TO: THE CHAIRMAN
OF THE SCIENTIFIC JURY

OPINION

by Prof. Dr. Ivanka Istalianova Dimova, MD, PhD
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of the Dissertation of Dr. Mari Ara Khachmerian-Andreeva on the topic "Genetic Counseling in Patients with a Probable Tumor-Predisposing syndrome", presented for the award of the Educational and Scientific Degree "Doctor"
Doctoral program: Medical genetics
Professional field: 7.1. Medicine
Field of higher education: 7. Healthcare and sports
Scientific supervisors: Assoc. Prof. Dr. Maria Levkova, PhD and Assoc. Prof. Dr. Eleonora Dimitrova, PhD
The selection of a member of the scientific jury is in accordance with Order No. R-109-394/25.09.2025 of the Rector of the Medical University - Varna. The review is in accordance with the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADRB) and the Regulations for its implementation, as well as with the Regulations for the Development of the Academic Staff of the Medical University - Varna.

The dissertation submitted to me for review entitled "Genetic Counseling in Patients with a Probable Tumor-Predisposing syndrome" by Dr. Mari Ara Khachmerian-Andreeva, PhD student at the Department of Medical Genetics, Medical University – Varna, covers 103 standard typewritten pages and contains 15 figures and 3 tables. There are 161 references, of which 157 (98%) are in English and 132 (82 %) are publications from the last ten years. There are also 3 appendices to the dissertation: Appendix 1, presenting the Order for genetic analysis and the Informed Consent for genetic testing; Appendix 2, containing Links with detailed information from the performing laboratory about the genes analyzed in the panel testing, possibilities and limitations and Appendix 3 with Online sources for information.
The dissertation is devoted to genetic counseling in patients with a probable tumor-predisposing syndrome. In recent decades, the basis of genetic predisposition in many cancer syndromes has been clarified, and in modern healthcare in many countries, genetic diagnostic screening programs have been introduced to study highly penetrant/high-risk genes that are

mutated in familial cases of cancer. Mutational analysis of these genes is of great importance for genetic counseling, helps to increase the chance of survival, determines the prognosis of carriers, and directs to the most appropriate and personalized prophylactic and therapeutic measures. Subsequent genetic studies helped to discover additional genes with moderate risk, and genome-wide association studies identified other low-penetrance alleles associated with predisposition to cancer syndromes. It became clear that genes associated with certain cancer syndromes carry a high risk for a certain tumor type, but also have low penetrance and carry a low to moderate risk for the development of other tumor types. Next-generation sequencing, based on gene panels with a focus on tumor-associated genes, is the most commonly used platform at present due to its lower cost and significantly more complete information compared to individual direct gene sequencing. Its use in diagnostic practice requires careful clinical and genetic work-up, which forms the basis of oncogenetic medical-genetic consultation.

Medical genetic counseling (MGC) is of key importance in the management of patients with tumor-predisposing syndromes, as it provides a comprehensive approach to the assessment of hereditary risk, diagnosis and prevention of oncological diseases. It provides the link between genetic information and clinical decision-making, allowing early prevention, personalized therapy and improved quality of life for affected individuals and their families. Given the complex nature of tumors and the complexity in the interpretation of multiple genetic variants detected in next-generation sequencing, oncogenetic MGC is a specific, highly specialized and responsible activity with important, specific aspects that need to be addressed.

In this sense, **the topic of the dissertation is particularly relevant and of great importance for medical-genetic diagnostics and clinical behavior in patients with a probable tumor-predisposing syndrome, as well as of great health and social importance.**

The dissertation is written according to the generally accepted principle and consists of a Literature Review (30 pages), Aim and Objectives (1 page), Materials and Methods (5 pages), Results and Discussion (30 pages), and finally a Conclusion and directions for future work, the most important Conclusions and Contributions are drawn.

The literature review is informative, presenting a model for characterizing tumor-predisposing syndromes, including a clinical spectrum, molecular mechanisms and recommendations for clinical behavior of established guidelines from oncological societies and networks worldwide. The second part of the review examines the features of genetic counseling in the era of new-generation sequencing. **The goal of the dissertation is logically derived**, namely - to make an "epidemiological assessment and study of the effectiveness of medical-genetic counseling as a prophylactic approach in individuals with a probable tumor-

predisposing syndrome, based on the experience of the Laboratory of Medical Genetics - Varna for a period of five years".

To achieve this goal, the doctoral student sets the following tasks: 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 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 MCG in the multidisciplinary approach for diagnostic clarification and provision of care for patients with probable TPS, including the contribution of the MCG to the informed choice of the patient, his psychosocial adaptation and compliance with recommendations for follow-up and prevention; 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.

The section “Results” presents: the epidemiological characteristics of patients who underwent MCG for the assessment of a potential TPS and after a visit for biomarker diagnostics; age and gender characteristics; risk factors and referral units. The low percentage of 4.3-7.5% found for the part of oncogenetic consultations in the total number of MCGs emphasizes the need to optimize the approach and promote it among clinicians and the public. Then, it presents the data for the patients who underwent subsequent DNA analysis with a new- generation sequencing – 76 in total, with only 1 from the group consulted for biomarker testing. This means that less than 1% of patients who undergo oncogenetic biomarker testing undergo subsequent testing for germline mutations. I find some weakness in the attached Order and Informed Consent, which nowhere mention the new- generation sequencing and its capabilities. No order and informed consent for the biomarker studies being conducted have been presented. Can the doctoral student derive recommendations and criteria for including patients from biomarker diagnostics in subsequent germline analysis through an expanded tumor panel?

The results of the pathogenic/probably pathogenic variants found in the genes from the studied panels are presented – in total in 28% of the studied ones. Targeted sequencing was performed on 7 healthy relatives and pathogenic variants in the *BRCA1, CHEK2, MSH2, NBN* genes were found in 4 of them. I find it unnecessary to perform panel sequencing for cascade testing, since the analysis of the specific mutation found with a cheaper and simpler method is completely sufficient. Variants of unclear clinical significance were found in 25% of the studied patients.

Is segregation analysis offered in families as part of genetic counseling, especially in cases with new variants found and those with unclear clinical significance?

As a result of the dissertation work, 5 conclusions were drawn: the first concerns the patient profile and trends; the second is about the diagnostic success rate of the applied DNA analyses; the third emphasizes the need for periodic re- analysis of genomic data; the fourth determines the effectiveness and challenges of MHC; the fifth is focused on directions for improvement.

I define the dissertation work as health-social more than fundamental and this is by no means a disadvantage. The issues raised about the European Reference Networks, the national anti-cancer plan, preimplantation diagnostics in tumor-predisposing syndromes and all aspects of biobanking undoubtedly show the high public commitment of the doctoral student in the implementation and resolution of these issues. Regarding the frequent mention of the methods of payment for MCG and genetic tests in the dissertation, I would like to ask the doctoral student's opinion on the issue of the key participation of pharmaceutical companies in the payment of these tests, which is the current situation in Bulgaria. What regulation, in her opinion, should be adopted in Bulgaria in order to guarantee patients' access to these important and often decisive tests for diagnosis and treatment?

The data included in the dissertation work are presented in 3 scientific articles, with the doctoral student being the lead author in all of them - 2 of the articles are referenced in Scopus and have a quartile Q2 . He has presented 3 reports at scientific forums in Bulgaria and 5 participations in international conferences and congresses.

I have reviewed the abstract draft and believe that it faithfully reflects the data and contributions of the dissertation.

CONCLUSION: The dissertation work of Dr. Mari Ara Khachmerian -Andreeva is dedicated to a very important topic, namely – medical-genetic counseling related to genetic predisposition to tumors. The key moments in this process are outlined and directions are proposed for optimizing the approach and building a sustainable system for handling genomic data in the era of personalized medicine. Having all this in mind, I strongly recommend that the esteemed jury award **Dr. Mari Ara Khachmerian -Andreeva** the scientific and educational degree of DOCTOR.

03.11.2025

Reviewer:

Prof. Dr. Ivanka Dimova, MD