Assessment of PhD dissertation by mgr Rezvan Noroozi entitled: „Investigating the role of genetic and epigenetic variation in facial skin and scalp hair aging and DNA-based prediction of age-related human appearance traits".

In the dissertation PhD candidate presents results of study aiming to investigate the association of genetic and epigenetic makeup of the individuals with traits reflecting the visible signs of aging. The epigenetic research in broadly understood longevity field is currently attracting increasing interest of the researchers. Especially after assessment of the aging based on the accumulation of the epigenetic changes has been shown to better reflect influence of the environment on the aging of the individuals than chronological aging. Thus, the subject of research undertaken by the PhD candidate is very relevant and the thesis addresses important research questions.

Formal assessment

The dissertation is 115 pages long including title page and the list of references. The text starts with abstract in English and which are followed by the acknowledgements, copy right statement and table of contents. The body of the text is structured into five chapters including: introduction, theoretical background, materials and methods, results and discussion. Appendixes and the list of references, end the dissertation. The structure of the text is standard for this type of the dissertations.

Assessment of key parts of the dissertation

The abstract: This section consists of almost 1500 words, which in my opinion, is too long for the standard abstract expected in the case of both the thesis and research papers. The text is rather description of the thesis rationale, contents and short discussion of analyses that were performed, than the abstract of the presented work. For example, the description of the stages of the project built around hypotheses (pages 6 and 7). Overall given the length of this section, introduction of indexing of specific parts in this section would significantly increase the clarity of the section and make it easier to read. The abstract ends with accurate in my opinion selection of the **key words**. This part the dissertation ends with the detailed and adequate table of contents and glossary of the thesis.

Subsequent part of the thesis is divided into five chapters.

Chapter 1: contains one page introduction to the thesis, which in principle is and abstract of the thesis that in short and adequately summarizes the content of the dissertation.

Chapter 2: Consist of 13 pages long description of the state-of-the-art upon which the thesis is built. This part contains in my opinion sufficient for this type of dissertation summery of the current knowledge in the field. The text is subdivided and indexed in the way that makes it easy to read and follow. The content of this section clearly shows that PhD candidate has acquired high level and up to date knowledge that is necessary to successfully preform research in the field. Moreover, the structure of the text shows that the candidate is able to logically and coherently present scientific argument, which is a skill indicating high research maturity of the candidate.

The section at a few points lacks references but that is probably attributed to simple overlooking. The description to figure one is not coherent with the Figure. The description of the figure should contain references (indexing) to specific parts of the figure especially that figure illustrates rather complicated

concept. Also, a minor issue is that this section ends with research question which would resonate better with the reader if it was presented in separate section.

Chapter 3: This section contains concise and adequate to this type of text description of the methods and materials used in the experimental work presented in the thesis. The text shows that PhD candidate is familiar with standards used for presentation of the materials and methods in scientific writing. However, as the use of M-values in analysis of methylation microarray data is still controversial, I would like to ask PhD candidate to elaborate during the defense, incentive to use these values, challenges with the interpretation of the results of the analysis that utilizes this measurement and explain the relation of this measurements to methylation levels expressed in the beta - values scale. Also, as a minor criticism, I would suggest including in this section description of the geographic diversity of the recruited population as different living environments may introduce variation in epigenetic measurements.

Chapter 4: This section consists of eight subsections describing results of the analysis performed by the PhD candidate. Overall, the subsections are in logical order and the results are presented according to the standards of the scientific writing.

Specifically: **Section 4.1** describes interesting results illustrating discrepancies between measurements of the epigenetic age, which may originate from the data processing. This section also contains comparison of performance of various epigenetic clocks to PCA-clocks. However, the incentive for the use of this comparison as well as and limitations of the PCA-clocks methodology is not discussed, neither here nor in the introduction. In the discussion author writes, "the PC clocks were developed to serve as an alternative method to address the potential noise in age estimation and enhance the reliability of the results obtained from DNAm age clocks" but this text still does not explain why methodology of PCA-clocks is used as reference to other methods. The readers cannot be faced with need to perform literature searches to follow data presented in the thesis. Thus, I would like PhD candidate to address this criticism during public defense.

Section 4.2 describes simple but very interesting analysis of correlation among different DNAm age and epigenetic age acceleration measurement methodologies. These are important results illustrating the limitations of different methodologies used in the field.

In **Section 4.3** author continues with presentation of statistical analysis of age-related EVCs with specific categories of data collected in the project. Presented analyses are methodologically correct but the results in this section in my opinion are reported only party according to the standards of scientific publication. The key to clear presentation of this type of results is quantification of the changes that is statistically significant. Reporting of only p-value in the text is not informative unless reported alongside with the measurement of the difference that had been shown to be statistically significant. In this way the reader is able to assess the significance of the findings in the context of the biology of described phenomenon. PhD candidate does report regression coefficients and odds ratios in the tables, but does not interpret them. I would like the author to address this interpretation during the defense. I do understand that interpretation of the identified differences may be at some instances challenging but in my opinion the PhD candidate should make that attempt.

In **Sections: 4.4. and 4.5** author reports results of GWAS results and SNPs associated with EVCs and epigenetic age association measurements. The results are reported according to standards of scientific work.

In **Section 4.6** author describes results of EWAS analysis which in principle are performed correctly according to chosen methodology. However, (as I have already mentioned in review of Materials and Methods) the justification of use of methodology based on M values is missing in the thesis and should be addressed during the defense. That justification is necessary for the reader to understand, for example, the interpretation of the "Ave. Meth." value from Table 10 as it is not clear from the table,

what is the unit of this measurement and how does this measurement reflect methylation difference between cases and controls.

Section 4.7 describes analysis aiming to elaborate the biological context of the identified epigenetic and genetic markers according to standard GSEA methodology and results here are presented according to the accepted scientific standards. In the description of Table 11, term DMSs is used, it is easy to understand what the term means but this term is not consistent with the text.

The last section of the results (**Section 4.8**) describes development of the prediction model for perceived age, wrinkle area and full-face wrinkle. PhD candidate has chosen a specific methodology for the models' development and in general correctly performed the data inference and reported the data according to the standard. This section regardless of criticism of used statistical methods which may represent different data analysis practices, clearly shown that PhD candidate has developed a high-level proficiency in use of not only bioinformatics but also statistical models and is prepared to scientific work at the level of postdoctoral fellow.

Chapter 5 consists of 16-page long discussion of the results. Minor criticism here is that the subsections of the discussion are not indexed what makes this section not consistent with other parts of the dissertation. Overall, in this section PhD candidate discusses the results of the analysis performed in the context of previously published data and uses correct references to previously published work. The level of the discussion is appropriate for a PhD candidate and shows extensive knowledge of the research in the field.

The discussion is structured into six sections and ends with short conclusion. In the first and the second section author discussed the origins of the discrepancies in age estimation calculations received with different methodologies. The section is rather descriptive and would benefit greatly if author added here discussion of the informed speculations as to what is the origin of identified discrepancies. Author touches here on discussion of the influence of technological noise on calculations but that noise would affect all methodologies uniformly as all calculations were performed on identical raw data.

In subsequent section the association of lifestyle and EAA measures with EVCs is discussed. This part is also very descriptive in my opinion. PhD candidate should have elaborated more here on for example origins of found in the study "higher degree of facial aging in male" or reasons for reported in the thesis association of the perceived aging with the socioeconomic status. The data presented in this thesis in my opinion allow for example to test association with UV light exposure and type of job beyond making only speculation and reference to previous studies.

The fourth section of the discussion contains discussion of putative biological function of the identified genetic variants that the author identified to be associated with measurements of aging. The discussion is well written and based on the literature data what again highlights authors knowledge of the literature in the field. Importantly and due to the fact that the literature data do not confirm the results of author's experiments, the author does acknowledge in this section the need for validation of the findings discussed in this section in future independent studies.

In the fifth section of the discussion author sufficiently describes the results of EWAS analysis in the context of the literature data. However, I would be more careful in drawing conclusions from the ontology term analysis and I would suggest validation of the GO analysis with other GSEA tools based on different databases such as for example: GREAT or FUMA GWAS, before making conclusions.

The last section of the discussion summarizes the main parameters of the prediction models for the selected aging related EVC build on data collected in the study and discusses those models in the context of previous models reported in the literature. Author concludes this section suggesting that despite of use of stat-of-the-art methodology to develop the models, the predictive value of the models has limitations and needs to be validated in future independent studies, this again indicates scientific

maturity of PhD candidate and critical approach to one's results that is necessary in research work. The discussion ends with short conclusions.
The last two sections of the dissertations are **Appendixes** that include figures and tables adequately referenced in the text and **the list of bibliography**. The bibliography list is, however, not edited according to consistent reference style. Also, citations of the references in the text are not presented in uniform output. Author should have used one of the reference editing tools for example EndNote to properly build this section. Especially that proficiency in use of bibliography editing tools is necessary for authors future research work and publications of research results.
The discussion ends with short conclusion summarizing the thesis.

Final remarks:
It is my pleasure to conclude that the level of scientific knowledge presented by PhD candidate Rezvan Noroozi in evaluated dissertation is sufficient to be awarded a PhD degree. Despite a minor criticism the substance of the thesis shows that expertise of PhD candidate is extensive and accompanied with the knowledge of the literature in the field. PhD candidate can in a logic, coherent and clear way discuss scientific phenomena and communicate the results of the undertaken studies at the level appropriate for scientific writing. I am confident that with that knowledge PhD candidate is prepared to undertake and successfully lead research as a postdoctoral fellow.

Stwierdzam, że oceniana rozprawa doktorska mgr Rezvan Noroozi, zatytułowanej: „Investigating the role of genetic and epigenetic variation in facial skin and scalp hair aging and DNA-based prediction of age-related human appearance traits". spełnia wymagania ustawy z Dz. U. z 2018 r., poz. 1668 z późniejszymi zmianami. Prawo o Szkolnictwie Wyższym i Nauce i może być skierowane do dalszych etapów postępowania doktorskiego.

Your sincerely / Z poważaniem

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