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REVIEW
From

PROF. LYUDMILA BONCHEVA ANGELOVA, DM, PhD

DEPARTMENT OF MEDICAL GENETICS

Specialty / scientific degree – Professor of Medical Genetics, Doctor of Medicine
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ABOUT:

Dissertation on the topic **"Genetic counseling in patients with probable tumor-predisposing syndrome"**

Author: Chief Assistant Professor Dr. Mari Ara Hachmeriyan-Andreeva, PhD - Administrative Assistant, Medical University of Varna Department of Medical Genetics and University Hospital "St. Marina" EAD Varna, 1 Hr. Smirnenski St.

Form of study – **self-**study, Department of Medical Genetics , Medical University of Varna, enrolled by doctoral student program **" Medical Genetics "** for acquisition of **the ONS "Doctor"** , Field of higher education: 7. Health and sports Professional field: 7.1 Medicine for awarding the scientific and educational degree "doctor".

Scientific supervisors: *Associate Professor Maria Levkova, Ph.D.*
Assoc. Prof. Dr. Eleonora Dimitrova, MD.

Procedure:

According to Order No. R-109-394 / 25.09.2025 of the Rector of MU - Varna, I am appointed as an internal member of the Scientific Jury, and based on Protocol No. 1 / 08.10. 202 5 years from the first (on-line) meeting I have been appointed as the chairperson of the scientific jury and reviewer of the dissertation.

Dr. Hhachmeriyan is enrolled as a doctoral student in an independent form of training with a term of 3 years with (Order R-109-284 / 07. 08. 202 4 of the Deputy Rector of MU-Varna) taking into account Report with Entry No. 102-1638/12.06.2024 by *Prof. Dr. Lyudmila Angelova, MD, Head of the Department of Medical Genetics on the need for a second scientific supervisor with a clinical specialty given the interdisciplinary nature of the topic* . The training is organized and conducted at the Department of Medical Genetics of the Medical University of Varna, where the doctoral student is an employee and therefore exempt from paying a fee, according to a decision of the Academic Council (Protocol No. 9/29.07.2024).

The stages of the doctoral studies for admission to the public defense have been met] the doctoral student has been directed to finalize the procedure after *the internal defense* at a meeting of the Department Council of the Department of "MEDICAL GENETICS" in accordance with Art.61, para.1 and para.2 of the Regulations for the Development of the Academic Staff at MU - Varna. The dissertation was prepared at the Department of Medical Genetics of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna; Medical Genetics Laboratory at UMBA L "St. Marina" EAD Varna.

During the defense procedure, Dr. Khachmerian presented the necessary materials, according to the requirements of the Medical University of Sofia Varna.

This review was developed and presented according to the requirements of The Act on the Development of the Academic Staff of the Republic of Bulgaria /LAADRB/, The Regulations for the Implementation of the Law on the Protection of the Rights of Persons with Disabilities and the Regulations for the Development of the Academic Staff at the Medical University /MU/ - Varna. I declare that I have no conflict of interest with the author of the dissertation.

Brief biographical data and professional qualifications

Dr. Mari Hachmeriyan was born on 05.03.1986 in Varna. In 2005, she graduated from the V Language High School "Ioan Exarch" with main subjects English and information technologies. In 2011 she graduated from the University of Medicine in Varna (Diploma No. 001521/22.11.2011). Since 2012 (February) she has been an assistant professor in the Department of Medical Genetics and assistant physician in the Laboratory of Medical Genetics of the University Hospital "St. Marina" Varna. In 2015, she obtained a master's degree in "Molecular Biology and Biotechnology" at the University of Paisiy Hilendarski (Diploma No. MB-12/15.10.2015). In 2017 (*September), s*he obtained the "Doctor" qualification in the specialty "Genetics" in the Field of Higher Education: 4. Natural Sciences, Mathematics and Informatics, Professional Direction: 4.3. Biological Sciences on the topic "Maternal Biochemical Screening as a Method for Prenatal Genetic Prophylaxis" scientific supervisor Prof. Dr. L. Angelova, MD. In 2017 *(December), s*he obtained the specialty "Medical Genetics" (Diploma No. 3926 with reg. No. 021463/20.02.2018, Medical University of Varna). Since 2018 and currently she is a senior assistant at the Department of Medical Genetics of the Medical University. Since 2019 (June) she is the Head of the Laboratory of Medical Genetics, University Hospital "St. Marina" Varna.

Dr. Hachmeriyan builds teaching skills for physicians (Bulgarian and English language training), pharmacists and "Nursing" specialists, as well as laboratory and consulting competencies for laboratory work. She attends thematic courses (dysmorphology, basics of medical genetics, oncogenetics, and reproductive genetics) in England, Italy, the Netherlands, Turkey, and Poland. She presents data on 48 full-text publications; 71 contributions published in abstracts; participation in four research projects. Her scientific interests are in the field of molecular genetic methods for the diagnosis of rare genetic diseases and common socially significant diseases with hereditary predisposition.

Human Genetics and Genomics; the European Society of Human Genetics (ESHG), the European Cytogenetics Association (ECA), and the Bulgarian Pediatric Association.

Structure of the dissertation

The dissertation is presented in a total of 103 pages, contains 15 figures and 3 tables: table of contents (2 pages), abbreviations (1 page), introduction (2 pages), literature review (32 pages), goal and objectives (1 page), material and methods (6 pages), results (15 pages), discussion (20 pages) (conclusion and directions for future work (3 pages), conclusions (1 page), contributions (1 page), bibliography (12 pages), Appendices 1,2,3 (6 pages), bibliography (12)

The abstract, 55 standard pages, is written in accordance with the dissertation work. The members of the scientific jury are pointed; Prof. Alexey Savov, D.B. for technical reasons, replaced an external member Assoc. Prof. Dr. Elitsa Becheva.

Note: The scientific work is structured in the 8 standard sections, in an acceptable ratio. Scientific publications and communications on the topic of the dissertation are not included, but they are reflected in the Abstract.

Relevance of the topic of the dissertation

The dissertation submitted for review addresses *a topical* topic related to the need for modern molecular - genetic diagnostic evaluation in patients with genetic predisposition to malignant syndromes. It concerns socially significant diseases for which risk assessment and the possibility of prevention are of key importance. The emphasis is on the active activity of medical-genetic consultation and approaches to reach at-risk patients, while discussing the possibilities and limitations of currently applied genetic studies.

In the Literature Review, the author thoroughly systematizes the scientific information on the topic under development, such as: 1) Tumor-predisposing syndromes (epidemiology, significance, classification, general characteristics of selected syndromes, inheritance models) and 2) Genetic-diagnostic methods for identification (genetic counseling, molecular-genetic methods for diagnosis, analysis and reanalysis of data, recontact).

Bibliography shows the most up-to-date, including 2025, sources of information on the topic; five by Bulgarian authors are included (one in Latin and four in Cyrillic).

I accept some technical incompleteness (No. 95 and No. 96) and repetitions (No. 40 and No. 153), (No. 99 and No. 100), (No. 101) as a result of a lack of time for the author, and not as ignorance. Actually 158 (not 161) sources and websites are cited.

The summary systematizes the scientific data and logically leads to the construction of *a Working Hypothesis* with sound arguments for the choice of the topic being developed. "Insufficient studies on the effect of medical-genetic consultations in patients with suspected tumor-predisposing syndrome in our country and the need to assess their effectiveness against the background of a growing demand for genetic services."

The aim of the dissertation work is logically derived from the literature facts and working hypothesis – To conduct an epidemiological assessment and study of the effectiveness of medical-genetic consultation as a prophylactic approach in individuals with probable tumor-predisposing syndrome (TPS), based on the experience of the Laboratory of Medical Genetics -

Varna for a period of five years. To achieve this aim, **five tasks** have been formulated, which are logically connected and in a synthesized form.

1. To distinguish patients with a probable tumor-predisposing syndrome, registered in the medical-genetic counseling office for a period of 5 years, and to characterize them (epidemiologically and analytically) by indications for referral, presence of a positive family history.
2. To collect, summarize, and analyze the results of laboratory genetic studies conducted both in the Laboratory of Medical Genetics - Varna and in structures outside it.
3. To reinterpret genetic data from previously conducted analyses and to assess the need for periodic reanalysis of genetic analysis data.
4. To assess the role of the MHC in the multidisciplinary approach to diagnostic clarification and care provision in patients with probable TPS, including the contribution of the MHC to informed patient choice, psychosocial adaptation, and adherence to follow-up and prevention recommendations.
5. To develop guidelines for improving the organization and conduct of MCG in patients with probable TPS within the framework of overall multidisciplinary patient care.

I pay particular attention to the fourth task (related to the place of the MGC in providing care for patients with Tumor-Predisposing Syndrome in our country) and the fifth task, summarizing the data for deriving guidelines for work with these patients.

Material and methods.

The study is retrospective in nature and reflects the experience of the Medical Genetics Laboratory at St. Marina University Hospital - Varna in the period January 2020 - December 2024. The patient contingent is registered in the Medical Genetics Consulting Office of the laboratory and includes:

- **154** registered patients underwent MCG to *discuss the risk* of an underlying tumor-predisposing syndrome over a 5-year period.
- **157** people underwent *additional consultation* regarding the risk of an underlying tumor-predisposing syndrome for a 2-year period 2023 – December 2024.
- **76** people who *had DNA analysis* for tumor-predisposing syndrome in collaborating laboratories within the 5-year period.

The methods are:

1. **Primary and subsequent medical-genetic consultation** according to the steps of the process:

- *Genealogy and medical history* with inclusion and exclusion criteria in patient selection and indication for referral, use of information from the hospital electronic database. All data are protected in accordance with confidentiality requirements and GDPR after receiving informed consent *(Appendix 1*) for participation in the study, approved by the EC at the Medical University of Varna, protocol No. 9/31.01.2025.
- *Discussion of the possibilities for laboratory genetic diagnostics* (Appendix 2); scope of the analyses, accessibility (including financing) importance for subsequent behavior for both the index patient and his relatives); discussion of the result, interpretation of the reported variants and formulation of recommendations from the laboratory analysis performed.

2. **Laboratory methods**

Peripheral blood collected in EDTA tubes, DNA isolated from whole blood, buccal mucosa. In some cases, tumor tissue samples stored as formalin-fixed, paraffin-embedded (FFPE) blocks have also been used. Targeted panel or whole-exome sequencing (NGS in external laboratories certified according to ISO15189 and/or Clinical Laboratory Improvement Amendments (CLIA) or College of American Pathologists (CAP) in:

- **25 patients** tested with the Invitae Multi-Cancer Panel (84 genes) (Invitae)
- **34 patients** tested with Blueprint Genetics Hereditary Cancer Panel (160 genes) (Blueprint Genetics)
- **14 patients** tested with Blueprint Genetics Hereditary Cancer Panel (28 genes) (Blueprint Genetics)
- **3** patients studied with WES (Blueprint Genetics) in cases with complex clinical presentation

3. **Bioinformatic methods** for *reanalysis and reinterpretation* of variants classified as variants of uncertain significance (VUS) (platform - https://franklin.genoox.com - Franklin by Genoox (last accessed 03.2025)
4. **Online-based programs** and information resources *for risk assessment* through online resources and tools: (Good practice recommendations: ESMO, ASCO, NCCN recommendations for the management of tumor-predisposing syndromes); Risk assessment models CanRisk (www.canrisk.org) for breast and ovarian cancer; Genetic data analysis (Franklin Genoxx (www.franklin.genoox.com) - a platform for analysis of VCF files and interpretation of genetic variants.
5. **Methods for statistical data processing** - a statistical panel with a set of 5 types of statistical methods was used according to the assessment of dependencies, a personal work of the doctoral student. (correlation analysis, Fisher's exact test, analysis of variance, t-test, significance level.

It is appropriate to indicate the number of patients in this section who have been analyzed with bioinformatic reanalysis methods and online risk assessment programs.

The results are presented correctly in a separate section, separate from the discussion, in response to the tasks set; they are illustrated with 14 figures and 3 tables, the application of which does not duplicate the text. According to the type of questions, tasks to be solved and the studies conducted, the results are grouped in:

- Descriptive – epidemiological characteristics of 154 patients who underwent MCG *for evaluation of a potential TPS* (number, age, gender, risk factor, referral unit), and of 157 patients who underwent *additional MCG after laboratory* diagnosis, as well as a comparative assessment of the characteristics of the two groups of patients who underwent MCG. Significant differences between the two groups are detected in demographics, motivation, and referral unit.
- Of interest are descriptive-epidemiological characteristics of patients who underwent DNA analysis (number, age, and gender, referring unit, scope and indications, revealed pathology). Of all 311 patients who underwent MGK, only 76 (24%) had DNA analysis, almost all after primary medical-genetic consultation (the first analyzed group). Statistically significantly more patients are female, younger, directly referred by a hospital unit, own financing of laboratory diagnostics from a preferably broad panel of genes associated with predisposition to cancer.

I consider Table 1 very useful; it reflects the revealed (probably) pathogenic variants in the context of the phenotype and tumor-predisposing syndrome in 21 individuals out of 76 studied. The diagnostic success rate of the InVitae laboratory is comparatively higher than that of

BlueprintGenetics, despite the higher number of reported VUS from the former, probably also due to the earlier years of the possibility of referral to InVitae. (Labcorp Genetics since 2024).

It is noteworthy that limitations in bioinformatics tools preclude a comprehensive reassessment. However, the focus on reclassifying reported variants of uncertain significance (VUS) has led to some experience in reanalyzing 68 VCF SNP files, yielding important observations.

I accept the results and their statistical processing as adequate to the set goals and objectives.

The discussion is the main section for every dissertation, in which the doctoral student analyzes the results obtained in the context of his knowledge from the literature review and seeks logical analysis in evaluation and comparability.

There are two subsections: a) a parallel discussion of the descriptive-epidemiological characteristics of the two groups who underwent genetic testing; and b) a detailed discussion of the patients who underwent genetic testing based on results and a commentary on the reinterpretation of the results.

In her discussion, the doctoral student analyzed the difficulties associated with clinical indication for testing; proposed laboratory testing for adequate gene coverage; recognition and diagnosis of rarer syndromes; overlapping/ atypical phenotype or time of onset, but with an increased risk of malignant diseases. The expansion of testing at-risk relatives (only 7 individuals out of 36 (19%) at-risk relatives underwent MCG and subsequent targeted testing is a problem. These psychosocial aspects are particularly important when testing healthy individuals and are an additional obstacle to the financial burden for them.

The overall diagnostic approach is commented on - it should combine different technologies and analytical strategies (tools) according to the clinical phonotype and the type of mutation sought. The use of different versions of software, reference genomes, filtering criteria or annotation tools may lead to the discovery of variants.

The author summarizes that this dissertation study provides confirmatory data from practice on the clinical benefit, the need for periodic reanalysis of genomic data as standard practice in patients with TPS and pleads for increasing awareness and education among all participants – clinicians, geneticists, patients – about the dynamic nature of genomic information. The patient's disease outcome addresses the need for public awareness for effective prevention and improvement of mass health culture in general.

A detailed and up-to-date main section of the dissertation.

The scientific work conclusions are based on the data obtained and meet the set goals and objectives.

1. Patient profile and trends: There is an increasing number of medical genetic consultations and DNA analyses performed in the three groups of patients analyzed. Patients who visit the MCG for evaluation for potential TPS and proceed with DNA analysis are mainly young women (<50 years old) with a positive personal or family history, residents of the city of Varna and referred by a physician,

2. Characteristics of genetic testing: The diagnostic success rate of the applied DNA analyses is 28%, with the preferred type of analysis being an extended gene panel.

3. Need for data reanalysis: Periodic reanalysis of genomic data is clinically necessary, given the high proportion of initially reported variants of unclear significance (25%).

4. Effectiveness and challenges of MCG: A critically low percentage of DNA tests performed after a free hospital consultation, after MCG for biomarker diagnostics, and relatives who undertook cascade screening has been found.

5. Directions for improvement: Improving oncogenetic services requires an integrated approach: increasing educational activities, expanding access through telegenetics and financial support, and building bioinformatics capacity for data storage, analysis, and reanalysis.

The last conclusion (5. Directions for improvement) is in fact presented as a separative topic of the dissertation 7.Conclusion and directions for future work.

I accept the author's self-assessment of **the contributions** of the dissertation work, organized into 2 groups.

Contributions of an original nature

1. The dissertation represents the first systematic study of the practice of medical-genetic counseling in patients with diverse tumor-predisposing syndromes in Bulgaria.

2. The paper provides epidemiological and clinical characteristics for age-sex distribution, risk factors, referral units, types of genetic tests performed, and frequency of identified pathogenic variants.

3. The paper analyzes the results of the genetic studies conducted, assessing the frequency of detection of pathogenic variants, VUS and the need for reanalysis of the data.

Contributions of a practically applied nature

1. The dissertation confirms the key role of the MCG in identifying at-risk patients with probable TPS. It emphasizes the importance of the clinician and other factors outside the MCG in patient behavior and participation in subsequent genetic analysis.

2. The dissertation identifies key challenges facing MCG in patients with TPS in Bulgaria, as a starting point for specific guidelines for improving health resource planning and organizing genetic services.

Works related to the dissertation (presented in the Abstract)

Dr. Khachmerian has presented sufficient materials on her scientific work: 3 full-text publications in scientific journals; 5 participations with abstracts (2 in print); 3 reports in our country without abstracts. The publications are current and with leading authorship:

a) Hachmeriyan M, Levkova M, Yahya D, Stoyanova M and Dimitrova E (2025) Genetic counseling for hereditary cancer syndromes: a 5-year experience from a single center in Bulgaria. *Oncol. Rev.* 19:1605606. doi: 10.3389/or.2025.1605606 ;

b) Hachmeriyan, M., Levkova, M., Yahya, D., Stoyanova, M., Dimitrova, E. Ethical and Psychosocial Issues Associated with Genetic Testing for Hereditary Tumor Predisposition Syndromes. Healthcare 2025, 13, 880. https://doi.org/10.3390/ healthcare13080880.

c) Mari Hachmeriyan, Mariya Levkova, Milena Stoyanova, Dinnar Yahya, Eleonora Dimitrova. Genetic Tumor Risk Syndromes: Current Aspects of Diagnosis and Genetic Counseling. Scripta Scientifica Medica. 57,1 (Apr. 2025), 7–16. DOI: https://doi.org/10.14748/myc3na25.

Critical notes, comments and recommendations for the dissertation:

Individual remarks and recommendations end each section of my review.

My general view of the scientific work is that it essentially presents the acquired consultative and analytical experience of the doctoral student for molecular genetic examination of patients with a probable tumor-predisposing syndrome in support of clinical practice. The author skillfully interprets the results in the context of dynamically changing genomic databases, data that are still

insufficient as publications for our country. Therefore, I sincerely recommend her scientific development in the field of oncology.

I have known Dr. Hachmeriyan since she joined the Laboratory and Department of Medical Genetics in 2012. As an assistant and specialist, she has very good theoretical and practical knowledge in the field of medical genetics, which she uses daily in her practice as a medical specialist and teacher. Defense of a previous research paper in the Field of Higher Education 4, has imapcted for acquiring scientific literacy on her current dissertation work in accelerated finalization.

Conclusion:

The dissertation corresponds to the requirements for obtaining the ONS "Doctor". It shows that the author possesses theoretical knowledge and skills in the doctoral program "Medical Genetics" and demonstrates the ability to analyze and independently conduct scientific research, covering the scientometric criteria of the PRAS of MU-Varna. Due to the above, I give my positive assessment and vote to Dr. Mari Ara Hachmeriyan-Andreeva for awarding the ONS "Doctor" in the Field of Higher Education: 7. Health and Sports , Professional Field: 7.1 Medicine .

Заличено на основание чл. 5, §1, б. „В“ от Регламент (ЕС) 2016/679

23. 10. 2025 **Reviewer**

PROF. DR. LYUDMILA ANGELOVA, DM