Opinion

From Professor Dr. Violeta Mihova Iotova, Ph.D
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Subject: dissertation of Dr. Milena Petrova Stoyanova, PhD student in full-time education at the Department of Medical Genetics, MU - Varna, for the acquisition of the scientific-educational degree "doctor", in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3 Biological sciences, doctoral program "Genetics".

On the basis of Order No. R-109-329 / 01.08.2022 of the Rector of the MU - Varna and with the decision of the Scientific Jury (Protocol No. 1/ 09.08.2022) I have been appointed to prepare an opinion on the dissertation work of topic **"GENETIC-DIAGNOSTIC STUDY IN CHILDREN WITH HEREDITARY PATHOLOGY WHO WERE CONSULTED AT THE GENETIC COUNSELING OFFICE"** for the acquisition of a scientific-educational degree "doctor" in professional direction 4.3 Biological sciences, scientific specialty "Genetics".

The opinion was prepared according to the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its Implementation at the Medical University - Varna.

Biographical data.

Dr. Milena Stoyanova was born in 1978. After completing her secondary education in Yambol, she studied medicine from 1996 to 2002 at the Medical University in Varna. From 2003 to 2009 he specialized in "Pediatrics" at the Department of Pediatrics of the Medical University - Varna. In 2010, Dr. Stoyanova successfully acquired a specialty in Pediatrics and, after a competition, held the academic position of "assistant Professor" at the then Department of Pediatrics and Medical Genetics, and later at the newly formed Department of Medical Genetics. In 2012, she started working as a pediatrician in the Laboratory of Medical Genetics at the UMBAL "St. Marina" - Varna, and after successfully acquiring a specialty in genetics in 2018 - as a medical geneticist at the same structure. After joining the Laboratory of Medical Genetics and the relevant Department, Dr. Milena Stoyanova is actively engaged in teaching activities, with the study and workload for the last 3 years exceeding the uniform state requirements. She is interested and works actively in the field of science and in particular, the development of applied genetics in medicine. She has participated in 5 scientific projects for the "Science" Fund and the Ministry of Education and Culture, two of which are directly related to the topic of the scientific work submitted for defense.

Dr. Stoyanova has so far participated in 5 international and 3 national trainings and courses focused on the modern principles of clinical genetics. She is a member of the Bulgarian Society of Human Genetics and Genomics, the Bulgarian Pediatric Association, the European Society of Human Genetics (ESHG), the European Cytogenetic Association (ECA). In July 2018, after a competition, she was enrolled as a full-time doctoral student on the

subject of this dissertation, with the supervisor Prof. Lyudmila Angelova, Ph.D., Head of the Department of Medical Genetics.

1. Assessment of topicality of the dissertation:

The present work is the first in our country to present and evaluate the contribution of the overall activity of a genetic structure operating in a third-level university hospital in the service of pediatric patients over a long period of time. Against the background of the increasing importance and fastly developing methods for genetic diagnosis, as well as the need to develop the knowledge and skills of clinicians in the direction of the correct selection of a research method in cooperation with a clinical geneticist; expanding the activities of multidisciplinary teams in the service especially of patients with rare diseases (by definition - with genetic etiology) and the rapid development of this discipline in the world, the work is extremely relevant. Last but not least, the still high cost of achieving a genetic diagnosis requires a more targeted research, the basis for which is created by the present scientific study.

2. Structure of the scientific work.

The dissertation is well structured, with all the main parts presented:

- *Literature review (35 page).* The literature review is logical and a consistent exposition of the terminology in the field of medical clinical genetics, a systematic presentation of the approach to the phenotyping of patients suitable for genetic testing, as well as the history, organization and essence of genetic counseling in the world and in our country. In the entire overview, both the exact and critical interpretation of the literature data by the doctoral student, as well as the pragmatic-clinical approach, based on the extensive experience gained over a period of more than 10 years, are distinct. This is undoubtedly the basis of the formulation of the aim of the present study.
- *Aim and tasks (1 page).* 5 main tasks are clearly formulated, fully meeting the set aim of the study.
- *Setting the basis of own studies (10 pages).* In essence, the study is a 10-year retrospective analysis of the activity of the Clinical Genetics Laboratory of UMBAL "St. Marina" based on searches from the hospital's clinics, external users and individual patients in cooperation with other Bulgarian and international genetic structures and laboratories. The organization of the research is presented competently and comprehensively, as the main contribution is the personal participation of the PhD student in most of the activities carried out. The work was carried out according to the rules of good scientific practice with the permission of the Committee on Ethics of Scientific Research at the MU-Varna. The successive steps of the analyzes are explained in detail, as the doctoral student independently or under supervision prepared the samples for research and carried out all collaborations for their preparation.

- *Results (41 pages).* In more than 40 pages, 17 tables and 34 graphs, the PhD student presented the obtained results. The sequence of the assigned tasks was observed and the concept of the scientific development was followed. The provided text is comprehensible and shows the competence of the author. For the first time in our country, the organization and development of the overall activity of a genetic structure in a highly developed multi-profile university hospital over a long period of time (10 years) is presented in detail longitudinally. The very good visualization and the logical tabular presentation of the results are impressive. From a pediatric point of view, the evidence of expanding the activity of the pediatric structures of the hospital, as well as increasing the diagnostic accuracy in this long period, incl. regarding congenital metabolic disorders. Importantly, there is evidence of strict adherence to modern diagnostic guidelines for a number of pediatric diagnoses in which genetic testing is increasingly mandatory. It is the key to proper care and a clear results from the treatment of genetic diseases. The huge variety of diagnoses and subspecialties with which the doctoral student and the Laboratory work reflect the characteristics of the activity of the patient-referring units. Collaboration with external academic or highly specialized genetic laboratories not only increases diagnostic precision, but also serves as a learning mechanism in the course of clinical laboratory activity.

- *Discussion (28 pages).* In clear and precise literary language, showing the excellent training of Dr. Stoyanova, she very consistently presents the assessment of her scientific results and compares them with the available literary sources, incl. with a critical analysis of the phenotyping tools used. Her proactive approach to analyzing how pediatric patients reaching genetic evaluation can be increased (e.g. from the Pediatric Intensive Care Unit and the Pediatric Psychiatric Clinic) is impressive. There are the modesty, openness, collaboration, and in-depth analysis required of a young scientist regarding the limitations of the current work, as well as at every stage - directions for future improvement of both clinical and medico-genetic work. Elements of economic analysis, so important to the still expensive genetic research, are present in places. It is extremely satisfying to see the progress of this highly specialized activity such as genetic diagnostics, incl. with provision within the framework of available funding in the hospital and in the country. Representing genetic testing, still unavailable due to its high cost and specific competence requirements, as part of the limitations to reach a higher rate of diagnosis, is particularly valuable for future analyses. Last but not least, the doctoral student pays attention to the training of medical specialists to understand the possibilities of different methods and to correctly evaluate the results obtained, stressing that more and more often in the future there will be a need for training and that working in multidisciplinary teams with joint discussion will become increasingly important in the coming years.

- *Conclusions and contributions of the dissertation work (5 pages).* A total of 5 conclusions have been consistently, clearly and comprehensively formulated in agreement with the tasks set, with 12 sub-points. All of them are original to the

national scientific space, since to date the available studies do not cover similar groups of patients and a similar descriptive-epidemiological evaluation of genetic studies has not been done. Based on the conclusions and with a high degree of analysis, 8 contributions have been formulated, 3 of which are original, 3 confirmatory and 2 scientific-applied. 6 publications are presented - 3 full-text (1 article in English in a refereed journal published in Bulgaria, 2 articles in Bulgarian) and 3 participations (2 participations in international conferences in English, published in an abstract and 1 participation in a Bulgarian conference with international participation).

- *Appendices (2 pages).* This part of the scientific work is valuable for future scientific comparisons and as an example of good scientific practice, because it presents the work sheet of the Laboratory of Medical Genetics.
- *References (16 pages)* - 246 sources, of which 25 are in Cyrillic. The PhD student shows skillful handling of published sources and correctly cites them. A total of 146 (59.3%) publications are from the last 10 years, which makes the bibliography extremely contemporary. No significant repetitions are found in the citation.

3. Evaluation of the results:

The results of the present dissertation are in-depth, scientifically based, of great importance for practice, especially for pediatric practice at tertiary university units. The competent assessment of the doctoral student and the visible own opinion on the presented results are impressive, as well as the correct attitude towards collaboration with European structures (European Networks for Rare Diseases and our associated Expert centers) and global laboratories, a carrier of innovation and quality in modern genetics.

4. Assessment of Contributions:

The contributions are original, with distinct scientific and practical significance, especially for native science and practice – as I emphasized, at tertiary university units. The most important thing, I think, is the creation of a base on which to build future work and to make comparisons about the progress of clinical genetics' own achievements or of other structures willing to self-analyse. An applied contribution, in addition to the rightly pointed out by Dr. Stoyanova, is the possibility that the current data can be used by policy makers in the field of health care improvement, which cannot happen without following the rapid development in the diagnostic genetic palette.

5. Critical Notes:

The present dissertation work is an important contribution to native science and modern diagnostic and therapeutic practice. He is an example that, even with insufficient funding and an unequal position compared to the international level of medical genetics at the moment, hard work, a firm and dignified attitude to everyday clinical problems and excellent collaboration with clinicians from different structures can lead to valuable scientific results and outline the way for further development. The dissertation is the result of joint work and an excellent certificate for the scientific supervisor and for the entire

Department and Laboratory of Medical Genetics. I have no critical remarks about Dr. Stoyanova, I just wish she was more and more confident in her competence and in her own irrevocable value as a significant collaborator in the diagnosis of genetic abnormalities.

6. Conclusion:

The dissertation of Dr. Milena Stoyanova is an excellent example of a retrospective "audit" type study with a scientific evaluation of the significant modern achievements in a dynamically developing science with a direct and ever wider contribution to daily clinical practice. The design of the study is adequate to the set tasks and is based on the capabilities of the Laboratory of Medical Genetics, whose chronological rapid development is excellently presented. The contribution of the doctoral student is clearly distinguishable, although the work to achieve the results is the responsibility of the entire Laboratory. Own results are correctly stated, without unnecessary details and without going beyond the personal contribution. As a pediatrician, I cannot miss to report the results of this dissertation giving a digital expression of the distinct development in the pediatric diagnostic and treatment activity at our units, undoubtedly related to the ever better collaboration.

All this gives me reason to confidently express my **positive opinion** and to recommend to the respected Scientific Jury to award Dr. Milena Petrova Stoyanova the scientific and educational degree "Doctor".

03.10.2022
Varna

/Prof. Dr. V. Iotova, PhD, DSc/