REVIEW

By: Professor Daniela Mircheva Avdjieva-Tzavella, MD, PhD, Department of Clinical Genetics, University Pediatric Hospital, Medical University-Sofia,
Member of a Scientific Jury according to the Order of the Rector of Medical University - Varna - R– 109-362/28.10.2024

Regarding: dissertation "Metabolic risk and body composition in children born small for gestational age due to existing genetic syndromes (Prader-Willi, Silver-Russel and others)" for the award of the educational and scientific degree "Doctor" to Dr. Nikolinka Yordanova Peykova
Field of higher education: 7. Health and Sport; Professional Field 7.1 Medicine; Scientific specialty "Paediatrics"
Form of doctoral studies: full-time, Department of Paediatrics at the Medical University of Varna
Scientific supervisor: Prof. Dr. Violeta Iotova, MD, Department of Pediatrics, Medical University of Varna

Biographical data about the PhD student

Dr. Nikolinka Yordanova graduated from the Mathematical High School "Atanas Radev" in Yambol. In 2015 she graduated in Medicine at Medical University - Varna. In March 2016 she started specialization in pediatric endocrinology and metabolic diseases at the 1st Children's Clinic of the University Hospital " St. Marina "-Varna. In 2019 she won a competition for a full-time PhD student at the Department of Paediatrics at MU - Varna on the topic "Metabolic risk and body composition in children born small for gestational age with existing genetic syndromes (Prader-Willi, Silver-Russell, etc.)". In 2020 she successfully completed a specialty fellowship in pediatric endocrinology and metabolic diseases. Her clinical and research interests are mainly in rare endocrine diseases. She leads a multidisciplinary team for the care of patients with Prader-Willi and Silver-Russel syndrome at the Centre of Expertise in Rare Endocrine Diseases, which is part of the European Reference Network for Rare Endocrine Diseases (Endo-ERN). Since 2020 she is a visiting assistant professor at the Department of Pediatrics at MU-Varna. In 2022 she is doing a specialisation at Erasmus MC Sophia Children's Hospital, Rotterdam, The Netherlands in the field of specific care of patients with rare imprinting syndromes, funded by Endo-ERN. She

has been involved in two research projects - "Advantages of modern technologies in glucose monitoring in children and adults with type 1 diabetes mellitus" and „Comparative analysis of biomarkers, empirical and commercial models to predict the effect of growth hormone treatment in childhood". She is a member of the Bulgarian Paediatric Association, Bulgarian National Society of Paediatric Endocrinology (BNSDE), Varna Society of Paediatric Endocrinology (VAPED) and European Society of Paediatric Endocrinology (ESPE). She is fluent in English and German.

The dissertation submitted for my opinion is structured in accordance with the Academic Staff Development Act in the Republic of Bulgaria. The dissertation covers 158 standard pages, of which 110 pages are main text and contains all the main sections - introduction (1 p.), literature review (37 p.), background, aim and objectives (2 p.), materials and methods (10 p.), results and discussion (54 p.), conclusions and contributions (5 p.). The reference list includes 382 sources, 7 in Bulgarian. The dissertation contains 8 figures, 30 tables and 7 appendices.

Relevance of the topic

Prader-Willi and Silver-Russel syndromes are relatively well known to paediatricians as they are relatively common rare disorders and have a characteristic phenotype pointing to the diagnosis. Both syndromes are a consequence of genomic imprinting. Both diseases have a high incidence of children born small for gestational age. For Silver-Russel syndrome, it is well known that low birth weight is associated with increased risk of cardiovascular disease, hypertension, dyslipidemia, obesity, insulin resistance, and type 2 diabetes mellitus. Another common feature of both conditions is the lower percentage of muscle mass compared to healthy controls. Treatment with recombinant human growth hormone (rhGH), available in Bulgaria for many years in both groups of patients results in an increase in the percentage of muscle mass. No published study has been found in the literature to establish a correlation between the reduced amount of muscle mass and metabolic profile in these patients. In Bulgaria, the metabolic profile of patients with Prader-Willi and Silver-Russel syndromes has not been assessed so far. There is a paucity of worldwide data on the correlations of muscle mass with metabolic status in these patients.

In recent years, much work has been done to improve the diagnosis, treatment and care of patients with rare diseases. The social relevance of the problem is undeniable due to their large number (more than 6000 nosological entities described so far), affecting millions of people worldwide, all united by their common problems such as late diagnosis, difficult access to medical specialists, lack of therapy and social integration. The development of international rare disease networks has contributed significantly to improving collaboration between medical professionals and to improving the care of patients with rare diseases worldwide.

The growing need to form multidisciplinary teams of different highly specialized specialists, to develop national collaborations in the field of rare diseases and to individualize the management according to the specific needs of each patient make this thesis extremely relevant from a practical and scientific point of view.

Knowledge of the problem

The literature review is presented in a detailed and informative manner, based on 285 sources, about 50% of which are from the last 10 years, and is divided into two main parts, devoted respectively to Prader-Willi syndrome and Silver-Russel syndrome. For both syndromes, the main features are discussed, including incidence, etiology, genetic variants and laboratory methods for their diagnosis, available international consensus, causes of reduced life expectancy, and a historical overview of the development of knowledge about these diseases in Bulgaria and worldwide. In Prader-Willi syndrome, the putative mechanisms of hyperphagia and obesity are discussed.

Endocrine features and treatment approaches for both conditions are presented. In Prader-Willi syndrome, this part of the review includes short stature and indications, dose regimen and side effects of rhGH treatment, hypogonadism in both sexes and hormone replacement therapy options, causes of reduced bone mineral density and osteoporosis, presence of metabolic syndrome and obesity, hypothyroidism and L-Thyroxine treatment. An important issue in patients with Prader-Willi syndrome is adrenal insufficiency and corticosteroid replacement, as central adrenal insufficiency has been described as a possible cause of sudden death in patients with this disease due to inadequately small adrenal gland sizes found in them post-mortem at autopsy. The endocrine disorders in Silver-Russel syndrome, hypoglycaemia, short stature and treatment with

rhGH, early puberty and treatment with GnRH analogue, metabolic syndrome and increased cardiovascular risk are also presented.

The review discusses in detail some of the more important adipokines and the literature data on their association with the metabolic profile in Prader-Willi and Silver-Russel syndromes, with scarce information on the latter disease in this aspect. The first adipokine described, leptin, regulates food intake, body mass and reproductive function and plays a role in fetal growth, proinflammatory immune responses, angiogenesis and lipolysis. Because leptin acts at the level of the hypothalamus by suppressing appetite, controlling energy expenditure and body weight there have been many studies of its role in Prader-Willi syndrome. However, the results of these analyses are conflicting and further studies are needed to further clarify the role of leptin in the pathogenesis of metabolic risk in this disease. Contradictory results have also been obtained comparing leptin levels in those born SGA with those born AGA, finding higher, similar and, in most studies, lower leptin levels in the former group. The levels and its relationship with growth parameters in the group of children born SGA were investigated and an inverse correlation was found between leptin concentration and response to rhGH treatment. This correlation could be used as a marker to predict the treatment effect in SGA children without postnatal catch-up in growth - the higher the leptin level, the better the response to rhGH treatment.

Adiponectin is another representative of adipokines, which inhibits gluconeogenesis in the liver, increases insulin sensitivity, stimulates the oxidation of fatty acids synthesized in the muscles and has anti-inflammatory function. A number of publications have shown that Prader-Willi syndrome patients have higher levels relative to obese controls with the same BMI. It has been suggested that children with this syndrome are more insulin sensitive than obese and similar to lean controls. According to some studies, adiponectin levels are lower in SGA infants compared to children born AGA.

This review presents the known evidence from the literature regarding the level and function of Irisin in Prader-Willi and Silver-Russel syndromes. It was found that patients with Prader-Willi syndrome have lower Irisin compared to obese controls, and this is more pronounced in children with the deletion form. Data on plasma Irisin levels in patients with Silver-Russel syndrome are not found in the literature, and are mixed in SGA children.

The dissertation discusses the differences in body composition of SGA children with and without postnatal catch-up in growth and AGA children, and the effect of rhGH treatment on this parameter.

A place in the review is also devoted to studies conducted on quality of life and psychosocial aspects in patients with Prader-Willi and Silver-Russel syndromes, topics that are increasingly becoming the subject of interest for a large number of researchers.

It is clear from the literature review that the dissertator is familiar with the current state of the art. The material is presented concisely, accurately and is very well structured.

Aim and objectives

The aim of the thesis is formulated briefly and clearly - To assess cardiovascular and metabolic risk in patients with Prader-Willi and Silver-Russel syndromes according to their fat and muscle mass. To achieve the stated aim, four objectives were formulated, which include: Evaluation of the clinical and laboratory profile of patients with PWS and SRS who are followed up at the Varna Centre of Expertise in Rare Endocrine Diseases; Determination of the prevalence of metabolic and cardiovascular disorders in patients with PWS and SRS in comparison with sex-, age- and partially BMI-matched healthy controls; Analysis of body composition in patients with PWS and SRS and its relationship with cardiovascular and metabolic parameters; Assessment of quality of life in these two groups of patients. The objectives were in line with the aim of the study.

Materials and methods

This dissertation combines the results of two parallel transversal case-control substudies, the first comparing 25 patients with genetically proven Prader-Willi syndrome and the second 18 patients with a genetic and/or clinical diagnosis of Silver-Russel syndrome with 39 sex-, age-, and BMI-matched healthy controls. All but three patients were treated with rhGH.

An interview was conducted to collect anamnestic data with patients and controls, and two separate options were adapted for the patient group, tailored to the specifics of the respective disease. A questionnaire designed to investigate quality of life adapted for patients with mental retardation was given to the patient group.

The clinical evaluation of the patients including anthropometry (height, weight and abdominal circumference), a thorough examination of organs and systems including measurement of blood pressure, heart rate, stages of pubertal development according to the

Tanner scale and pathological deviations in somatic status entering into the main characteristic features of both diseases, shows a good command of physical examination and knowledge of the features of Prader-Willi and Silver-Russel syndromes.

A range of biochemical and hormonal investigations were also performed, including blood glucose, fasting insulin and in the course of a standard oral glucose tolerance test, lipid profile, uric acid, CRP, glycated haemoglobin, cortisol, SHBG, leptin, high molecular weight adiponectin, irisin, IGF-1 and IGFBP-3.

To determine body composition and measure the amounts of fat-free (muscle), fat and bone mass, a DXA scan was performed on the participants.

The chosen research methods allowed achieving objective results in response to the aim and objectives set in the thesis.

Results and Discussion

In this dissertation, for the first time in Bulgaria, a study of body composition in Prader-Willi and Silver-Russel syndromes is conducted, and the discovery of correlations between the different indices would help to derive an algorithm for metabolic risk assessment in these patients. Analysis of body composition in patients with Prader-Willi syndrome showed lower bone density and lower height-adjusted fat-free mass (BMI/height) compared with controls, There was a positive correlation of both measures of adipose tissue volume (g) and adipose tissue volume (%) with leptin, as well as a negative association of both of the above measures with SHBG. In patients with Silver-Russel syndrome, the analysis of body composition and its relationship with the metabolic indices studied showed no significant differences compared to healthy controls.

Studies performed in patients with Prader-Willi syndrome showed a more favorable metabolic profile with significantly lower CRP, uric acid, insulin and HOMA-IR values, significantly higher SHBG levels and a more favorable lipid profile compared to the control group.

Higher values of systolic and diastolic blood pressure were found either separately or in combination with the decrease in HMW adiponectin levels. The reported significant linear relationship between abdominal circumference and systolic and diastolic arterial pressures has not been previously described in the literature and could be used as a predictor for the

demonstrated. The more favorable metabolic profile in these patients was confirmed by the findings of a lower relative proportion of patients with impaired glucose tolerance, lower absolute values of systolic and diastolic blood pressure, and a lower relative proportion of patients with systolic and diastolic hypertension.

The recorded overweight in 1/5 of patients with Prader-Willi syndrome and generalized obesity in almost half, confirms the well-known need to include specialized psychologists and nutritionists in the multidisciplinary teams following these children.

Irisin studied in the patient group showed a significant negative correlation only with serum triglycerides, confirming the association between its levels and the amount of adipose tissue (g and %).

Studies performed in patients with Silver-Russel syndrome showed a worsened metabolic profile with a significantly higher uric acid value, significantly lower serum SHBG levels, a trend towards a worsened glucose homeostasis profile, a higher mean CRP value, a higher relative proportion of patients with systolic and diastolic hypertension, and a higher heart rate. There was a direct correlation between BMI, abdominal circumference and leptin level, a significant inverse correlation with SHBG as well as a significantly higher irisin concentration compared to controls.

This thesis includes quality of life assessment, which is one of the most current trends in modern medicine. The study showed that Prader-Willi syndrome patients with high quality of life scores also had lower abdominal circumference, insulin, HOMA-IR and triglycerides compared to those with low scores. This is evidence that growth hormone therapy initiated early and administered at adequate doses also positively affects the quality of life of Prader-Willi syndrome patients, as it provides their more favorable metabolic profile. The benefit of rhGH therapy in terms of quality of life is also found in the other group, that of patients with Silver-Russel syndrome, where those with higher IGF-1 levels, higher height, lower insulin, HOMA-IR, CRP, leptin and adiposity (%) values are more likely to be affected.

The discussion is developed adequately to the results obtained and in accordance with the literature. An in-depth and critical analysis of outcomes in patients with Prader-Willi and Silver-Russel syndromes is performed. In his discussions, the doctoral student demonstrates creative

maturity, excellent command of methodology, and the ability to thoroughly and objectively interpret scientific results.

Critical Notes: 50% (n=9) of patients with Silver-Russel syndrome enrolled in the study had a positive genetic result. Conducting additional molecular analyses in patients without a genetically verified diagnosis would help to exclude other diseases with a similar phenotype and would enrich this work.

In the **conclusion**, the results are summarized, which define the necessity in daily clinical practice of a systematic assessment of anthropometric indicators, physical status, metabolic and hormonal profile, body composition and quality of life in patients with Prader-Willi and Silver-Russel syndromes in order to prevent obesity and its metabolic complications in the former, as well as for their early detection in the high-risk group of patients with the latter disease. The PhD student's suggestion of some markers of increased metabolic risk that could be applied in clinical practice in the routine follow-up of these two groups of patients - abdominal circumference, high molecular weight adiponectin levels, leptin, uric acid, CRP and SHBG - has a definite practical application and allows optimizing the diagnostic process.

Dr. Nikolinka Yordanova formulates the **main conclusions** of the dissertation and the **contributions** (original and confirmatory), deriving them logically from the results obtained in the dissertation. They correspond to the set tasks and are a consequence of the formulated aim of the present work. The study is the first worldwide to indicate an association between the increase in abdominal circumference in both groups of patients and the decrease in high molecular weight adiponectin levels in Prader-Willi syndrome with a higher risk of hypertension.

The results of the dissertation work are reflected in 2 articles and have been presented at 2 international scientific forums.

The layout of the dissertation is clear, the terms are used correctly, the text is structured logically correct, the figures and tables are precisely explained.

The abstract contains 99 pages, many figures and tables. It is written according to the requirements of the relevant regulations and reflects the main results, conclusions and contributions achieved in the thesis.

Conclusion

The dissertation contains scientific and applied results, which represent an original contribution to science and meet 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 internal criteria of MU - Varna. The submitted materials and dissertation results fully comply with the specific requirements of MU - Varna.

The dissertation shows that the PhD student Dr. Nikolinka Yordanova possesses in-depth theoretical knowledge, high professionalism and demonstrates qualities and skills for independent scientific research.

Because of the above, I confidently give my positive evaluation for the conducted research, 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. Nikolinka Yordanova Peykova in the doctoral program in the scientific specialty "Pediatrics".

23.11.2024 **Prof. D. Avdjieva-Tzavella**