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REVIEW OF PHD THESIS

BY ANNA LIGĘZKA

Title of the work: ***Clinical and basic investigations on aldose reductase inhibitors in Congenital Disorders of Glycosylation***

Supervisors: **Eva Morava-Kozicz**, M.D. Ph.D. and **Wirginia Krzyściak**, Ph.D. Affiliations: Mayo Clinic, Department of Clinical Genomics, in Rochester, Minnesota, USA (under supervision Prof. Eva Morava-Kozicz), and in Department of Medical Diagnostics, Pharmaceutical Faculty, Jagiellonian University Collegium Medicum in Cracow (under supervision of Wirginia Krzyściak, Ph.D.)

Congenital Disorders of Glycosylation (CDG) are a group of genetically determined disorders caused by abnormalities in the course of N- and O-glycosylation of proteins and lipids. About 150 clinical syndromes, which differ in their presentation and clinical course, have been identified, and most of them are passed down in the form of autosomal dominant inheritance. The first published case of protein N-glycosylation disorder was the case of a patient with phosphomannomutase 2 deficiency (PMM2-CDG) described in 1980 by prof. Jaak Jaeken. It is the most commonly diagnosed inherited disorder of glycosylation. The dominant symptoms include: retardation of psychomotor development, intellectual disability, cerebellar hypoplasia, craniofacial dysmorphism, inverted nipples, abnormal distribution of adipose tissue, liver fibrosis or hypertrophic cardiomyopathy.

The diagnostic method that allows to identify protein N-glycosylation disorders is the assessment of the profile of transferrin isoforms with the use of the isoelectric focusing. Currently, molecular diagnostic techniques also are important, and the more common use of next-generation sequencing (NGS) has enabled the recognition of new defects. Moreover, in congenital disorders of glycosylation there is no causal treatment, and the used therapies are symptomatic or preventive.

I in my opinion, the research carried out by Anna Ligęzka, MSc, under the supervision of Prof. Eva Morava-Kozicz and dr hab. Wirginia Krzyściak is very interesting both for scientific and potentially clinical reasons due to the determination of laboratory biomarkers for PMM2-CDG, which correlate with disease progression, as well as the choice of a new therapeutic target.

The doctoral dissertation of Anna Ligęzka, MSc, titled: Clinical and basic investigations on aldose reductase inhibitors in Congenital Disorders of Glycosylation, was based on the research conducted in the international cooperation between the Department of Medical Diagnostics of Pharmaceutical Faculty at the Jagiellonian University, Collegium Medicum, in Cracow and the Department of Clinical Genomics at Mayo Clinic in Rochester, Minnesota, the USA.

The dissertation, which has a layout typical for doctoral theses, was prepared on the basis of a monothematic cycle of three articles, two of which (papers 1 and 3) are published scientific papers, and one is a "preprint article" (paper 2). The latter, titled "Coagulation abnormalities in a prospective cohort of 50 patients with PMM2-congenital disorder of glycosylation", was published only after the doctoral dissertation was submitted for review (De Graef D, Ligezka AN, Rezents J, Mazza GL, Preston G, Schwartz K, Krzysciak W, Lam C, Edmondson AC, Johnsen C, Kozicz T, Morava E. Mol Genet Metab. 2023, 9;139(2):107606. doi: 10.1016/j.ymgme.2023.107606). Hence, it seems that it would be more appropriate to include this article in the cycle only after its publication.

The articles constituting the described series are multicenter publications that were published in the years 2021 - 2023 in the following journals: *Annals of Neurology* (the Author indicates 2022, and in PubMed we can find different information — Ligezka AN, Radenkovic S, Saraswat M, Garapati K, Ranatunga W, Krzysciak W, Yanaihara H, Preston G, Brucker W, McGovern RM, Reid JM, Cassiman D, Muthusamy K, Johnsen C, Mercimek-Andrews S, Larson A, Lam C, Edmondson AC, Ghesquière B, Witters P, Raymond K, Oglesbee D, Pandey A, Perlstein EO, Kozicz T, Morava E. Sorbitol is a severity biomarker for PMM2-CDG with therapeutic implications. Annals of Neurology **2021**; 90(6): 887-900, doi: 10.1002/ana.26245), *Molecular Genetics and Metabolism* (2023) and once again in *Molecular Genetics and Metabolism* (the Author indicates 2021, and in PubMed we can find a different year of publishing: Ligezka AN, Mohamed A, Pascoal C, Dos Reis Ferreira V, Boyer S, Lam C, Edmondson A, Krzysciak W, Golebiowski R, Perez-Ortiz J, Morava E. Patient-reported outcomes and quality of life in PMM2-CDG. Molecular Genetics and Metabolism **2022**; 136(2): 145-151, doi: 10.1016/j.ymgme.2022.04.002).

Anna Ligęzka is the first author of two papers, and the second author of one. However, it is difficult to determine the percentage of participation of the PhD student in writing these articles, considering that in the first paper (26 authors) Anna Ligęzka, MSc, was indicated as the person editing the manuscript. In the second article (12 authors) she was responsible for data collection, data evaluation, reviewing and editing the manuscript. Whereas, in the third paper (11 authors) she was responsible for data preparation and analysis, interpretation of data, drafting and revising the article.

It should be emphasized that all the discussed articles were published in the above-mentioned prestigious scientific journals, indexed in the interdisciplinary database Journal Citation Reports, with an impressive total value of the impact factor of 15.478 for two papers, and 19.682 for three of them. The score of the Polish Ministry of Science and Education is 300 for two publications, while 400 refers to three articles. The above indicates that the articles in question, which are the basis for applying for a doctoral degree, have previously been subjected to highly demanding review processes, and met the requirements of world-renowned scientific journals.

Given that there is a lack of both effective diagnostic biomarkers and effective therapies of PMM2-CDG, and there are not proper standards to better understand the current functionality of the PMM2-CDG patients, the research was undertaken with the following goals:

- to evaluate the effect of epalrestat (carboxylic acid derivative and a non-competitive and reversible aldose reductase inhibitor) on the PMM enzyme activity in PMM-deficient fibroblasts and leveraged multiplexed proteomics, and glycoproteomics to investigate global cellular N-glycosylation in epalrestat-treated PMM-deficient fibroblasts;
- to assess urinary polyol excretion in a cohort of 24 individuals with PMM2-CDG;
- to evaluate safety and efficacy of oral epalrestat therapy in a child with PMM2-CDG;
- to investigate coagulation abnormalities in the group of 50 patients with genetically confirmed diagnosis of PMM2-CDG;
- to use (for the first time) the Patient-Reported Outcomes Measurement Information System (PROMIS) as a tool to measure patient-reported outcomes (PROs) in the PMM2-CDG.

These goals were achieved through the use of numerous research methods and techniques, including: cell cultures, spectrophotometric measurements, immunoblotting, reverse transcription quantitative polymerase chain reaction, Western blot, gas chromatography/mass spectrometry, reverse phase liquid chromatography, liquid chromatography-tandem mass spectrometry as well as International Cooperative Ataxia Rating Scale (ICARS), Nijmegen Progression CDG Rating Scale (NPCRS) and Patient Reported Outcomes Measurement Information System (PROMIS).

As a result of the conducted research, the author states that

1. Epalrestat increases phosphomannomutase enzyme activity an improves global glycosylation profile in vitro. The evaluated drug had no effect on mRNA expression of cellular markers of N-glycosylation such as intercellular adhesion molecule-1 (ICAM-1) and lysosome-associated membrane glycoprotein-2 (LAMP-2), while it improved their glycosylation, as well as glycosylation of other glycopeptides. The beneficial effects of epalrestat on glycosylation were further supported by a clinical and glycosylation improvement in a single PMM2-CDG patient during a 12-month oral epalrestat treatment.

I would like to ask for providing the information on the side effects of the use of epalrestat.

2. Global glycoproteomic characterization of samples of individuals with PMM2-CDG is an emerging paradigm capable of enhancing our understanding of PMM2-CDG and providing quantitative, clinical laboratory-based biomarkers for assessing therapy response.
3. Urine polyol levels were normal (erythritol, arabinitol, ribitol, and galactitol) except for sorbitol and mannitol in most patients with PMM2-CDG, but only sorbitol levels correlated with the presence of peripheral neuropathy, and CDG severity rating scale. Therefore, the urine concentration of sorbitol was proposed as a new potential biomarker for assessing the severity of the disease and monitoring therapeutic efficacy in clinical trials.
4. In the course of PMM2-CDG, there is an imbalance in the coagulation and fibrinolysis system. The activities of prothrombotic and antithrombotic agents are abnormal in >80% of PMM2-CDG patients. The most frequently abnormal pro- and anticoagulant parameters were AT, PC, PT, INR, and FXI, and AT deficiency was especially evident. Antithrombin III and factor XI activities were proposed as potential biomarkers to estimate the risk of thrombotic episodes in trials.
5. Patient-Reported Outcomes Measurement Information System (PROMIS) compared with the standard severity tool, i.e. Nijmegen Progression CDG Rating Scale (NPCRS), seems to be a more effective tool for future clinical trials, which is due to the inclusion of patient/family data for the analysis.

In conclusion, I would like to declare that the doctoral dissertation "Clinical and basic investigations on aldose reductase inhibitors in Congenital Disorders of Glycosylation" submitted to me for review is an original, independent scientific achievement of Ms. Anna Ligęzka, MSc, who is the candidate for the scientific degree of PhD in Pharmaceutical Sciences. In the reviewed dissertation, the PhD student focused on a very current and important subject, demonstrated knowledge of the research tools which should be employed, and the ability to critically analyze the obtained results.

Anna Ligęzka's doctoral dissertation meets the formal and substantive requirements of the Law on Higher Education and Science published on the 20th of July 2018 (section V, chapters 1 and 2, art. 186, 187 points 1-4). Based on this, I am applying to the Council of the Discipline of Pharmaceutical Sciences of the Jagiellonian University Collegium Medicum to accept this dissertation and allow Anna Ligęzka to the next stages of her doctoral development.

Katarzyna Barbara Winsz-Szczotka

Elektronicznie podpisany przez Katarzyna Barbara Winsz-Szczotka
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