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REVIEW

REGARDING Competition for the occupation of the academic position **"Associate Professor"** in the field of higher education 4. Natural sciences, mathematics and informatics; professional direction 4.3 Biological Sciences, specialty "Medical Genetics" for the needs of the Department of "Medical Genetics", Faculty of Medicine (0.5) and the Laboratory of Medical Genetics at UMBAL "Sveta Marina" EAD - Varna (1.0) announced in SG No. 83/03.10.2023.

REVIEWER: Prof. Ludmila Angelova, MD, Ph.D., Head of the Medical Genetics Department; Specialty and scientific degree – Professor of Medical Genetics, MD; Medical University "Prof. Dr. Paraskev Stoyanov" Varna, Faculty of Medicine. Member of the Scientific Jury (SJ) under Order no P-109-515 / 30.11.2023 of the Rector of the MU – Varna; appointed as a Chairman of the SJ and internal reviewer according to Protocol No. 1/12.12.2023 of the Meeting of the SJ.

CANDIDATES: Maria Kostadinova Levkova, MD,PhD – only candidate
The candidate's documents are prepared in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), its regulation and the Regulations for the Development of the Academic Staff (PRAS) of the University of Varna.

I. Brief biographical and career data

- Born on 17.12. **1991** in the city of Varna
- In **2010,** she graduated from "First Language High School" Varna - with intensive study of English and German
- In **2016**, she graduated from the Medical University "Prof. Dr. Paraskev Stoyanov"-Varna master's degree, specialty "Medicine", *top of the class*
- In **2016 - 01. 2017** she worked as a "part-time assistant", discipline Medical Genetics, Department "Medical Genetics" of the University of Varna
- In February **2017**, she was appointed by law (PRAS-topper of the graduating class) as a full-time "assistant" in the Department of Medical Genetics of the University of Varna, and as a specialist in Medical Genetics in the Laboratory of Medical Genetics, UMBAL "St. Marina" EAD - Varna
- In **2018 - 2021,** she is a full-time doctoral student in the same department
- In **2021**, she majored in "Medical Genetics" and defended her PhD thesis.
- Since **2021** and currently working as a "chief assistant" with a specialty in Medical Genetics, in the Department of Medical Genetics, Medical University of Varna and as a physician specialist in Medical Genetics in the Laboratory of Medical Genetics, UMBAL "St. Marina"-Varna.
- Dr. Levkova is a member of the Bulgarian Society of Human Genetics and the European Society of Human Genetics (ESHG).

II. Research activity

The activity was evaluated in accordance with the minimum national requirements for ASSISTANT PROFESSOR based on Art.26 of ZRASRB dated 19.07.2022 and the regulations for its application, as well as the PRAS of the Varna University of Medical Sciences in force under Protocol No. 57/21.11.2022. The numbering of the peer-reviewed scientific categories follows the Academic reference for publications, citations and scientific profile of the candidate, prepared and certified by the Medical Library of the Medical University of Varna.

To have according to **indicator A (50 points)** an acquired educational and scientific degree **"doctor"** in the specialty of the announced competition; in the field of higher education 4. Natural sciences, mathematics and informatics; professional direction 4.3 Biological sciences, specialty "Genetics". The criterion is **met (50 points)** - successfully defended dissertation "Molecular-genetic and immunological biomarkers in the medical-genetic counseling of families with infertility" (Diploma No. 432 /20.05.2021, MU Varna). According to the Academic reference to the dissertation, 3 publications related to the subject of acquisition for the enrollment of the doctoral student (to May 2018) are presented; in the present procedure they are not subject to review. In all of them, the candidate is the lead author.

To have according to group **B indicators** – a minimum of 100 points obtained from habilitation thesis or equivalent scientific publications that are referenced and indexed in world-famous databases with scientific information (*Web of Science and Scopus*). Indicator B4 with a total of 107 points **fulfills** the criterion.The author has specified five up-to-date articles equivalent to a habilitation thesis, referenced in *Scopus and Web of Science*, four of them published in prestigious high-profile journals with *impact factor: Cancers, Biomedicines, Genes, Andrology.* An extended reference to the scientific contributions of these publications, prepared in accordance with item, is attached. An excellent impression is made by the leading or second place of co-authorship, as well as in-depth molecular genetic knowledge in published meta-analyses and reviews of socially significant diseases - infertility and carcinogenesis. I accept them as "Scientific activity, with scientific articles equivalent to a monograph, evaluated as "real publications... in foreign scientific journals" (PRAS of the MU Varna - Appendix 2, 1a), and not as "Scientific activity (PRAS of the MU Varna, Appendix 2, 2a)

To have according to group of **indicators G (G5-10)** a minimum of **200** points, as at least 60 of them to have been acquired from indicator **G7.** Criterion **met,** all by indicator **G7,** a total of **216** points. These are 15 scientific publications in full text, in publications, referenced and indexed in world-famous databases with scientific information (Web of Science and Scopus), 7 of them foreign (not only English-language) journals, incl. **4** with an **impact factor.** They are up-to-date (2019-2023) related to diverse areas of clinical genetics with the candidate's lead authorship. Two full text publications in scientific journals, beyond the minimum scientometric requirements are attached.

The candidate's summary publication activity of 22 *full-text* scientific works is analyzed according to:

- Language – 17 papers in English, 5 papers in Bulgarian.
- Type of Scientific Journal – refereed - 20 papers. Non-refereed - 2 items.
- Authorship – first author - 9 items, second author 6 items, third and subsequent 7 items.

These publications carry **an impact factor** of the journals in which IF 28.743 published them

Doctor Levkova is an active participant with various scientific events, published in *summary* in:

- 10 foreign forums, presents 27 reports/posters, incl. 13 pcs. in prestigious genetic journals with an IF impact factor (not featured)
- 8 national forums with international participation with 15 reports.

This scientific publication activity affects the citability of the data:

To have according to **indicators D (D11),** a minimum of **50** points from Citations in scientific publications, monographs and collective volumes and patents, referenced and indexed in world-famous databases with scientific information (*Web of Science and Scopus*). 50 points meet the criterion. From the search made only in Scopus and Web databases of Science in the Academic Reference from the Medical Library of the University of Varna is visible List of citations covering the minimum requirements for occupying the academic position "associate professor" dated 06.11.2023. It indicates **25 citations** of the candidate's articles. This mobile index is growing daily both due to the high interest in molecular genetic pilot studies, meta-analyses in the field of socio-communicable diseases, and due to the extreme relevance of the latest publications related to the era of next-generation sequencing. I would suggest looking for citations in Bulgarian sources that would also reflect the candidate's contribution as a representative of Bulgarian medical genetic diagnostics and clinical application.

An additional asset that *is not required* for the acquisition of the scientific title in the Higher Education Area 4 (therefore not currently reviewed) is the candidate's participation and experience as an administrative and financial officer in several national and institutionally funded research projects under European programs or through the Science Fund of the University of Varna.

Scientific achievements and contributions

Dr. Maria Levkova's scientific interests are focused both on molecular medicine and on the related medico-genetic counseling activity. To a significant extent, the scientific contributions have the theoretical character of meta-analyses and reviews with further development and enrichment of existing knowledge and methods; another part shows practical results, personal work (or leading co-authorship) of the candidate. Current scientific topics are presented in the publications and grouped in two main directions: highly specialized laboratory diagnostic activity of knowledge and application of modern genomic technologies, and multi-step process of medical genetic counseling, following strictly specific principles of the activity.

The reviewer, in the main fields of application, summarizes the scientific contributions:

1. Genetic studies of common, socially significant (complex) diseases
   - Reproductive genetics
   - Cancer genetics
   - Genetic predisposition to vascular disorders
   - Intellectual disability and autism spectrum
2. Genetic (laboratory and consultative) diagnostics in clinical practice

Regarding genetic studies of socially significant diseases

- *Reproductive genetics*

Dr. Levkova defends her PhD thesis "Molecular-genetic and immunological biomarkers in the medical-genetic counseling of families" in 2021. It reflects a serious innovative approach from molecular medicine in Bulgarian patients with male and female infertility (5T variant in the CFTR gene, Y microdeletions, polymorphic variants in regulatory genes of the immune system - the HLA-G gene and the TNF-alpha gene.

The continuation of this scientific interest in reproductive genetics was followed by serious studies related to the evaluation of molecular genetic mutations and polymorphic variants in men with impaired spermatogenesis. For the first time among Bulgarian patients, the gr/gr microdeletion in the Y chromosome was also investigated, which shows, albeit in a limited sample, that this variant is probably not related to impaired spermatogenesis, but rather is a polymorphic marker without clinical significance **(G7.4.).** The importance of microdeletions in the AZFa, AZFb and AZFc regions of the Y chromosome as a cause of reduced sperm concentration in the ejaculate is confirmed, which necessitates their search in patients with azoospermia and severe oligozoospermia (less than 1×106/ml). Original article presents the pilot study and meta-analysis demonstrating the IVS8-5T variant in CFTR as a risk factor for impaired spermatogenesis (OR = 2.84, $p < 0.05$) **(G7.12.).** In this regard, the need to evaluate molecular genetic mutations and polymorphic variants in the CFTR gene in men with non-obstructive azoospermia and severe oligozoospermia (less than 1×106/ml) has been confirmed. Dr. Levkova performed *a systematic review* and *meta-analysis* of information on the carriage of pathogenic variants, discovered and described 15 genes responsible for the production of structural components of dynein and case-control dynein-assembly factors that may contribute to male infertility. The genes examined in this study have been proposed as suitable targets for research into the etiology of sperm motility problems **(B4.3).**

Through research in the Genetic Research Registry maintained by the National Center for Biotechnology Information (NCBI), for the first time an analysis of the applied target panels of genes associated with male sterility in impaired spermatogenesis **(B4.5.)** was conducted. The 11 established laboratories offering targeted sequencing for male infertility tested 230 different genes; only the CFTR gene was identified, and for 65 (28.26%) there is no reliable data regarding their role in impaired spermatogenesis. In the absence of formal working protocols, clinicians are advised to use a stepwise approach of karyotype testing, Y microdeletions and testing with a targeted panel of genes in the second step of the analytical process. Results of a large-scale study on the significance of chromosomal polymorphisms among 1733 patients with reproductive disorders **(G7.11.).** A high statistical significance of a higher proportion of chromosomal polymorphisms was reported in patients with reproductive failures compared to controls; a detailed analysis was performed by sex, type of disorder and type of chromosomal polymorphism.

- *Genetics of colorectal carcinoma*

A systematic review was conducted to present the characteristics of circular RNAs (circRNAs) and their role as potential prognostic and diagnostic biomarkers in Colorectal Carcinoma. It is established that, depending on their localization, they perform different biological functions, including regulation of the expression of various genes, transcription and modulation of alternative splicing **(B4.1.).** Data are revealed for over-activated circular RNAs with oncogenic functions (stimulation of cell proliferation, invasion/migration, and metastasis) that simultaneously suppress apoptosis, as well as circular RNAs possessing more than one oncogenic function. Not only the potential role of oncogenic circular RNAs in the pathogenesis of CRC and their use as suitable new drug targets in the personalized treatment of CRC patients is indicated, but also the need to search for

new, more reliable biomarkers for early diagnosis, metastatic prognosis and resistance to conventional therapies as a huge challenge to modern medical practice.

A study investigating different SNPs in relation to colon cancer risk, prognosis and response to treatment is related to the data of different meta-analyses and the possible reasons for the contradictions in the results of different research groups. It summarizes the need for large-scale case-control studies involving participants of different ethnic origins to establish the role of miRNAs in the etiology of CRC **(C4.2.).** According to own results, three miRNA - SNPs - miR-146a rs2910164, miR-27a rs895819 and miR-608 rs 4919510 – show a promising role as prognostic and diagnostic biomarkers for CRC. Their participation in the control of basic cellular processes such as proliferation, differentiation, migration, angiogenesis and apoptosis makes them attractive objects for new therapeutic approaches, and their secretion in extracellular fluids is a suitable biomarker for evaluating the progression and metastasis of tumors.

- *Genetic aspects of vascular disorders*

For the first time, a meta-analysis was conducted in our country to clarify the role of hereditary predisposition to thrombophilia in the etiology of ischemic strokes in young people, in relation to factor V Leiden (FVL), factor II , prothrombin (PT), protein C (PC), protein S (PS), antithrombin (AT) in international databases of a total of 104 studies **(B4.4.).** All investigated markers, FVL, PT, PC, and PS and AT, were significantly associated with an increased risk of ischemic stroke in young people. A genetic panel for congenital thrombophilia is recommended to be included in the routine testing of young stroke survivors in order to improve the quality of care for these patients. Subgroup analyzes performed according to geographic location, sex ratio, and selection criteria did not identify significant differences between groups, but different geographic location was assessed as a likely source of the heterogeneity found.

The role of polymorphic variants for hereditary predisposition to thrombophilia - factor V (FV) Leiden G1691A, factor II (F II) G20210A, plasminogen activator inhibitor (PAI) 4G/5G and methylenetetrahydrofolate reductase (MTHFR) C677T in patients with repeated spontaneous abortions is studied. It is established that patients with a history of vascular disorders have a significantly higher frequency of the FV Leiden variant; it remains the strongest risk factor among those studied for vascular disorders and recurrent miscarriage **(D7.1.)** There was no statistical difference between the analyzed patients regarding the other three polymorphic markers.

- *Genetic aspects of intellectual disability and the autistic spectrum*

For the first time in our country, a molecular-genetic selective screening for fragile X syndrome was conducted among 52 high-risk children with intellectual disability/developmental delay/autistic behavior; syndrome in 5.7% of cases **(G7.9.).** It illustrates the role of screening programs in making the correct diagnosis and medical genetic counseling of the family easier.
A systematic review of the role of the gut microbiome in the etiology of autism spectrum disorders identified the ten bacterial genera for which there is the most available literature evidence to be significantly different between studied autistic patients and control subjects **(G7.15.).** The genera Bacteroides, Bifidobacterium, Clostridium, Coprococcus, Faecalibacterium, Lachnospira, Prevotella, Ruminococcus, Streptococcus and Blautia show the most evidence that their fluctuations in the gastrointestinal tract are associated with the etiology of autism. It is likely that

both increased levels of harmful bacteria and decreased levels of beneficial bacteria affect the symptoms of autism.

Regarding genetic (laboratory and consultative) diagnostics in clinical practice

- *Laboratory*

For the first time in our country, the results of a large-scale cytogenetic study of 1,781 children with suspected chromosomal pathology are described and analyzed, based on the data for a 10-year period of time (**D7.10.**). The revealed pathological karyotype in 15% of them show that cytogenetic analysis has an important role in the diagnostic process in detecting mosaicism and balanced rearrangements. Even in the presence of an indisputable clinical phenotype, laboratory evidence may also reveal an unexpected finding of a chromosomal aberration (**G7.5.**). The leading role of comparative genomic hybridization is the diagnosis of children with unexplained retardation in intellectual development and autistic behavior **(G7.8.).**

Clinical cases of rare diseases -the contributions related to clinical cases, the conducted overview analyzes, international consensuses regarding frequency, pathogenesis, and diagnostic difficulties are described in a propaedeutic way. Publications include Kabuki syndrome (**D7.2.**), Bardet-Biedl syndrome (**D7.6.**),congenital myasthenic syndrome **(D7.14.),** Cornelia de Lange (**D7.7.**).

- *medical genetic counseling (MGC)*

The essence of medico-genetic counseling as a diagnostic process was examined; the review is aimed at general practitioners (**D7.3.**) due to the need for knowledge in the detection of rare diseases related to the advancement of genomic technologies. Important patient cases are presented in which next-generation sequencing methods reveal variants with so-called VUS - unclear clinical significance in genes relevant to the clinical picture of patients. It creates consultative problems and raises the question of when they can be discussed as a cause of the observed genetic condition (**D7.13.**). Other publications illustrate the challenges of MGC in families with rare, fatal genetic diseases and the need for biobanking (add. publication). As an active form of medico-genetic counseling, an innovative survey of 500 women of childbearing age about awareness of genetic prevention approaches offered during pregnancy is of interest (add. publication).

III. Educational - teaching activity

Dr. Levkova's total assistant experience is 6 years and 8 months at the time of submitting the documents (currently 7 years). She was appointed (topper of the graduating class) as a full-time "assistant" in the "Medical Genetics" Department of the Medical University of Varna in February 2017; and chief administrative assistant in 2021. For the period of 4 active academic years (in the last 2 she was on maternity leave), she covered and exceeded the specified horarium of *academic hours of* practical *seminars* in the discipline "Medical Genetics". These were **216 to 276** hours per yeasr for different specialties: Medicine (BEO and AEO), Pharmacy, "Midwife", "Nurse", "Medical Laboratory Technician" (Attached reference from the Deputy Rector for Academic Activities of Varna University). From the beginning of the 2023/2024 academic year, she was included in *the lecture* course for Bulgarian and Engtlish speeking medical students.

The candidate actively participates in the development and implementation of course topics, knows and uses the Blackboard platform for on - line teaching, developing presentations with animated videos for this purpose. Dr. Levkova has been *an administrative* assistant since 2021.

IV. Diagnostic (laboratory and consulting) activity

The candidate has a total work experience of 6 years and 8 months at the time of submission of documents (currently 7 years), and all her professional experience is in *the field of Medical Genetics.* From her admission, she worked as a trainee, later as a specialist in Medical Genetics in the Laboratory of Medical Genetics, UMBAL "St. Marina" EAD - Varna. During her short work experience, the candidate actively participated in both laboratory and medical genetic counseling activities.

- In 2021, she acquired a specialty in Medical Genetics (Certificate of a recognized specialty registration no. 024861 / 07.01.2021 Series of Medical Genetics – 2021)
- In the same year, 2021, he successfully passed the exam for Medical Genetics and Genomics for qualification in European countries (European Board in Medical Genetics (EBMG) The European Certificate in Medical Genetics and Genomics (ECMGG).

The candidate specialized in Bertinoro, Italy (28th Course in Medical Genetics, 2016); Varna IVth , VIth (ISPAD Postgraduate Course "Changing Diabetes "; Diabetes and rare diseases, 2017,2021); Athens, Greece (Basics in Human Genetic Diagnostics – A Course for CLGs in education, 2017); Giessen, Germany (6th International Workshop, Molecular Andrology, 2019); Bertinoro, Italy (33rd Course on Clinical Genomics and NGS, Bertinoro, 2021); remotely in Virtual workshop: MLPA Raw Data Evaluation and Troubleshooting, (2021); ESHG Digital Course on Hereditary Cancer, (2021).

SUMMARY OF THE ACTIVITIES OF MARIA LEVKOVA, MD, PhD

Scientific - I present a synthesized table of indicators for occupying the academic position of ASSOCENT PROFESSOR in PRAS at the University of Varna (2018, add. 11.2022), in accordance with the criteria of the Regulations for the implementation of ZRASRB for Minimum national requirements for the scientific and teaching activities of candidates in the Professional direction 4.3. Biological sciences (Appendix SG, No. 15, 2019, supplement. 07. 2022.)

A group of metrics	Indicator	Number of points under ZRASRB	Minimum requirements under ZRASRB	Points (accordir to the reviewer
A	1 . Dissertation work for the award of the ONS "Doctor"	50	**50**	**50**
IN	4 . Habilitation work - scientific publications = **scientific publications** in publications that are referenced and indexed in world-famous databases with scientific information (Web of Science and Scopus)	25 for publ. in Q1; 20 for publ. in Q2; 15 for publ. in Q3; 12 for publ. in Q4; 10 for publ. in edition with SJR without IF	**100**	**107**
D	7. Scientific publications, in publications referenced and indexed in world-famous databases with scientific information (Web of Science and Scopus)	25 for publ. in Q1; 20 for publ. in Q2; 15 for publ. in Q3; 12 for publ. in Q4; 10 for publ. in edition with SJR without IF	**200 (≥ 60 points from indicator 7)**	**216**
D	**11.** Citations in scientific publications, monographs and collective volumes and patents referenced and indexed in world-renowned databases of scientific information (Web of Science and Scopus)	2 per citation number	**50**	**50 (25 pcs)**

Teaching activity - as an assistant since February 2017, (administerred to the department as a topper of the class, and chief administrative assistant (2021), the candidate shows persistence and responsibility in her activities. She fulfills and exceeds the academic horary (252 study hours on average); defends a dissertation for the acquisition of an educational and scientific degree "Doctor" on time with criteria fulfilled ahead of schedule (report according to Attestation cards).

Clinical activity - an important asset for the candidate's competences is the specialty in Medical Genetics (2021) and a recognized qualification in Medical Genetics and Genomics in European countries (The European Certificate in Medical Genetics and Genomics (2021). I appreciate the hospital activity (laboratory analyzes and genetic consultations) of the candidate as a highly specialized clinical activity in the *Medical Genetics specialty*, regardless of the criteria of Professional Direction 4.3 Biological Sciences.

My **personal impressions** of the candidate, as an assistant and doctoral student in our department, are related to consistency and purposefulness, accurate scientific language and analyst, loved by students, teacher, respected colleague in our team.

CLOSING

Dr. Levkova has excellent professional training, in-depth scientific knowledge and volume of research production, teaching experience and practical skills in the field of Medical Genetics, proven in a short period. She, unconditionally, fulfills all state requirements for holding the position for which she is applying.

Evaluating all this, I find it reasonable to give a positive review and to propose to the respected members of the Scientific Jury to award Dr. Maria Kostadinova Levkova, db, the academic position of "Docent" in the field of higher education 4. Natural sciences, mathematics and informatics; professional direction 4.3 Biological Sciences, specialty "Medical Genetics".

Evaluating all of this, I find it reasonable to give a positive review and to propose to the respected members of the Scientific Jury to award Dr. Maria Kostadinova Levkova, PhD, the academic position of "Docent" in the field of higher education 4. Natural sciences, mathematics and informatics; professional direction 4.3 Biological Sciences, specialty "Medical Genetics"

16.02.2024 **REVIEWER:**

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

/PROF. L.ANGELOVA, MD, PhD/