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**Opinion on the Doctoral Dissertation for the title of Doctor of Medicine of**

**Mr. Przemyslaw Czajkowski**

*“Associations between common FTO gene polymorphisms and diet, and their impact on obesity as well as its metabolic consequences”*

supervised by

**Edyta Adamska-Patruno**, PhD, Associate Professor from Clinical Research Support Centre at the Medical

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Obesity is a very complex (heterogenous) disease, but it's been known that there is a hereditary component within. But the fact that this association exists does not help us to assume that there is clinical knowledge about how to manage such information in the quest for Personalized, Predictive, Participatory, Precision, and Preventive (P5) treatments. The Dissertation of Mr Przemyslaw Czajkowski is a new step in the long journey to achieve that goal.

The dissertation consists of 83 pages of a typescript. It is based upon two scientific articles published in peer-reviewed, open access Journals. It includes a 20-pages overview of the research performed, including an Introduction, Aims, Materials & Methods, Results, Conclusions and Bibliography. There are also one-page Summaries in English and Polish. A copy of the two articles which comprise the doctoral dissertation is embedded. They are articles published in 2020 and 2021, in the journals *Nutrients* and *Antioxidants*, respectively (combined IF is 13.392, 240 MNiSW points). The dissertation also includes a copy of the Bioethics Committee Consent, and information about the nature of participation of all authors of the

respective articles, as well as the academic achievements of the two articles included in the dissertation, the 11 articles signed by the PhD candidate but not included in the dissertation (IF=20.308; MNiSW points=486), the list of conference abstracts, and the list of scientific articles not included in the dissertation.

The dissertation deals with the association of the main macronutrients of the diet (carbohydrate, protein and fat in the first article, and fibre in the second) with a particular genetic trait (common polymorphisms of the FTO gene) and their impact on health-related anthropometric (BMI, visceral fat, etc) and circulating lipids (cholesterol, LDL, HDL, etc.). For evaluating the dietary intake the PhD candidate has had access to data from volunteers selected from the 1000PLUS Cohort Study. All subjects were submitted to several tests: anthropometric and body composition, blood analysis (biochemical analysis) including the oral glucose tolerance test (OGTT), and genetic analysis. Moreover, part of the subjects completed the questionnaire for physical activity and a 3-day food intake diary. According to the food intake data, the subjects were classified upon their consumption of dietary protein, fat, carbohydrate and fibre. I must emphasize that one of the strengths of the dissertation is the access to a well-characterized cohort of subjects, with solid data collected about their status and lifestyle. Accordingly, the candidate shows a large set of results which are only a selection of all the possible combinations of statistical comparisons that might have been performed. One can only imagine how much time has been devoted to performing all the analysis, but for sure it has been long and carefully revised.

As this thesis is article-based, the peer-review process should be granted, and therefore Originality and Innovation, Scientific and Technical Merits, Planning and Methodology should have been already evaluated in the publication process by the reviewers and editors. After reading the dissertation, it is my strong belief that such aspects have been already taken into consideration. I will comment therefore the most important issues that I have raised while reading the dissertation.

Both articles share a common structure, with an Introduction describing the state of the art about current knowledge on obesity and variants of the FTO gene, and the possible association with dietary macronutrients. In the first article, protein, fat and carbohydrate intake was studied, whereas fibre was the matter of study in the second. Materials and Methods sections are very similar in both studies, as the cohort was the same, the selected subjects were common, and they recorded data for the same anthropometric and biochemical parameters. Nevertheless, there are two differences between both studies: although there were 819 subjects selected in both studies (and the descriptors are common, so I assume they were the same subjects) 490 completed the food intake diary in the first study, whereas 622 completed it in the second. Another difference is that although 4 polymorphisms were common between both articles (rs3751812, rs8050136, rs8044769 and rs9939609), in the second article rs6499640 and rs7190492 were also included. rs9939609 was in linkage disequilibrium with rs8050136, so in both articles results only for one (rs8050136) is shown. Although in the respective articles there is no need for comparison, in my opinion, some explanation is needed in the dissertation (as they are shown together) to explain the differences in the number of studied polymorphisms and in the number of subjects that completed the questionnaire, to avoid the thoughts of cherry-picking.

I consider it worth mentioning the complete description of the statistical analysis applied, because most of the numerical analysis is applied to the previous evaluation of the distribution of the data and is therefore not shown, but is compulsory to obtain reliable results.

In both articles, results are presented as tables and box-plot figures. Most of the information in tables 2 and 3 of article 1 is common to table 1 of article 2, as well as table 4 of article 1 and table 2 of article 2, where the means and standard deviation of all parameters are studied, grouped by SNPs of the polymorphisms (3 & 5 polymorphisms, respectively). The p-value (Holm-adjusted Kruskal-Wallis or ANOVA) of the significance within each polymorphism is also included. The results are also shown in a set of box plots of the different parameters, comparing the values of the SNPs according to the intake of each of the macronutrients studied. The box plots include also the significance of the difference between SNPs within each macronutrient intake level. The Results section includes a thorough comprehensive description of all the results comprised in the tables and box-plot figures, organised by genetic variants, as the tables and figures.

Both articles include a detailed discussion of the results obtained, strongly focused on the possible consequences that the results could have for personalized dietary recommendations to subjects carrying each of the studied variants. Of particular interest, the PhD candidate highlights as “surprisingly” the cholesterol results of the subjects carrying two variants of two polymorphisms, paradoxically higher when subjects reported higher fibre intake, together with higher LDL-cholesterol. In both articles, the discussion includes specific dietary recommendations (protein, fat, carbohydrate in article 1, fibre in article 2) for carriers of each genotype. In both articles, the conclusions of the articles state that the genome study is useful to develop customized diet recommendations to prevent obesity and metabolic complications.

It is also noteworthy that the articles include an honest statement about the limitations of the study, such as the self-reported intake, the single-ethnicity of the study, or without stratification for age. Nevertheless, during the public defence of the candidate, I would also like to ask him to discuss some aspects or consequences of his study which are interesting:

- Has the association of polymorphisms in FTO simultaneously with polymorphisms in other genes (e.g. MC4R) been studied?
- Are there alternatives to the questionnaires (dietary biomarkers in the blood) that could be measured to confirm/reject subjective results?
- Could it be possible to evaluate (in the 1000PLUS Cohort or a new study) the association of the variants with other determinants of obesity such as individual psychology (anxiety, depression, food craving, etc)?
- Will you try to relate the findings in the present dissertation with all the metabolomics studies that have been already performed in this Cohort?
- Could it be possible to evaluate the association with a particular gut microbiome?
- Moreover, although not directly related to the results described and discussed, I have missed some comments about the relationship of the variants with a possible gain or loss of function of the product of the FTO gene, the N6-methyladenine demethylase

To summarize, I would like to point out that the presented study adds valuable information to tackle a matter of concern. I congratulate the PhD candidate on the published work, based on deep analysis of a large set of data, taking always into consideration the limitations of the study. In my opinion, the Dissertation “Associations between common FTO gene polymorphisms and diet, and their impact on

obesity as well as its metabolic consequences” presented by Przemyslaw Czajkowski satisfies all requirements for the title of Doctor of Medicine.

Given all above, I hereby recommend that, if all the regulations of the Polish law and the specific rules of the Medical University of Bialystok regarding the doctoral qualification are fulfilled, the Dissertation of Przemyslaw Czajkowski is accepted for the further proceedings of the doctoral conferment by the Research Council of the Medical University of Bialystok.

Madrid, 21<sup>st</sup> November 2022

A handwritten signature in blue ink, appearing to read 'Francisco Javier Rupérez', with a long horizontal flourish extending to the right.

Francisco Javier Rupérez