REVIEW

from Acad., Prof. Dr. DRAGA IVANOVA TONCHEVA - MITEVA, PhD, DSci, MU Sofia, elected as a member of the scientific jury by order of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, in connection with an announcement in the Official Gazette, no. 83/03.10.2023 competition for the academic position "Associate Professor" in the specialty "Medical Genetics", professional direction 4.3. Biological sciences, field of higher education 4. Natural sciences, mathematics and informatics, in a full-time position for the Laboratory of Medical Genetics at UMBAL "Sveta Marina" EAD - Varna.

Common part

Dr. Maria Kostadinova Levkova was admitted to participate in the announced competition for associate professor. The review of its documents showed that the procedure for disclosure and announcement of the competition was followed. The materials for the competition have been prepared in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its Application at UMBAL "Sveta Marina" EAD - Varna.

Brief biographical data

Dr. Maria Kostadinova Levkova was born in the city of Varna. She graduated from the "First Language High School" in 2010, studying English and German languages. In 2016, she obtained an educational and qualification degree of Master of Medicine at the Medical University "Prof. Dr. Paraskev Stoyanov", Faculty of Medicine in the city of Varna (Diploma, Registration No. 004258/11.11.2016). Successfully defended a dissertation on the topic "Molecular genetics and immunological biomarkers in the medico-genetic counseling of families with infertility" as a full-time doctoral student at the Department of "Medical Genetics" MU - Varna from 2018 to 2021 (Diploma No. 432/20.05.2021).

Her professional path began as a specialist in Medical Genetics in the Laboratory of Medical Genetics, UMBAL "St. Marina" - Varna (2017-2021) and as a medical specialist with an acquired clinical specialty in "Medical Genetics" from 2021 (Certificate of recognized specialty #4606, Registration #024861/01.07.2021).

She went through all stages of development from "part-time assistant" in the Department of Medical Genetics at the MU "Prof. Dr. Paraskev Stoyanov", city of Varna (2016 - 01.2017), then assistant (2017-2021) and chief assistant from 2021.

Specializations and professional skills

Dr. Maria Levkova is a specialist in medical genetics, with a high qualification, which she further develops by participating in numerous short-term courses for postgraduate training, on novelties in rare genetic diseases and hereditary predispositions, diagnostic approaches, clinical genomics, etc.:

- "Medical Genetics", Remote Training Centre, MU Varna, Bertinoro, Italy (2016);
- IV ISPAD Postgraduate Course "Changing Diabetes", Varna (2017);
- Mucopolysaccharidosis II School, Varna (2017);
- Basis in Human Genetic Diagnostics – A course for CLGs in education, Athens, Greece (2017);
- 6th International Workshop, Molecular Andrology, Giessen, Germany (2019);
- 33rd Course on Clinical Genomics and NGS, Bertinoro (2021)
- Virtual workshop: MLPA Raw Data Evaluation and Troubleshooting (2021)
- ESHG Digital Course on Hereditary Cancer (2021)
- VIth ISPAD/VAPES Postgraduate Course and Meeting "Diabetes and rare diseases" (2021)

Dr. Maria Levkova successfully passed the exam for the European Certificate in Medical Genetics and Genomics (ECMGG - European Certificate in Medical Genetics and Genomics) (2021) and for the clinical specialty in Medical Genetics, MU Varna in the same year.

Dr. Levkova possesses prominent qualities for scientific activity and is a desirable researcher who participates in the implementation of scientific tasks under projects of the University of Varna: project #18011 "Study of molecular genetic factors in the CFTR gene in men with primary infertility"; project #18011 "Molecular-genetic analysis in children with unexplained rare genetic diseases"; project #19035 "Study of balanced subtelomeric rearrangements by molecular cytogenetic analysis in patients with combined reproductive problems"; project No. 19024 "Identify the reasons for the high epidemiological activity of chronic kidney disease in the municipality of Dulgopol, region Varna"; project #BG-RRP-2.004-0009-CO2 in two

scientific groups on "Application of innovative technologies for the management of oncological and rare diseases" and "Increasing translational achievements in medicine".

Scientific works

General review of scientific works

Chief assistant Dr. Maria Levkova, PhD participated in the competition for associate professor with 22 publications (17 in English and 5 in Bulgarian). She is the lead author in nine, second - in six and next co-author in 7 articles. Of the total number of articles, 20 were published in refereed journals (Web of Science, Scopus) and 2 in non-refereed ones. The total impact factor (IF) of the journals in which the candidate has publications is 28.743. The articles are from the last 5 years and have been cited 25 times in scientific publications. It is likely that this citation index will increase in the coming years.

The academic report on the publications, citations and scientific profiles of Dr. Maria Levkova, prepared by Mrs. Rumyana Radeva, Director of the Library, proves that her data exceed the minimum indicators for occupying the academic position of "associate professor" in field 4. Natural sciences, mathematics and informatics.

Dr. Levkova participated in 37 international and national scientific events. She has presented the results of her research at 22 international congresses/conferences: 51st European Society of Human Genetics (ESHG) Conference, June 16-19, 2018, Milan, Italy (3); 53rd European Society of Human Genetics (ESHG) Conference, June 6–9, 2020, Virtual Conference (3); 54th European Society of Human Genetics (ESHG) Conference, June 12–15, 2021, Virtual Conference (4); 25 International Conference on Prenatal Diagnosis and Therapy, 6-8 June 2021, Virtual Conference (3); 55th European Society of Human Genetics (ESHG) Conference, June 11-14, 2022, Vienna, Austria, Hybrid conference (2); 26th International Conference on Prenatal Diagnosis and Therapy, 19-23 June 2022, Montreal, Canada, Hybrid conference (1); 12th Balkan Congress on Human Genetics, 8-10 Sept 2017, Plovdiv, Bulgaria (8); Nephro Update 2019, 20.09.-22.09.2019, Prague (1); 6th International Workshop Molecular Andrology, 24-26 September, Giessen, Germany 2019 (1); 13th European Cytogenomics Conference (ECC), 3 – 5 July 2021, Virtual Conference (1).

Dr. Levkova participated in 15 national scientific events with reports/posters: Fifth Black Sea symposium for young scientists in biomedicine (BSYSB), April 6-9, 2017, Varna, Bulgaria (1); Sixth Black Sea symposium for young scientists in biomedicine (BSYSB), April 12-15, 2018, Varna, Bulgaria (3); XIV National Congress of Pediatrics (with international participation), 27-

30.09.2018, Borovets, Bulgaria (1); X National Conference on Rare Diseases and Orphan Medicines, 13-15.09.2019, Plovdiv, Bulgaria (2); Scientific and practical conference "Day of Rare Diseases in Bulgaria", February 28-29, 2020, hl-l Metropolitan, Sofia (2); XI National Conference on Rare Diseases and Orphan Medicines, September 11-12, 2020, Plovdiv, Bulgaria (2); 30th Annual Assembly of IMAB and 7th Annual Meeting of Alumni Club at Medical University Varna, 18 – 21 October 2020, Varna, Bulgaria (3); XII National Conference on Rare Diseases and Orphan Drugs, September 10-11, 2021, Plovdiv, Bulgaria (1).

Evaluation of scientific contributions

Dr. Levkova's scientific contributions are in the following four main areas: Genetic factors for impaired reproduction; Pediatric Genetics and Dysmorphology; Neurogenetics and Medical Genetic Counseling.

Genetic factors for impaired reproduction

Dr. Levkova's scientific interests are focused on a very important and socially significant problem of the role of molecular genetics and immunological biomarkers in infertility of women with recurrent miscarriages (research on the 14 bp insertion/deletion variant in the *HLA-G* gene and on -308 GA variant in the *TNF*-alpha gene) and in men with impaired spermatogenesis (Y microdeletions, delF508, R117H mutations and 5T variant in the *CFTR* gene).

This issue is explored in her dissertation. The obtained results from cases/controls have a contributing character and significance for the medico-genetic counseling of families with impaired reproduction. Among the spectrum of divergent data on the incidence of Y microdeletions worldwide and nationally, Dr. Levkova's data confirm the role of microdeletions in the AZFa, AZFb, and AZFc regions of the long arm of the Y chromosome as a cause of reduced ejaculate sperm concentration in carriers them A significant difference between the control and experimental groups was demonstrated in the distribution of genotypes for the IVS8(n)T variant in the CFTR gene. Dr. Levkova's results do not prove a role for the -308 GA variant in the TNF-alpha gene as a risk factor for spontaneous abortions, but reveal an association of a 14 bp insertional variant in the HLA-G gene with spontaneous abortions. The positive results of the study show that certain molecular markers could be used in the medico-genetic consultation of patients with reproductive problems.

Research into the causes of impaired spermatogenesis has been continued through funding under project #18011 "Study of molecular genetic factors in the CFTR gene in men with

primary infertility". The results are reflected in three publications (G7.12, G7.11, G7.4) A potential marker 5T variant in the CFTR gene was revealed. A meta-analysis has confirmed a role of the IVS8 - 5 T variant in sperm production disorders. Evidence is provided that the gr/gr microdeletion in the Y chromosome is a polymorphic variant of no clinical significance.

Pediatric genetics and dysmorphology

Dr. Levkova has interesting achievements and the largest number of publications in the field of medical genetics in pediatrics (Г7.9, Г7.10, Г7.2, Г7.6, Г7.7, Г7.8, Г7.5, Г7 .15). Rare genetic diseases (Kabuki syndrome, Bardet-Biedl syndrome, Cornelia de Lange syndrome, patient with double aneuploidy - trisomy 21 and three X chromosomes, 16p11.2 duplication syndrome) are defined, their phenotypes and approaches to diagnostics.

The obtained results have both a scientific and a scientific-applied nature. A reasoned proposal is made for selective screening for fragile X syndrome (FXS) among high-risk children with intellectual disability/developmental delay/autistic behavior. On the basis of a summary analysis of 10 years of results of chromosomal studies of children with a phenotype suspicious for chromosomal pathology, a high frequency of pathological karyotypes (15%) has been demonstrated.

Of particular note are the results of a meta-analysis of a total of 34 studies of the gastrointestinal microbiome in children with autism spectrum syndrome (Human Fertility. 2021 Mar 6:1-0., IF 2.161, Q3). Reliable data have been obtained on the association of bacterial genera Bacteroides, Bifidobacterium, Clostridium, Coprococcus, Faecalibacterium, Lachnospira, Prevotella, Ruminococcus, Streptococcus and Blautia with the etiology of autism. It is suggested that future research should continue to determine how their levels of variation affect clinical aspects of autism.

Neurogenetics

A patient with a rare genetic disease - congenital myasthenic syndrome (CMS) is described, with a detailed clinical profile of the disease, which unfolds gradually over time and with good long-term therapeutic control of symptoms. The need for a personalized approach when working with patients with rare diseases is emphasized (Journal of the American Association of Nurse Practitioners, pp.10-1097, IF 1.495, Q2).

The results of a meta-analysis of 104 studies to clarify the role of hereditary predisposition to thrombophilia in the etiology of ischemic strokes in young people have a high scientific and practical value (Genes, 2022, 13, 2081., IF 4.141, Q2). Results prove that the studied genetic

markers are risk factors for ischemic stroke (FVL OR = 1.74; PT OR = 1.95; PC OR = 10.20; PS OR = 1.74; AT OR = 3.47; $p < 0.05$). They are proposed with a preventive purpose as biomarkers for research in young people at increased risk of ischemic stroke.

Medical-genetic counseling

The issue of the diagnostic and predictive value of VUSs is very important and numerous research groups are working on their clinical characterization. New NGS technologies often reveal variants for which there are conflicting data on their clinical significance.

Dr Levkova analyzed the role of variants of unclear significance (VUS) in three rare diseases: Charcot-Marie-Tooth type 1C, familial Mediterranean fever and Wilson's disease. In the first case, a VUS was detected in the *LITAF* gene, in the second case, a VUS was determined in the *MEFV* gene, and in the third patient, two heterozygous variants were detected in the *ATP7B* gene. Dr. Levkova's opinion is that VUSs could have an etiological role if they are located in a gene associated with a disease clinic and there is a strong genotype/phenotype correlation (Journal of the American Association of Nurse Practitioners. 2022 Jun 22; 10-1097, IF 1.370, Q2).

Patients with rare diseases often do not reach a genetic diagnosis, which is a major problem for the genetic counselor. Biobanking of samples from undiagnosed deceased patients are rare genetic diseases, can solve problems of medico-genetic counseling of their relatives.

Scientific and scientific-applied contributions

- ✓ A molecular genetic cause of impaired spermatogenesis associated with variant IVS8 - 5 T in the *CFTR* gene has been proven;
- ✓ Microdeletion gr/gr in the Y chromosome is defined as a polymorphic marker without clinical significance;
- ✓ The role of microdeletions in the AZFa, AZFb and AZFc regions of the Y chromosome as a cause of reduced sperm concentration has been confirmed;
- ✓ Chromosome disorders and variants of chromosomal polymorphisms were determined in patients with reproductive disorders;
- ✓ A 14 bp insertion in the *HLA-G* gene was found to be associated with a risk of spontaneous abortion. Polymorphism - 308 GA in the *TNF* - alpha gene does not increase the risk of pregnancy termination;
- ✓ Fragile X chromosome has been confirmed to be diagnosed with a high frequency (15%) among children with intellectual disability/developmental delay/ and autistic behavior;

- ✓ Phenotypic variations of rare genetic syndromes have been proven in Bulgarian patients, which illustrate the need to develop an international consensus for the given disease;
- ✓ Clinical heterogeneity of a rare clinical case of congenital myasthenic syndrome is described, which helps in the differential diagnosis with other diseases;
- ✓ Biomarkers have been proposed for research in young people with an increased risk of ischemic stroke with a preventive goal;
- ✓ It has been hypothesized that variants of unclear significance (VUS) could have an etiological role if they are located in genes associated with clinical disease.

Teaching experience

Dr. Levkova has a teaching experience of 6 years and 08 months. 12 days, calculated as of 25.10.2023 (04 years, 09 months, 11 days as an assistant in the Department of Medical Genetics, Varna University and 01 years, 11 months, 01 days as a chief assistant in the same department). She conducted exercises for medical genetics students in various specialties: medicine (BEO and AEO), pharmacy, midwives, nurses, medical laboratory assistants.

Her study load is, respectively, by year: 275 hours (2017-2018), 242 hours (2018-2019), 216 hours (2019-2020) and 276 hours (2020-2021). She was on maternity leave for the next two years.

Conclusion

Dr. Maria Kostadinova Levkova is a bright creative person. She has remarkable scientific and scientific-applied achievements, reflected in prestigious international journals, with an impact factor with results cited. Dr. Levkova applies innovative technologies in her research. She implements inovative methods in clinical genetics approaches for phenotyping, molecular diagnostics, genetic methods for the analysis of hereditary diseases and predispositions.

Her participation in a large number of projects is respectable.

Professionally, she is a university teacher of medical genetics to students of various specialties, with wide erudition and modern knowledge obtained in international courses.

Dr. Levkova meets all the criteria for holding the academic position "Associate Professor". She has a defended doctoral dissertation, serious publications in international journals with an impact factor, has achieved significant results of a scientific and scientific-applied nature, has original contributions in various fields of medical genetics, etc.

Based on these data, I strongly recommend to the respected Scientific Jury to award Dr. Maria Kostadinova Levkova the academic position of "Docent" in the specialty "Medical Genetics", professional direction 4.3. Biological sciences, field of higher education 4. Natural sciences, mathematics and informatics, in a full-time position for the Laboratory of Medical Genetics at UMBAL "Sveta Marina" EAD - Varna.

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Academician, Prof. Dr. Draga Toncheva, PhD, DSci