopathic infantile hypercalcemia. The prevalent mutation in these patients was *E143del*. Early laboratory and genetic differentiation between *SLC34A1* (NaPi-IIa) and *CYP24A1* (24-hydroxylase) mutation carriers is very important for appropriate targeted therapy in patients with IIH [13].

Venko Filipce MD. University Department of Neurosurgery Skopje, gave a talk on the selective and superselective angiography of Moyamoya (MMD) disease angioarchitecture. An angiographic investigation with injections through selective and superselective catheterizations was done in 6 children with MMD. The use of high quality selective and superselective angiography enabled the demonstration of the specific microangiographic anatomy of the moyamoya anastomotic network [14, 15].

In summary, this year RD meeting was exciting and productive on a wide range of diseases and on a novel insights on diagnosis and treatment. New methods are expanding our capabilities for a fast and precise diagnosis. Novel knowledge offers better distinction on whom to treat with which medications (e.g. steroid dependent nephrotic syndrome). Novel diseases or variants are published (segmental overgrowth). And most importantly new treatments are created, while patients in Macedonia are beginning to be treated with proper medications for their RDs (Gaucher's disease).

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Резиме

ЧЕТВРТИ СОСТАНОК ЗА РЕТКИ БОЛЕСТИ ВО ЈУГОИСТОЧНА ЕВРОПА (ЈИЕ) – СКОПЈЕ, МАКЕДОНИЈА (14 ноември 2015)

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Четвртиот состанок за ретки болести во Југоисточна Европа (ЈИЕ) се одржа во Скопје, во Македонската академија на науките и уметностите (МАНУ), на 14 ноември 2015 година. Фокусот беше ставен на метаболичките, ретки мозочни болести, како и реткиот дисморфичен синдром. Авторите на извештајот особено сакаат да истакнат дека една од главните цели на состанокот, имено, да им се помогне во лекувањето на пациентите со ретки болести, започна да дава плод. Предавањето за имуносахарид, кандидат на лек за третман на GM1 ганглиозидоза и мукополисахаридоза IVB (болест Моркио тип В), беше инспиративно. До денес нема достапен третман за овие пациенти. DORPHAN го прикажа развитокот на иминосакхарид за третман на GM1-ганглиозидоза и мукополисахаридоза IVB.

Особено беше интересно предавањето за последните случувања во лабораториската дијагноза на мукополисахаридозите (MPS), кои ја покриваат лабораториската дијагноза на болести

на MPS со стратегија на клиничко испитување, биохемиска анализа на примероци на урина, ензимски тестови и генетска карактеризација на основните мутации. Беа развиени нови техники, вклучувајќи анализа на уринарните гликозаминоглигани со тандем масена спектрометрија, минијатуризирани ензимски тестови и нови синтетички супстрати за ензимските анализи со употреба на исушени капки крв. Беа прикажани изводливоста и исплатливоста на овие методи со скрининг-програми на новороденчињата.

Невромускулните ретки болести, а особено фамилијарната амилоидна полиневропатија (FAP), беа тема на предавањето на бугарските колеги. Дијагнозата, скринингот и улогата на микроглијата, исто така, беа теми од посебен интерес.

Како заклучок, состанокот за ретки болести оваа година беше возбудлив и продуктивен за широк спектар болести и за нов увид во нивната дијагноза и третманот. Новите методи ги прошируваат нашите способности за брза и

прецизна дијагноза. Новите знаења нудат подобро правење дистинкција во однос на третирањето со лекови (на пример, стероидно зависен нефротски синдром). Новите болести или варијанти се објавени (сегментален прекумерен раст).

Авторите на извештајот особено сакаат да наведат дека главната цел – да им се помогне на пациентите со ретки болести почна да дава резултати. Имено, Фондот за здравствено осигурување на Македонија прв пат ги третира пациентите со болеста Gaucher. Се надеваме дека бројот на пациенти што се лекуваат од болеста Gaucher и бројот на пациенти со други ретки болести, кои се третираат, ќе продолжи да расте.

Клучни зборови: ретки болести, МАНУ, мукополисахаридоза, сегментален прекумерен раст, стероидно зависен нефротски синдром, невромускулни ретки болести, фамилијарна амилоидна полиневропатија (FAP), фенилкетонурија, Pompe