REVIEW

regarding PhD study:

„Genetic Diagnostic Study among Pediatric Patients with Hereditary Pathology Who Received Genetic Counselling"

for award of educational and scientific degree "Doctor"

in the area of higher education: 4. Natural sciences, mathematics and informatics:

professional direction: 4.3. Biological science;

scientific specialty: "Genetics"

Author:	Milena Petrova Stoyanova
Research supervisor:	Prof. Lyudmila Angelova, MD, PhD
Reviewer:	Prof. Savina Petrova Hadjidekova, MD, PhD Head of the Department of Medical Genetics, Medical University of Sofia

1. General presentation of the procedure and the PhD student

The review was prepared according to Order RK109-329/01.08.2022 of the Rector of the Medical University of Varna and Protocol 1/09.08.2022 from the I-st session of the Scientific Jury.

The presented set of materials are in accordance with the Procedure for the acquisition of the educational and scientific degree "Doctor" in MU of Varna.

The documents presented by the applicant are in accordance with the requirements of the regulation for the acquisition of the educational and scientific degree "Doctor" and the Rules of the Medical University of Varna.

2. Brief data from the professional biography of the candidate

The PhD student Milena Petrova Stoyanova graduated in 2002 as a "master" in medicine at the Faculty of Medicine of the Medical University "Prof. Dr. Paraskev Stoyanov" Varna. In 2009 – acquired a specialty in pediatrics, and in 2018 a specialty in medical genetics at the same

university. Since 2018, she has been enrolled as a full-time PhD student in the scientific field "Genetics" with scientific supervisor Prof. Dr. Ludmila Angelova.

From 2010 to the present, she holds the position of assistant professor at the Department of Medical Genetics, Medical University "Prof. Dr. Paraskev Stoyanov" Varna. In the period June 2010 - June 2018, she has worked as a pediatrician in the Laboratory of Medical Genetics at the Medical University "Prof. Dr. Paraskev Stoyanov" Varna in the area of medical diagnostics and genetic counselling.

Milena Stoyanova is a member of two international scientific organizations - the European Society of Human Genetics (ESHG) and the European Cytogenetic Association (ECA), as well as two Bulgarian ones - the Bulgarian Society of Human Genetics and Genomics and the Bulgarian Pediatric Association.

The candidate is a co-author of 26 full-text scientific publications and participates in five scientific projects.

She is fluent in written and spoken English.

3. Relevance and significance of the topic

The dissertation work of Milena Stoyanova was prepared in the "Medical Genetics" department, Medical Faculty, Medical University of Varna and summarizes the ten-year data from the activity of the Laboratory of Medical Genetics at "St. Marina" Hospital - Varna in the genetic counselling and research of children with suspected hereditary pathology.

The current dissertation is dedicated to an extremely serious problem in medico-social aspect - genetic diseases and congenital anomalies in paediatric patients, as well as to the important role of the genetic consultant. Congenital anomalies represent a heavy burden for affected families and society as a whole and have a direct negative effect on fertility, increase morbidity and mortality. The incidence of congenital developmental defects is 3-5% of all births. About 2,500 new patients with a genetic disease are born in Bulgaria every year. The extremely high cost of treatment and care of affected individuals is paid by every member of society through the budget, social and health insurance. In developed countries, congenital anomalies are the leading cause of fetal, neonatal and child mortality and morbidity. Twenty percent of child mortality is due to congenital anomalies - a percentage that is constantly increasing. Morbidity and disability of surviving children is a serious medico-social problem. Approximately 25% of hospitalized patients are due to congenital anomalies of various types. Thanks to the rapid development of genomic technologies in recent years, the genetic diagnosis is being clarified for more and more patients, and the number of patients who need medical genetic counseling

is increasing. And here the role of the genetic counsellor stands out, whose task is to direct the patient to the correct diagnostic test and to give detailed information to the patient and his family about the disease itself, the risk for the offspring and siblings, the prognosis, treatment options and prenatal diagnosis. Therefore, in the dissertation, a special focus is placed on the need for genetic counselling. The present work summarizes the experience of the Laboratory of Medical Genetics at UMBAL "St. Marina" - Varna in the genetic counselling and research of childhood patients with suspected hereditary pathology for a period of ten years.

4. A review on the structure and content of the dissertation

The dissertation is consistent with the usual appereance of work of this type, according to the standards. It includes 149 pages, containing: Introduction - 2 pages, Literature review - 35 pages, Aim and objectives - 1 page, Material and methods - 10 pages, Results - 41 pages, Discussion - 27 pages. , Conclusions - 4 pages and Contributions - 2 pages, Cited literature - 17 pages, Publications and scientific reports included in the dissertation work - 1 page, Appendix - 2 pages. The work is illustrated with 31 figures and 15 tables in the text . 246 literary sources in Bulgarian and Latin are cited. The fundamental nature of the subject allows the citation of older sources.

Literature review

The structure, content and volume of the literature review show excellent knowledge of the problem and comprehensively reflect the studies of the literature data, with a logical structure. The classification of the birth defects, etiology of diseases, and epidemiology are successively reviewed. The approaches to genetic diagnosis are described - genealogical method, physical examination, laboratory-genetic methods, and a comparative analysis of the resolution of genetic analyses is made. In addition, modern software and computer dysmorphology programs and sources used in the interpretation of clinical phenotypes and laboratory results are indicated. Special emphasis is placed on genetic counselling as a highly specialized activity in the diagnosis of genetic pathology.

The overview shows that the PhD student is very well versed in the subject she is working on. I give a high rating to the literature review not only because of the excellent knowledge and analysis of the literary data, but also because it is systematized and aimed at the specific tasks of the dissertation.

Aim and tasks

The dissertation aims at a descriptive-epidemiological evaluation and study of the effect of the activity of genetic counselling as an approach to clinical - genetic diagnosis in persons with

suspected hereditary pathology in childhood based on the experience of the University Genetics Center for a period of ten years.

For the fulfilment of this goal, 5 tasks have been set, which are formulated clearly and precisely.

1. To differentiate the contingent of patients aged 0 – 8 years, registered at the office for medical genetic counselling for a period of 10 years, to select patients with an unexplained/unproven disorder subject to diagnostic activity and present characteristics (descriptive-epidemiological and classification by referral indications).
2. To summarise and analyse the results of the conducted laboratory-genetic studies (within and outside the sectors of the university hospital structure) for evaluation of the diagnostic laboratory contribution in clarifying the aetiology of hereditary disorders in children.
3. To assess the overall activity of the MGC as the main structure for genetic assistance in the LMGV in the multidisciplinary diagnostic process of revealing the genetic aetiology of diseases and predispositions in childhood patients.
4. To analyse the role of the MGC's active genetic diagnostic (laboratory and advisory) activity in a selected group of patients with an unexplained/unverified disease status for evaluation of the genetic unit's participation and contribution.
5. To provide guidelines for improving the approach for conducting a clinical-genetic evaluation of a hereditary disease in children as a part of the overall multidisciplinary care for the patient in the clinical-laboratory practice.

Methods

The following research methods were used in the implementation of the above-mentioned objective:

- Clinical methods including pedigree method and family history, physical examination
- Laboratory methods, including cytogenetic method, molecular genetic (DNA) methods - DNA isolation, dTP-PCR (direct triplet-primed PCR) and melting curve analysis (screening for fragile X-chromosome syndrome, FRAXA), Multiplex Ligation-dependent Probe Amplification (MLPA), Real-Time PCR. The above mentioned methods were carried out in the Laboratory of Medical Genetics at UMBAL "St. Marina" - Varna. In addition, to clarify the diagnosis, genetic analyzes were carried out in external laboratories - selective metabolic screening/enzyme analysis; microarray analysis; Sanger sequencing, Next Generation Sequencing, Methylation-specific MLPA, Methylation-specific PCR.

- Analyzes based on dysmorphology databases and software, London Medical Databases/ Winter-Baraister Dysmopfology Database online-based programs - OMIM (Online Mendelian Inheritance in Man), THE PHENOMIZER, FACE2GENE, ORPHA.NET, Monarch Initiative, POSSUM
- Methods for statistical data processing - non-parametric analyses, regression analysis, graphical analysis, Graph Pad Prism 9

From the review of the experimental techniques, it can be concluded that in the process of her studies, the PhD student developed herself as a highly qualified medical geneticist. The applied methods are adequate to fulfil the set goals and tasks.

Materials

The material for the dissertation summarizes the activities of the Laboratory of Medical Genetics - Varna (LMGV), MU-Varna for a ten-year period from January 2011 to December 2020, including a total of 3,124 pediatric patients, aged 0 to 18 years (mean age 5.9 years). Of them, 1,855 (59.4%) are male, respectively the remaining 1,269 (40.6%) - female (M:F = 1.5:1). The patients went through the Medical genetic office for counselling and/or genetic analysis. Biological samples were taken for cytogenetic analisis, for biochemical, enzymatic or molecular genetic analyses.

A distinctive feature of the dissertation work is the integral approach to patient selection performed by a highly qualified team of pediatricians, neonatologists, hematologists, endocrinologists, neurologists, psychiatrists, dysmorphologists and geneticists.

Results and discussion

The analysis of the results is presented in 2 subsections: 1) Discussion of the descriptive epidemiological characteristics of the patients included in the study; 2) Discussion of results of performed genetic and metabolic/enzymatic studies, including analysis of results of conventional cytogenetic analysis, DNA analysis for monogenic diseases and results of active diagnostic activity.

Based on the indication for carrying out MGK, patients are divided into 6 groups - group I - 971 (31.1%) - suspected chromosomal disease, group II - suspected monogenic (non-metabolic) disease, group III - with a suspected hereditary metabolic disease - 148 (4.7%), group IV - with a leading clinical phenotype of single or multiple congenital anomalies/unclear
dysmorphic syndrome, with or without intellectual disability, group V - with a leading clinical phenotype of an developmental delay, ID, behavioural disorders – autism spectrum disorders; group VI – others.

Data analysis shows that the most common indication for genetic counselling is suspected dysmorphic syndrome/multiple congenital anomalies with or without intellectual disability - almost 50% of all referrals.

In 13.9% of the patients the underlying pathology was established by cytogenetic analysis, with the most frequently diagnosed disorder being trisomy 21. Visibly balanced chromosomal rearrangements were found in 26 individuals.

The DNA analysis contributed to the diagnosis of cystic fibrosis in 21 patients, and another 20 carriers were revealed presenting characteristic symptoms. Other diagnosed disorders were Wilson's disease, beta-thalassemia, Gilbert's syndrome and predisposition to celiac disease and hereditary thrombophilia. In addition, 43 patients were screened for pharmacogenetic defects. FRAXA syndrome was diagnosed in 5.7% of patients. The MLPA analysis revealed the diagnosis in 13.9% of the patients referred for examination. 44 patients were examined with microarray analysis, and pathogenic results were found in 19 children (43.2%).

Through DNA sequencing (classical and new generation) were diagnosed patients with suspected RAS-opathies, imprinting diseases, autosomal dominant polycystic kidney disease, rare and ultra-rare diseases such as Beare-Stevenson cutis gyrate syndrome, Hutchinson-Gilford progeria, Cartilage-hair hypoplasia, disease of Dent, Lowe syndrome, etc.

Selective metabolic screening and/or enzyme analysis was performed in 126 pediatric patients and a diagnosis was made in 14 patients.

The present work represents a pilot research in the Republic of Bulgaria for descriptive-epidemiological characterization and assessment of the contribution of the activity of genetic structure in the service of a pediatric contingent. It summarizes valuable information about the distribution of genetic pathology among patients with hereditary diseases and predispositions and could serve as a basis for developing programs for detection, follow-up and treatment of patients, as well as for financing the diagnosis of genetic diseases. In addition, it provides valuable guidelines for the medico-genetic counseling of patients with genetic pathology.

Conclusions and contributions

Five conclusions have been formulated that reflect the results and fully meet the set tasks. Original, confirmatory and applied contributions are also presented. I agree with the author's assessment of the contributions of the dissertation work, and I would highlight mainly those of an original nature, especially regarding the creation of a basis for comparative population studies and planned actions in the field of medical care for patients with suspected genetic disorders.

5. Publications on the topic of the dissertation

In connection with the dissertation, the doctoral student has published three publications of which she is the first author. The candidate also presents 3 presentations with results on the topic of the dissertation. The presented scientific works fully cover the subject of the dissertation work and contain the results of the conducted research.

6. Evaluation of the thesis summary

The thesis summary is written on 65 pages, meets the main sections of the dissertation work and correctly reflects the regulatory requirements.

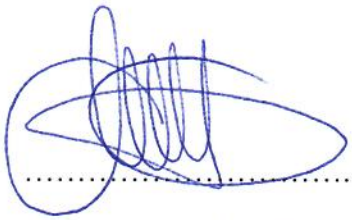
7. Conclusion

The dissertation work "Genetic Diagnostic Study among Pediatric Patients with Hereditary Pathology Who Received Genetic Counselling" is dedicated to an important medical problem and is an in-depth study. When discussing the results, the high professional maturity and scientific competence of the doctoral student make an impression. The conclusions and contributions in the dissertation have an applied value for the correct diagnosis of patients with genetic pathology. The work establishes the author as a responsible and reliable researcher who can independently conduct research at a high scientific level and interpret scientific results.

The dissertation meets the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulation for the Development of the Academic Staff at the MU of Varna for awarding the scientific and educational degree "Doctor".

In conclusion, I would like to mention that I personally know the PhD candidate Milena Petrova Stoyanova and I can say that she is a responsible professionalist with established authority among medical circles and has the respect of the genetics specialists in Bulgaria.

All this gives me a reason to vote for awarding the educational and scientific degree "Doctor" to Milena Petrova Stoyanova.



30 September 2022 Reviewer: .....

Sofia Prof. Savina Hadjidekova, MD, PhD