To the Chairman of the Scientific Jury,

appointed with Order No. №P-109-362/28.10.2024

of the Rector of the Medical Univerisity of Varna

and in accordance with Protocol № 1/4.11.2024

REVIEW

By assoc. prof. Mira Valentinova Siderova, MD, Ph.D.

Head of Clinic of Endocrinology and metabolic diseases,
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on the dissertation work

of Dr. Nikolinka Yordanova Petkova, a full-time doctoral student, on the topic "Metabolic risk and body composition in children born small for their gestational age due to existing genetic syndromes (Prader-Will, Silver-Russell and others)" for the award of the educational and scientific degree "doctor" in the professional field 7.1. Medicine, under the doctoral program "Pediatrics"
with scientific supervisor: Prof. Dr. Violeta Mihoha Yotova, PhD, DSc

1. General presentation of the procedure

The presented set of materials complies with the requirements of the procedure for acquiring the educational and scientific degree "doctor" according to the Regulations of MU - Varna and includes all necessary documents.

2. Brief biographical data of the doctoral student

Dr. Nikolinka Yordanova was born in 1990 in the city of Yambol, where in 2009 she completed her secondary education at the Atanas Radev Mathematics High School. In 2015 she graduated as a Master of Medicine at the Medical University - Varna. In 2016 she began specialization in pediatric endocrinology and metabolic diseases at the 1st Pediatric Clinic of the University Hospital "St. Marina", Varna, and in 2020 she successfully acquired a specialty. In 2019, she won a competition for a full-time doctoral student at the Department of Pediatrics at MU - Varna, with scientific supervisor Prof. Dr. Violeta Yotova. The doctoral student's clinical and scientific interests are in the field of rare endocrine diseases. She is the head of a multidisciplinary team for the care of patients with Prader-Willi and Silver-Russel syndrome at ECREB (Expert Center for Rare Endocrine Diseases), which is part of the European Reference Network for Rare Endocrine Diseases (Endo-ERN). Since

2020, she has been a part-time assistant at the Department of Pediatrics at MU-Varna. In 2022, she conducted a one-month specialization at Erasmus MC Sophia Children's Hospital, Rotterdam, the Netherlands in the field of specific care for patients with rare imprinting syndromes (Prader-Willi and Silver-Russell), funded by the European Reference Network for Rare Endocrine Diseases (Endo-ERN). Three-time recipient of grants from the International Prader-Willi Society (IPWSO) to attend international congresses in Europe and America (Cuba in 2019, Ireland in 2022 and Arizona in 2025). Member of BPA, BNSDE, VAPED and ESPE.

3. Topic relevance

Prader-Willi syndrome is a rare endocrine condition (incidence 1:20,000) characterized by marked muscle hypotonia in the neonatal period, excessive weight gain due to lack of satiety after 3-4 years of age, short stature, hypogonadism, and psychiatric abnormalities. Patients with Silver-Russell syndrome (incidence 1:30,000 to 1:100,000) are born small for their gestational age, do not catch up in growth postnatally, and have characteristic dysmorphism and body asymmetry.

The two conditions are linked by certain mechanisms of occurrence of the genetic defect. In both cases, genomic imprinting is involved. Other common features are low birth weight and the development of early metabolic complications. In patients with Silver-Russell syndrome (SRS), dependencies characteristic of the fetal hypothesis are observed - low birth weight is associated with an increased risk of cardiovascular diseases, hypertension, dyslipidemia, obesity, insulin resistance and type 2 diabetes mellitus. A lower percentage of muscle mass has been established in both syndromes compared to healthy controls. Treatment with recombinant human growth hormone (rhGH) in both groups of patients leads to an increase in the percentage of muscle mass, and to date, no published study has been found to establish a correlation between the reduced amount of muscle mass and the metabolic profile in these patients. In recent years, the concept of improving clinical care and knowledge in the field of rare diseases (such as both syndromes) has become an increasingly interesting and widespread part of modern science and practice. With the establishment of reference networks for rare endocrine diseases on a European and global scale, knowledge about rare diseases is increasing in the direction of improving care for these patients and increasing their duration and quality of life. These facts emphasize the relevance of the dissertation work.

4. Evaluation of the dissertation work

The dissertation is written on 158 pages, illustrated with 30 tables and 8 figures and is well structured in the following main sections: Title page (1 page); Table of contents (2 pages); Abbreviations used (3 pages); Introduction (1 page); Literature review (37 pages); Prerequisites (1 page); Aim and objectives (1 page); Materials and methods (10 pages); Results (27 pages); Discussion (25 pages); Conclusions (2 pages); Contributions (1 page); Conclusion (2 pages); References (20 pages). The bibliography contains 382 titles, of which 11 in Bulgarian and 371 in English.

5. Structure of the dissertation

The literature review is very well structured and includes two subsections with up-to-date information on the main characteristics of the two syndromes, covering the following topics: frequency, historical data, etiology and diagnostic methods, facial dysmorphism, causes of mortality, endocrine features, quality of life and psychosocial aspects. In patients with Prader-Willi (PWS), the mechanisms of hyperphagia and the characteristic type of obesity are described in detail, and in Silver-Russell (SRS) - belonging to the small for gestational age (SGA) cohort as an explanation for the features of their metabolic profiles. The standard metabolic indicators and body composition of both groups of patients relevant to future cardio-metabolic risk, as well as some adipomyokines, are examined. The doctoral student knows the state of the problem very well and has presented a creative assessment of the analyzed literary material.

The goal of the dissertation is clearly formulated, namely to assess the cardiovascular and metabolic risk in patients with PWS and SRS depending on their fat and muscle mass. To achieve this goal, 4 specific tasks have been set, well formulated and feasible to implement.

Participants and methods:

For the purposes of this dissertation, Dr. Yordanova conducted two parallel cross-sectional case-control sub-studies. A total of 25 patients with genetically proven PWS and 18 with genetically proven or clinical SRS were included, as well as 39 healthy controls, matched by gender, age and partly by BMI. The number of patients is sufficient considering the low frequency of both diseases and is subject to statistically valid conclusions. The inclusion and exclusion criteria are adequate for the purpose and design of the study. The clinical, laboratory, instrumental and questionnaire research methods are optimally selected. Adequate statistical analysis was used.

Results and discussion:

The results of the study are presented correctly separately for the two syndromes in 5 sections, well illustrated in tabular and graphical form, accompanied by adequate and objective commentary.

The first section presents the demographic, auxological and clinical characteristics of the participants. Weight, height, BMI, waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and pubertal development are compared and dependencies between the patient group and the control group are described.

In patients with **PWS**, no significant difference was found in the distribution by gender and age between the participants and the control group. The diagnosis was confirmed in all patients by genetic analysis. The data analysis showed significantly lower values of SBP and DBP in patients with PWS compared to controls. After controlling the analysis and BMI, in patients with PWS, a direct relationship was found between abdominal

circumference and SBP, as well as between abdominal circumference and DBP, i.e. the more pronounced accumulation of abdominal fat tissue in patients with syndromic obesity is associated with adverse hemodynamic changes, which are an independent predictor of CVD in adulthood.
When comparing the **SRS** group with the control participants, no significant difference was found in the distribution by gender and age. 50% of the patients with SRS had a genetic diagnosis. In bivariate analysis of the data for all participants, a strong direct correlation of abdominal circumference with SBP and DBP was demonstrated.

The second section presents the diagnostic criteria for both diseases and the family burden. Of the patients with PWS, 48.0% were born small for gestational age (SGA) compared to 12.5% in the controls, with PWS participants demonstrating a significantly lower birth weight. In the group of patients with PWS, an operative delivery was more often reported. In the families of patients with PWS, there is a significantly higher frequency of family history of CVD and MS compared to the control group.
94.4% of children with SRS were born SGA, compared to 0% of the matched controls, with the former demonstrating a significantly lower birth weight and height, with the presence of relative macrocephaly in more than 55% of the patients. It is demonstrated that in the families of SRS patients the frequency of CVD burden is lower than that in controls, while the presence of familial MS burden is insignificantly more frequent among syndromic participants.

The third section examines the biochemical and hormonal indicators in both groups. In patients with PWS, a more favorable metabolic profile was found - significantly lower serum insulin concentration and HOMA-IR, a more favorable lipid profile, significantly lower levels of CRP and uric acid, significantly higher levels of SHBG. A direct correlation of abdominal circumference with insulin levels, HOMA-IR, LDL-C, CRP, uric acid, IGF-1, leptin and an inverse correlation with SHBG and HMW adiponectin were proven, as well as a significant negative dependence of irisin with serum triglycerides.
In patients with SRS, the doctoral student reported a worse metabolic profile - significantly higher values of uric acid; significantly lower serum levels of SHBG. There is a tendency towards a worse profile of glucose homeostasis and a higher average value of CRP. A direct correlation was found between BMI, abdominal circumference and leptin level, as well as a significant inverse correlation with SHBG. Of interest is the measurement and analysis of the myoadipokine irisin and its significantly higher concentration in SRS compared to the corresponding controls.

The fourth section reflects the body composition indicators and the distribution of fat/fat-free mass. Significantly lower bone density in PWS patients despite the treatment with rhGH, as well as lower fat-free tissue content (lean mass), corrected for height, is demonstrated. In SRS patients, the analysis of body composition and its relationship with the studied metabolic indicators does not show significant differences compared to healthy controls.

The fifth section focuses on quality of life (QoL) in both patient groups. In a detailed stratification of only the PWS patients with high QoL scores, a reliable direct relationship was established between the calculated QoL scores and IGF-1 SDS. In patients with SRS, a significant correlation was demonstrated between the calculated QoL score with IGF-1 and fat free mass. In an analysis including only the participants with high QoL scores, a reliable direct relationship was established between the calculated QoL scores and bone mass, lean mass (g) and IGF-1.

The discussion is in-depth, summarizes the obtained data in the context of contemporary understandings of the problem and compares them with the results of other groups. The self-criticism of the doctoral student, pointing out some limitations/shortcomings of the dissertation work, is present.

The fifteen **conclusions** drawn (8 for the Prader-Willi group and 7 for Silver-Russell) are in accordance with the obtained results and follow the tasks set in the dissertation work.

The **contributions** of the dissertation work are of a confirmatory and scientifically applied nature. Most of them are innovative for Bulgaria, and some are also internationally.

Abstract of dissertation and publications related to the dissertation work
The abstract is fully sufficient in content and quality to present the main results achieved in the dissertation. The doctoral student has attached to the documentation 2 full-text publications related to the dissertation work, as well as 2 participations in a prestigious international forum with abstracts in refereed publications. The attached list meets the requirements for acquiring the educational and scientific degree "doctor".

6. Critical remarks and recommendations

I have no critical remarks or recommendations regarding the conducted scientific research and the materials provided to me.

In conclusion, the dissertation I reviewed, dedicated to rare endocrine diseases, reflects a thorough and valuable work, impressive with precise analysis. I believe that the work meets the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADRB), the Regulations for its implementation and the Regulations for the Development of the Academic Staff at MU-Varna for the acquisition of the scientific and educational degree "doctor" and I vote positively for the award of this degree to Dr. Nikolinka Yordanova Peykova.

Varna,
22nd Nov 2024

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Assoc. prof. Mira Siderova, MD, PhD