REVIEW

for award of educational and scientific degree "PhD"

in the area of higher education: 7. "Health and Sport", in the professional area 7.1. "Medicine" and scientific specialty "Medical Genetics"

Author: Nelly Nedyalkova Miteva-Marcheva, MD

Form of doctoral study: regular PhD student

Department: Pediatrics ana Medical genetics, Medical University of Plovdiv

Title: „ Pharmacogenetic Markers Associated With Drug Metabolism in Cancer Patient"

Research supervisor: Prof. Vili Stoyanova, 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 R-3700/13.12.2023of the Rector of the Medical University of Plovdiv.

The submitted set of materials complies with Article 70 (1) of Section I. Acquisition of educational and scientific degree "DOCTOR" and scientific degree "DOCTOR OF SCIENCES" at MU-Plovdiv; Regulations of MU-Plovdiv from 28.01.2021 and includes the following documents:

- Application to the Rector of MU-Plovdiv for the disclosure of the procedure for the defense of a dissertation
- CV in European format
- a notarized copy of a diploma of higher education
- orders for enrolment in a doctoral programme
- an order for conducting an examination from the individual plan and a corresponding protocol for passing the examination or doctoral minimum in the specialty
- protocol of the departmental council for the preliminary discussion of the dissertation and the decisions taken on the opening of the procedure and on the composition of the scientific jury
- dissertation

- abstract
- list of scientific publications on the topic of the dissertation
- copies of scientific publications
- list of participations in scientific forums
- declaration of originality and authenticity of the attached documents

The doctoral candidate has attached 3 publications, one of which is from participation in a scientific conference.

The materials submitted for the defence comply with the legal and procedural requirements.

2. Brief data from the professional biography of PhD student

The PhD student Dr. Neli Miteva-Marcheva in 2015 graduated as "Master" in Medicine at the Medical University of Plovdiv, and in 2019 - acquired a specialty in Pediatrics at the University Hospital "Puldin". Since 2020 she has specialized in Medical Genetics at the University Hospital "St. She has been studying genetics since 2020. Since 2019 till now she is an assistant professor at the Department of Pediatrics and Medical Genetics, Medical University of Plovdiv.

The candidate is co-author of 6 scientific publications. She has participated in three national conferences on emergency pediatrics (2015,2016 and 2017), a workshop on pediatric cardiology in Salzburg, a conference on pediatric endocrinology, ESHG Conference (2022 and 2023). She was ranked 1st for her scientific paper on "Involvement of nitric oxide in tacrine-induced smooth muscle responses" in the Science and Youth competition. The PhD student is in the research team of three research projects. He is a member of the BMA, the Bulgarian Society of Human Genetics and Genomics and the Society of Cardiologists in Bulgaria. He is fluent in written and spoken English, Spanish and Russian.

3. Relevance and significance of the topic

Modern personalized medicine is based on individual therapy determined on the basis of the results of genetic analyses carried out before the start of treatment. Personalized medicine is based on studies of rare monogenic defects and polymorphic variants in genes that are involved in the biotransformation of drugs - resorption, transport, binding to cell receptors, metabolism, excretion from the body. The goal is to identify the right drug, in the right dose, at the right time for the right patient, according to the genetic variants (biomarkers) identified.

The present dissertation draws attention to a significant health problem - the search for an individual approach to the treatment of patients suffering from cancer. Cancers are widespread,

socially significant and occupy an important role in human pathology. According to the World Health Organization (WHO), cancer is the second cause of death worldwide. The efficacy and outcomes of treatment of malignancies depend on various factors. The efficacy of the same drug administered in a therapeutic dose according to a standard protocol is known to vary between patients. This may be due to various reasons genetic factors, physiological/pathophysiological processes - environmental factors. Variants in certain genes may determine differences between individuals in the rate of drug resorption, transport, binding to cell receptors, biotransformation, formation of active substances and removal from the body. Pharmacogenetics and pharmacogenomics combine the capabilities of genetics and pharmacology to study the role of genetic variants in an individual's altered response (increased, decreased, or absent) to drug action. NGS technology has exceptional potential for application in pharmacogenomics as it allows the analysis of an entire genome, exome or panel of a large number of genes in a single reaction quickly, accurately and inexpensively.

4. Knowledge of the problem

The structure, content, and scope of the literature review demonstrate excellent knowledge of the problem and reflect a comprehensive exploration of the literature with a logical structure. The style is accessible, in literary Bulgarian.

The literature review is developed using literature sources mainly from the last 5-10 years.It is contemporary in content, competently and purposefully focused on the problem under study. At the beginning of the review, a historical account of the emergence, development and concept of pharmacogenetics is given. The terminology used is discussed and comprehensively explained. Basic concepts of pharmacogenetics and pharmacogenomics as the foundations of personalized medicine are briefly presented.

The PhD student focuses on the problem of genetic heterogeneity of tumors and the importance of the molecular profile of the tumor as a basis for personalized targeted therapy. The principles, possibilities and individual stages of different molecular techniques for the study of genetic markers of tumors - classical sequencing, denaturing high-performance liquid chromatography, single-strand conformational polymorphism analysis, next-generation sequencing (NGS) - are competently presented.

Data on the incidence of malignancies worldwide are provided. The principles of cancer treatment are outlined, with a detailed discussion of the most commonly used chemotherapeutic agents and their pharmacokinetics and dynamics. The groups of: fluoropyrimidines; irinotecan, platinum-containing cytostatics; taxanes; cyclophosphamide inhibitors; vascular endothelial growth factor inhibitors (VEGF inhibitors); etoposide are presented.

Literature data on studies of genetic biomarkers and targeted therapy in patients with carcinomas are summarized. On the basis of the outlined molecular heterogeneity of tumors, the PhD student successfully presents in a synthesized form data for new approaches to target therapy.

In the last part, the review concludes with a summary highlighting the importance of a personalized approach to ensure efficacy, reduce the incidence and severity of adverse drug reactions (ADRs) and preserve patients' quality of life as much as possible.

I commend the literature review not only for its excellent knowledge and analysis of the literature, but also because it demonstrates analytical thinking and the ability to generalize.

The literature review is systematic and generally shows a very good knowledge of the evidence, the results of the many studies in this field, and the analytical methods. This has enabled the formulation of the aim of the study and the resulting objectives.

Aim and objectives of the study

Aim
The aim of the thesis is to investigate germline pharmacogenetic variants associated with drug metabolism in cancer patients.

To accomplish the aim, the following objectives were set:

Objectives
1) To isolate gemomic/cell-free DNA from patients with non-small cell lung carcinoma (NSCLC), colorectal carcinoma (CRC), breast cancer (BC), and 40 healthy individuals.

2) To conduct target sequencing of a panel of genes in the isolated samples to analyze their genetic makeup.

3) To identify germline pharmacogenetic variants associated with the metabolism of chemotherapeutics used in treatment, utilizing the PharmGKB database.

4) To characterize the frequency and clinical significance of identified genetic polymorphisms.

5) To assess the frequency of genetic polymorphisms in the Bulgarian population in comparison to the general human population.

5. Research methodology

Methods

The following research methods were used in fulfilling the above objective:

1. Isolation of genomic DNA from blood;
2. Isolation of cell-free DNA from blood;
3. Next generation sequencing (NGS).

DNA extraction was performed using the QIAGEN QIAamp DNA Blood Mini Kit for purification of genomic DNA. Isolation of cell free DNA was performed with BioChain's cfPure® Cell Free DNA Extraction Kit. Isolated DNA (genomic and cell-free) was sequenced at Novogene Corporation Inc using next-generation sequencing. A target panel of 484 genes was applied to identify different genetic variations among the patients studied, i.e. single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs).

From the review of the experimental techniques, it can be concluded that in the process of his training he possessed the qualities of a qualified geneticist. A wide range of classical and modern molecular biological methods have been mastered and applied. It is obvious that the PhD student has sufficient molecular biological knowledge and the methods used are described and explained in detail. The methods applied are consistent with the aims and objectives.

Materials

In the initial selection 140 participants were included in the study, from which 90 individuals were finally selected. Of these, 50 were cancer patients (26 were diagnosed with colorectal cancer, 13 with non-small cell lung cancer, 11 with breast cancer) and 40 were healthy individuals. The selection of the study patients was based on the high prevalence of carcinomas, the incidence of adverse drug reactions among the patients, and the similar chemotherapeutics used in their therapy.

6. Characteristics and evaluation of the dissertation

The dissertation follows the usual structuring of a work of this type, according to the accepted standards of a dissertation for the degree of PhD. It comprises over 147 pages, including: Introduction - 2 p., Literature Review - 44 p., Aim and Objectives - 1 p., Materials and Methods - 8 p., Results - 29 p. and Discussion - 20 p., Conclusions and Contributions - 2 p., Literature Cited - 11 p., Appendices 3 p. The work is illustrated with 15 figures and 25 tables in the text. 212 references and 8 web-based sources are cited, all in Latin.

The dissertation shows a good layout and illustration, which deserves high praise.

The analysis of the newly generated sequencing results is presented in 6 subsections:

1. Patients with colorectal cancer
2. Patients with non-small cell lung cancer 2.
3. Patients with breast cancer
4. Patients according to chemotherapy
5. Comparing the frequency of genetic variants in the healthy patients with GnomAD (non-cancer patients)

6. Comparing the frequency of germline pharmacogenetic variants identified in the study group and healthy patients with the frequency of the same variants in the global database (GnomAD).

ADR in patient groups with different types of carcinomas are described. To comprehensively assess the clinical significance of genetic variants associated with the metabolism of the chemotherapeutics used, due to similarity in the chemotherapy regimens performed in the patients, and to meet the study objectives, participants were classified into seven groups:

1. Patients treated with platinum-containing chemotherapeutics (Oxaliplatin, Carboplatin, Cisplatin) - 35 patients;
2. Patients treated with pyrimidine analogues (5-Fluorouracil, Capecitabine) - 26 patients;
3. Patients treated with folinic acid (Leucovorin) - 21 patients;
4. Patients treated with EGFR inhibitors (Panitumumab, Cetuximab, Erlotinib) - 11 patients;
5. Patients treated with taxanes (Docetaxel, Paclitaxel) - 15 patients;
6. Patients treated with anthracyclines (Farmorubicin) - 8 patients;
7. Patients treated with ankylating agents (Cyclophosphamide) - 8 patients

In the analysis of data from the present work, single nucleotide polymorphisms in specific genes associated with drug metabolism of the chemotherapeutics used were found in more than one patient: *MTHFR*, *DPYD*, *CYP1B1*, *XPC*, *ABCG2*, *SLC22A2*, *SOD2*, *EGFR*, *ABCB1*, *ABCC2*, *GSTP1*, *ATM*, *SLCO1B3*, *TP53*, *XRCC1*, *ERCC2*. 23 germline pharmacogenetic variants were identified in 16 genes associated with the metabolism of chemotherapeutics leading to ADR: *MTHFR* c.1286A>C, *MTHFR* c.665C>T, *DPYD* c.2194G>A, *DPYD* c.1627 A>G, *DPYD* c.496A>G, *DPYD* c.85T>C, *CYP1B1* c.1294G>C, *XPC* c.2815C>A, *XPC* c.1496C>T, *ABCG2* c.421C>A, *SLC22A2* c.808T>G, *SOD2* c.47T>C, *EGFR* c.1562G>A, *ABCB1* c.2677 T>G, *ABCC2* c.1249G>A, *GSTP1* c.313A>G, *ATM* c.5557G>A, *SLCO1B3* c.334T>G, *SLCO1B3* c.699G>A, *TP53* c.215C>G, *XRCC1* c.1196A>G, *ERCC2* c.2251A>C, *ERCC2* c.934G>A. 3 germline pharmacogenetic variants in 3 genes (*SLC22A2* c.808 T>G, *DPYD* c.2194G>A, *XRCC1* c.1196A>G), and compared to the GnomAD healthy group, 4 germline pharmacogenetic variants in 4 genes were detected (*SLC22A2* c.808T>G, *DPYD* c.2194G>A, *XRCC1* c.1196A>G, *EGFR* c.1562G>A).

With a lower frequency among the patients compared to the group of 40 healthy subjects studied, 2 germline pharmacogenetic variants were detected in one gene (*XPC* c.2815C>A,

XPC c.1496C>T), and compared to the group of healthy subjects in GnomAD, only one germline pharmacogenetic variant was detected in one gene (*ERCC2* c.934G>A).

The following 6 germline pharmacogenetic variants in 6 genes were found with higher frequency in the Bulgarian population compared to the world population: *MTHFR* c.665C>T, *DPYD* c.2194G>A, XPC *c*.2815C>A, *EGFR* c.1562G>A, *XRCC1* c. 1196A>G, *ERCC2* c.2251A>C. The following 4 germline pharmacogenetic variants in 4 genes were found with lower frequency in the Bulgarian population compared to the world population: *MTHFR* c.1286A>C, *DPYD* c.85T>C, *ABCG2* c.421C>A, *ERCC2* c.934G>A.

Six conclusions are drawn and formulated accurately, which reflect the results and fully meet the objectives. I agree with the author's assessment of the contributions of the thesis, of which I find particularly valuable the identification in the Bulgarian population of the frequency of specific germline pharmacogenetic variants determining toxic effects of chemotherapy. This creates the prerequisites for the development of a genetic panel for patients with upcoming chemotherapy to select the right drug at the appropriate dose and minimal risk of ADR.

8. Evaluation of the publication related to disseration

In connection with the dissertation, the PhD student presents 3 publications written in English, of which he is the first author. Two of them have a high impact factor (IF 8.633; Q1and IF 1.4; Q4). One of them is from participation in a scientific conference. The presented scientific papers fully cover the dissertation topic and contain results of the conducted research. In addition, the dissertant has 6 poster presentations at the European Conference on Human Genetics in 2022 and 2023.

9. Personal participation of the PhD student

The PhD student was personally involved in collecting the data, summarizing, analyzing and describing it.

10. Abstract book

The abstract is 68 pages in length and reflects the main sections and results of the thesis. It is sufficiently informative and can be used as a source to familiarize oneself with the research results and the qualities of the dissertation

11. Critical remarks and recommendation

None.

12. Personal impressions and opinion

None.

13. Recommendations for future application of dissertation contributions and results

NGS technology has the potential to improve the discovery of new genetic variants associated with cancer, leading to better treatment decisions and different monitoring approaches. Analyzing a larger number of genes could allow the identification of rare variants specific to the Bulgarian population. The development of NGS panels tailored to the specific genetic profile of the Bulgarian population will allow the discovery of rare pharmacogenetic variants and the development of strategies to individualize therapy according to gene variants, optimize efficacy and minimize toxicity of drugs.

CONCLUSION

The dissertation work "Pharmacogenetic markers associated with drug metabolism in patients with oncological diseases" meets the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria (LADAB), the Regulations for the Implementation of the LADAB and the relevant Regulations of the Medical University - Plovdiv. The submitted materials and dissertation results fully comply with the specific requirements of MU - Plovdiv.

The work establishes the author as a responsible and reliable researcher who can independently conduct research and interpret the results obtained.

All this gives me a reason to give a positive assessment of the research conducted, presented by the above reviewed dissertation, abstract, results and contributions and I propose to the Honorable Scientific Jury to award the educational and scientific degree "Doctor" to Dr. Nely Nedyalkova Miteva-Marcheva in the doctoral program in "Medical Genetics"..

12 January 2022 Reviewer: .....

Sofia Prof. Savina Hadjidekova, MD, PhD