

# STATEMENT

**by Assoc. Prof. Dr. Vesela Ivancheva Lyubenova, MD, PhD, FEBO**

External member of Scientific Jury, appointed by order № P-109-1/05.01.2023 of Rector of Medical university Varna „Prof. Dr. Paraskev Stoyanov”

regarding

dissertation of Dr. Maria Rumenova Boyadzhieva, MD, for acquiring of an educational and scientific degree "Doctor (PhD)" in the field of scientific specialty "Ophthalmology", code 03.01.36.

Topic of the PhD thesis:

**"Live microstructural analysis of rare eye diseases with modern technologies"**

**Scientific tutor:** Corresponding member Prof. Dr. Christina Nikolova Grupcheva, MD, FEBO, FICO(Hon), FBCLA, FIACLE

## **Brief biographical data**

Dr. Maria Boyadzhieva was born on 30.12.1983. in Sofia. She finished her secondary education in 2002 in science and mathematics high school "Acad. Sergey P. Korolyov" Blagoevgrad, majoring in biology and English. In the period 2003-2009, she studied medicine at the Varna Medical University "Prof. Dr. Paraskev Stoyanov". Since 2009 she worked in Emergency medical care and Emergency department, and a year later became part of the team of Intensive Respiratory Department of "St. Marina" Hospital - Varna (today - Department for Invasive Treatment and Non-Invasive Ventilation - OILNV). In 2016 began to specialize in ophthalmology at the Specialized Hospital for Eye Diseases for Active Treatment-Varna Ltd. (SBOBAL-Varna). Since 2017, she has been working as a part-time assistant at the Department of Ophthalmology and Vision Sciences, and since 2018, after passing a competitive exam, she has been appointed as an assistant at the aforementioned department. In the same year, she was enrolled as a regular doctoral student at the Medical University - Varna. In 2020, she acquired a specialty in ophthalmology. Dr. Boyadzhieva continuously improves her qualifications by annually attending scientific meetings and conferences in the country and abroad. Participated in many courses and specializations, among which: Orthokeratology Course - Paris (2019), Summer School in Lincshoppin under the guidance of Prof. Nil Lagali (2017) and numerous trainings in Bulgaria. There are over 17 scientific publications in national and international journals, of which 3 are related to the dissertation work, and 19 citations. She is part of the author team of a textbook on eye diseases, edited by

Prof. Dr. Christina Grupcheva, MD. FEBO, FICO(Hon), FBCLA, FIACLE. Her professional interests are in the field of diseases of the anterior segment of the eye.

### **Timeliness of the problem**

Rare eye diseases are a challenge that is yet to be solved. Considering that these eye diseases affect about 6% of the world's human population, and have a negative effect on the lives of these patients, their loved ones and society, their importance is clear. Many of them are the result not only of genetic mutations, but also of the influence of various environmental factors. The study of rare eye diseases will open new horizons, both in diagnosis and in their treatment. Most patients with a rare disease have a long and complicated journey to the correct diagnosis. Approximately half of people with a suspected rare disease are undiagnosed, and at the same time, the diagnosis process takes an average of 5-6 years. The result is a waste of time, inappropriate therapies and impaired quality of life for patients.

### **Structure**

The dissertation contains 157 pages, including 14 tables and 46 figures. 142 literary sources are cited, of which 17 are in Cyrillic and 125 are in Latin. 11 chapters are presented, corresponding to the purpose and tasks and meeting the requirements for the layout of the dissertation work. Contents - 1 page, Introduction - 3 pages, Abbreviations used - 1 pages, Summary - 3 pages, List of figures and tables - 5 pages, Literature review - 36 pages, Purpose, tasks and hypotheses - 1 page. , Materials and methods- 31 pages. Results- 38 pages, Discussion- 7 pages, Conclusions- 2 pages, Contributions- 1 page, Bibliography- 8 pages, Appendices- 14 pages, List of publications- 1 page

### **Literature review**

An analysis and overview of Bulgarian and foreign scientific publications regarding rare eye diseases is presented. A definition of rare diseases is given according to the European Union, WHO, as well as in different countries. Due to the specificity of these diseases, progress can only be achieved with the combined efforts of all countries. Sources of information - online platforms, patient communities, international reference database (Orphanet) - were reviewed. Within the EU, the European Commission is creating a European reference network, the Clinical Patient Management System (CPMS).

Dr. Boyadzhieva presented the essence of genetic disorders, in particular Mendelian disorders, mitochondrial disorders and the clinical picture of some of them. In recent years, the diagnosis has been made with the methods of molecular genetic testing, which can support early diagnosis and better treatment. The dissertation presents new technologies that are applied in Bulgaria - microarray analysis, new generation sequencing since 2015 at the Center for Molecular Medicine at the Medical University of Sofia.

Advances in unraveling the genetic, molecular and cellular basis of rare eye diseases provide treatment options not only for this eye pathology but also for other common eye diseases. Gene therapy is a powerful and innovative approach using genetic material such as DNA or RNA to compensate for genetic mutations, provide the ability to produce potentially therapeutic substances, or induce an immune response to fight disease.

In the field of rare diseases, primary prevention aims to prevent the occurrence of a disease, ie to prevent the birth of an "affected" child. In recent years, more and more emphasis has been placed on the role of screening, in particular mass neonatal screening, as one of the most effective forms of secondary prevention, which enables early diagnosis and treatment of a number of serious diseases - children are examined a few days after birth, before symptoms of the disease appear. More than 50 diseases can be detected at an early stage through neonatal screening and timely treatment can be started. Mass neonatal screening is currently carried out in Bulgaria, covering about 90% of all newborns for 3 diseases - phenylketonuria, congenital hypothyroidism and congenital adrenal hyperplasia.

In the study - Current Status of Newborn Screening in Southeastern Europe from 2021, the obstacles to expanding newborn screening programs among the countries of Northeastern Europe (including Bulgaria) are the lack of financial resources, organization and political will.

Secondary prevention also includes prenatal screening and prenatal diagnostics, regulated in Ordinance No. 9 of 2019 on determining the package of health activities guaranteed by the NHIF budget and Ordinance No. 8 of 2016 on preventive examinations and dispensation. In the context of rare diseases, the purpose of prenatal screening diagnostics is to identify mothers at high risk of transmitting a rare disease (based on family history - birth of a child with a rare disease, a relative with a known or suspected genetic disease) or the detection of a fetus with a rare disease.

Due to the low prevalence of these diseases, knowledge is scarce, research is limited, access to medical expertise is rare, and the care offered is often inadequate. Dr. Boyadzhieva also presented the difficulties related to the development of safe and effective drugs, biological and medical devices for prevention, early diagnosis and treatment of these conditions - difficulties in attracting public and private funding for research activities, recruiting a sufficient number of scientific research and clinical trial participants.

Carrying out the therapy with expensive medicinal products for some is life-sustaining, for others it gives a chance to have a better quality of life, better education and social integration.

Bulgaria, as a country with the lowest per capita income among the countries in the European Union, faces many challenges in providing the most innovative medicines to its citizens. Bulgarian patients have relatively delayed access to innovative medicines.

In 2004, the "Fund for the treatment of children" center was established. It provides organizational and financial support for children up to 18 years of age who need diagnostic and treatment procedures, for which there are no conditions for carrying them out in Bulgaria. In the creation of the Fund, rare metabolic and exchange diseases in children were included, for which there are no specific methods for diagnosis and treatment in the Republic of Bulgaria - some mitochondrial diseases, peroxisomal diseases and those affecting peroxisomal oxidation.

In Europe, rare eye diseases (RED) are the leading cause of reduced vision and blindness in children and young people. To date, according to Orphanet, more than 900 rare eye diseases have been described.

Rare eye diseases are often bilateral, asymmetric and progressive. Early and correct diagnosis of rare eye diseases is essential for their prognosis. Timely intervention has a significant impact on the prognosis of many potentially blinding diseases. Innovations in the treatment of rare diseases of the eye are related to the discovery of the genetic defect and the possibility of creating a treatment that can affect the progression of the disease or even a definitive cure. Compared to other organs, the eye has a greater potential for gene therapy due to easy access, immuno-privileged status, presence of ocular barriers preventing exposure to other organs, and the ability to monitor the response to treatment by non-invasive techniques.

A new direction in the treatment is the use of different types of progenitor and stem cells that have been administered subretinally in animal models for the treatment of degenerative diseases of the retina, the subretinal administration of cells from retinal pigment epithelium (Human Embryonic Stem Cell Derived Retinal Pigmented Epithelial), obtained from human embryonic stem cells.

Thanks to the scientific and technological progress, today it is possible to carry out a large range of research at the tissue, molecular and biochemical level, as well as visualization and exact topographical localization of the damage (computer perimetry, optical coherence tomography, fluorescein angiography).

In 2018 the first center in Bulgaria for "non" medical care for people with rare diseases was created - Center for people with rare diseases, offering social services - information, medical rehabilitation, a platform for cognitive rehabilitation and psychological counseling, and in May 2022 - launched the first information telephone line (0700 300 97) for people with rare diseases for medical, legal and social support.

**Purpose:** The aim of the current study is to follow the path of patients with rare eye diseases, to evaluate the registration regime, to include eye diseases in the national rare disease registry and to create a model for a clinical registry to benefit daily ophthalmic practice.

To achieve the stated goal, the following tasks are set:

1. To carry out a review of the European rules and registration regimes for rare diseases and rare eye diseases. View the national register.
2. To assess the knowledge of rare diseases among ophthalmologists.
3. To create a model for the registration of a rare eye disease - Aniridia.
4. To make a clinical assessment of rare eye diseases according to the Orphanet classification.

5. To create a model for a publicly accessible national register for rare eye diseases in Bulgaria.
6. To develop a plan to create an expert center for rare eye diseases in Bulgaria.

## **Materials and methods**

**Object of the study:** The present study was conducted at the Department of Eye Diseases and Visual Sciences of the Medical University - Varna on the territory of the Specialized Hospital for Eye Diseases for Active Treatment - Varna and Medical University "prof. Dr. P.Stoyanov" Varna, for a period of 5 years. It carried out a thorough review of the published literature and publicly available data on rare diseases, including rare eye diseases, and the follow-up of patients with rare eye diseases.

Criteria for inclusion in the study:

- Patients with rare eye diseases, eye diseases, admitted for examination and/or hospitalization in SBOBAL - Varna;

Exclusion criteria:

- Children without a confirmed genetic diagnosis, not allowing follow-up according to the algorithm prepared during the study;

Study period:

- annual period from 2017 until 10.2022

## **Documentary method**

- A systematic and analytical review of the European rules and registration regimes for rare diseases and rare eye diseases was conducted.
- A review of the National Register for Rare Diseases was carried out.

**Survey method** - development and application of a questionnaire to test the knowledge of rare eye diseases among specialists and specialists in eye diseases:

A 16-item questionnaire assessing the awareness of ophthalmologists (eye specialists and specialists) regarding rare eye diseases. 15 of the questions asked have a suggested answer, and one - open. 76 persons were surveyed.

**Registration of a rare eye disease** - aniridia - the algorithm for registering this disease is presented

## **Creating a model for a publicly accessible national registry for rare eye diseases.**

- Building a local database management system (DBMS) architecture in a relational model with partial elements of a hierarchical structure;
- Defining the fields in the relevant tables with their keys and optimizing field types;
- Organization of a Remote system in the Cloud;



- Building a Socket mechanism for In time correction to the Cloud Server;
- Building a CRUD (Create Read Update Delete) local system;
- Real-time connection of the recording event via a socket to the Cloud Server;
- Cloud Server data recording, including a set of common rare disease registration data elements;
- Building a Soft Delete mechanism for a Delete operation with a zero flag set, to avoid physical deletion;

#### **Development of a plan to create an expert center for rare eye diseases in Bulgaria:**

- Formation of a team of medical specialists in the field of rare eye diseases and their training;
- Designation of a material and technical base in SBOBAL-Varna for the construction of an expert center for rare diseases on the territory of the medical institution;
- Building multidisciplinary teams - cooperation with a psychologist, the Department of Medical Genetics, other expert centers and reference networks, social service providers, etc.;
- Preparation of standards for work with patients with rare eye diseases and organization of the work process - development and implementation of good practices and algorithms for diagnosis, treatment, follow-up and rehabilitation;
- Creation and maintenance of an up-to-date information system for patients with rare eye diseases;
- Activities to popularize the activity of the expert center;
- Preparation and submission of an application to the Commission on Rare Diseases and the Ministry of Health for the designation of an expert center on rare eye diseases;

#### **Clinical methods** - clinical methods for evaluating rare eye diseases:

- Examination of visual acuity and color perception;
- Biomicroscopy of anterior eye segment and fundobiomicroscopy (stereophthalmoscopy with +90 D lens);
- Optical Coherence Tomography (OCT) by Zeiss Cirrus 5000 HD-OCT;
- Live laser-scanning confocal microscopy with the HRT Rostock Cornea module;
- Visual field examination with the Humphrey® Field Analyzer 3 (HFA™3);
- Photo documentation of with Zeiss Digital Fundus Camera - Visucam 524;
- Electroretinography (ERG);

An algorithm for the clinical evaluation of a rare disease of the anterior eye segment and posterior eye segment is presented

#### **Results**

The assessment of knowledge about rare diseases among ophthalmologists included 51 specialists and 23 trainees. The average age of the surveyed specialists is 39.5 years, and that of the specialists - 26.6 years. Specialists with academic affiliation - 74.36%. Almost half of the participants reported that they had met patients with RED. More than half of the respondents do not treat or refer such patients, and 39.47% treat RED. Of those treating rare eye diseases - 42.11% indicated that they treat all types of rare diseases, 30.31% - diseases of the anterior eye segment, 10.53% noted that they treat rare eye diseases of the posterior eye segment.

Those who do not treat persons with RED refer their patients to another colleague, a specialist in eye diseases - 63.33%, to a specialist in rare diseases - 26.67%, and 10% answered otherwise and added a doctor specializing in medical genetics .

According to 55.26% of those who completed the questionnaire, there is no reference center for rare eye diseases in Bulgaria. Almost 60% - 62.16% of specialists and specialists in eye diseases share that during their studies they had specific classes/lectures dedicated to the prevention, diagnosis and treatment of rare diseases (figure 32), but over 70% ( 79.48%) of those who took part in the questionnaire think that this training is not enough. over 60% of those who filled out the questionnaire (respectively 60.53% and 64.10%), Bulgarian doctors and ophthalmologists do not receive professional training for the prevention, diagnosis and treatment of rare diseases. The analysis of the questionnaires shows that over 80% of those who completed the questionnaires answered that they did not have a chance for specialized postgraduate training in the prevention, diagnosis and treatment of rare diseases

For the purposes of the study for the period 2017-2022. in SBOBAL-Varna, 39 patients with a rare eye disease were followed up. The patients participating in the study had rare diseases of the visual analyzer according to the Orphanet classification. And they are divided into the following groups:

- First group: Neuro-ophthalmological diseases - one patient with Usher syndrome;
- Second group: Rare diseases of the anterior segment of the eye - five patients with aniridia, one with Hereditary congenital endothelial dystrophy, one patient with Schneider dystrophy, one with Salzmann dystrophy, 1 with posterior polymorphic dystrophy, 3 with with dystrophy "Map- dot-fingerprint", 6th with Fuchs dystrophy, 5th with lattice dystrophy;
- Third group: Rare diseases of the posterior segment of the eye - six patients with retinitis pigmentosa, three patients with Stargard's disease, 2 with Best's disease, 2 with macular dystrophy;
- Fourth group: Uveitis - one with a patient with Vogt-Koyanagi-Harada;
- Fifth group: Diseases involving more than one structure - one patient with ocular pemphigus.

The average age of the followed patients was 37 years, with the youngest patient being 12 years old and the oldest being 62 years old. The female gender prevails in rare diseases of the anterior eye segment, and the male in rare diseases of the posterior eye segment.

Data from the study indicated that an inaccurate diagnosis led to futile medical interventions in 16% of participants, inappropriate medical treatment in 33%, and 10% received psychological help due to the assumption that the symptoms were psychosomatic. In more than 50% of the monitored and surveyed patients, the diagnosis was made before the age of 18. Nearly 74% of the patients who filled out the survey stated that they have relatives affected by the same disease. Only one of the patients followed in the current study had a genetically proven diagnosis, after referral by the treating ophthalmologist

More than 90% of those participating in the survey indicate that they receive information about their illness from the treating ophthalmologist. More than 90% of those participating in the survey indicate that they receive information about their illness from the treating ophthalmologist.

**Creation of a registration model for a rare eye disease - Aniridia**

In November 2022, the disease Aniridia was approved by the Commission for Rare Diseases and is included in the National Register of Rare Diseases, as well as Leber's hereditary optic neuropathy.

### **Development of a plan to create an expert center for rare eye diseases in Bulgaria.**

On the basis of the analysis and accumulated data, a model of a registry for rare eye diseases was created in SBOBAL - Varna. The goals of this model are to create a replicable data base that will serve to support the registration of rare eye diseases in the National Register of Rare Eye Diseases, to improve diagnosis and treatment, clinical trials and the creation of good practice. Initially, this registry includes two diseases - aniridia. For this purpose, a template will be created - a cloud-based file customized according to the requirements of the individual disease, including demographic data, clinical history, diagnoses, medications, medical and surgical procedures, tests and results of examinations, imaging, and other documentation that may be necessary for the individual condition.

### **Discussion**

Rare diseases are a priority of the health policies of countries worldwide. The lack of specific health policies for RH and the lack of expertise lead to delayed diagnosis and difficult access to health care. This in turn leads to additional physical and psychological suffering for patients and their families.

Although Bulgaria is one of the first countries with an adopted national plan/strategy for RB, today there is still a lack of a comprehensive and clear health policy regarding rare diseases. Questions related to the prevention, diagnosis and treatment of some rare diseases are partially represented in various national programs and laws, but without a comprehensive solution to the many problems of a medical, social and ethical nature. Dr. Boyadjieva also presented the factors that limit patients' access to specialized laboratory tests. Bulgaria is seriously lagging behind in the development of its expert activities and capacity in the field of rare diseases compared to the EU countries - among the 1500 expert centers approved by the EC on the territory of the EU and Norway, Bulgaria has only 7. The absence of Bulgarian medical facilities in 19 of the total of 24 therapeutic areas within the scope of the European Reference Networks, it deprives the Bulgarian specialists - clinical doctors and researchers - of cooperation with the expert centers in the composition of the ERM. Our country is one of the three countries (along with Luxembourg and Malta) officially named by the European Commission that did not participate in 2019. in the process of joining medical facilities, healthcare providers, hospitals within the European reference networks (Bulgaria does not broadcast a single hospital for participation in these 24 areas within the invitation to join issued by the European Commission in 2019).

Rare disease registries are an indispensable database for clinical trials in this field and represent a key tool for improving the care of patients with these diseases, creating treatment protocols and optimizing health care. They are an indispensable research tool, enabling the collection, consolidation and generalization of information from different geographical latitudes, at regional, national, European or global level.



In Bulgaria since 2015, there is a National Register of Rare Diseases. It does not include a patient with a rare eye disease. In addition to the human resources, to date there are only 20 expert centers for rare diseases in our country and they do not cover all rare diseases. In order to open an expert center for a given rare disease in Bulgaria, it is necessary for the disease in question to appear in the national list of rare diseases and an application approved by the Commission for Rare Diseases. On the other hand, the entry of a rare disease in the national list takes place after its approval by the Minister of Health - the Commission for Rare Diseases receives, examines and submits to the Minister of Health the submitted applications for the entry of a rare disease in the national list. This time process is not fixed and is often of long duration. On the other hand, the lack of accurate information (such as the total number of affected patients in Bulgaria, available affordable treatments and studies on therapies, etc.) for rare diseases that are currently outside the existing list is a prerequisite for the submitted applications for inclusion in the list not to be approved. With the lack of an expert center for rare eye diseases, Bulgaria remains invisible to European reference networks, accordingly, Bulgarian patients with rare eye diseases remain discriminated against, far from innovative therapies, diagnostics, experimental treatments, from the possibility of referral to other reference centers, etc., and our professionals cannot take advantage of the resources, professional mobility and training programs provided by the networks. Building a center of expertise for rare eye diseases would help the individual patient, the respective affected family to get up-to-date and accurate information about the disease itself and treatment options. Dr. Boyadzhieva also examines the role of electronic health records - part of the National Health Information System. Electronic medical records provide an easy and affordable way to collect a wide range of data and to enrich information about most diseases, including rare ones. Having electronically stored and coded data will provide an opportunity to improve medical care and service.

### **Conclusions:**

1. The study confirms the need to create an expert reference center for rare eye diseases.
2. Based on the conducted in-depth and analytical analysis of the data, the need to include more rare eye diseases in the National List of Rare Diseases has been proven.
3. The analysis of the European rules and registration regimes for rare diseases and rare eye diseases proved the need to build a new national program for rare diseases, supporting the National Register of patients with rare diseases and updating Regulation No. 16 of 2014. of the Ministry of Health in line with European legislation.
4. On the basis of the present study, it has been proven that the creation of an up-to-date, accessible and functional registry for rare eye diseases is not only possible, but can represent a step forward in epidemiological and clinical studies in this field - the lack of reliable epidemiological and clinical data, generated in local conditions, is a significant obstacle to effective planning and management of health care costs for any country, including Bulgaria.
5. The study confirms the need for the participation of Bulgaria and a Bulgarian expert center for eye diseases in the European reference networks for improving the care and diagnosis of rare eye diseases.

6. It is necessary to update the standard on "Medical genetics", Ordinance No. 26 of the Ministry of Health and the diagnostic algorithms and indications for conducting genetic and genomic research - the establishment of a precise molecular diagnosis for any genetic eye disease can only be achieved through genetic research and this will allow the clinician to stratify clinical risk in terms of prognosis, comorbidities, treatment options, and/or participation in clinical trials. The financing of diagnostic genetic tests now only takes place within the framework of Ordinance 26 of the Ministry of Health - children up to 18 years old. age are examined free of charge. For patients over 18 years of age with rare diseases, genetic diagnosis is practically not provided within the current clinical pathways, and these patients remain in a large number of cases undiagnosed and inadequately treated.
7. Need to improve prevention, diagnosis and care for patients with rare diseases, including rare eye diseases, as well as providing equal access to therapies with orphan drugs and innovative therapies for these patients in Bulgaria.
8. The results of the analysis of the questionnaires confirm the need to increase awareness of rare eye diseases among the population and medical specialists.
9. The present study proved the need to create algorithms for good practices and care for patients with rare eye diseases, including the preparation of files on rare eye diseases in Bulgarian, which would be accessible to specialists, patients, patient organizations, etc.
10. Based on the reviewed laws and analyzed data, we believe that it is imperative to build a comprehensive health policy for patients with rare diseases, including rare eye diseases, and make changes in social policy.

## **Contributions**

### **1. Contributions of a cognitive nature**

- 1.1. An in-depth and analytical literature review on rare diseases, including rare eye diseases, was made.
- 1.2. An in-depth and analytical review of the epidemiology and classification of rare eye diseases was made.
- 1.3. This study is the first initiative to improve the management of patients with rare eye diseases, by promoting the preparation of a registry for rare eye diseases, the creation of an expert center and a model of a rare eye disease for inclusion in the list of rare diseases of the Republic of Bulgaria.
- 1.4. A comparative analysis of health policies for rare diseases in European countries was made.

### **2. Contributions of a scientific nature**

- 2.1. For the first time in Bulgaria, a comparative study was conducted on the European and national policy for rare eye diseases.
- 2.2. For the first time, an algorithm was developed for the diagnosis and follow-up of patients with a rare eye disease.

2.3. For the first time, an algorithm was developed to include a rare eye disease in the rare disease register of the Republic of Bulgaria.

2.4. For the first time, an algorithm was developed for creating and registering an expert center for rare eye diseases.

### **3. Contributions of a confirmatory nature**

3.1. For the first time, the need for a registry for rare eye diseases was proven.

3.2. For the first time, the need to create an expert center for rare eye diseases was proven.

Dr. Boyadzhieva has 3 publications related to the dissertation.

This work is the first and most essential step towards the establishment of an expert center for rare eye diseases and the declaration of our desire to participate in ERN-EYE.

The dissertation is a completed scientific development and meets the scientific criteria indicated in the Regulations for the Implementation of the Law on the Development of the Academic Staff at MU-Varna

The scope of the dissertation, the topicality of the problem, as well as the in-depth analysis give me the reason to propose to the Scientific Jury to vote positively for awarding Dr. Maria Boyadzhieva the scientific degree "DOCTOR".

Reviewer: .....

/Assoc.Prof. Dr. Vesela Ivancheva, MD, PhD, FEBO/

