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Review of the PhD thesis by Ms. 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"

The explosion of the genome-wide association study (GWAS) discovery and applications across multiple disciplines have certainly influenced the research focused on prediction of external visible characteristics (EVCs) for forensic purposes. In particular, the GWAS has sped up the identification of single nucleotide polymorphisms (SNPs) and candidate genes associated with a number of complex traits of forensic interest, including pigmentation traits, hair morphology, male pattern hair loss, and, to a lesser extent, height and face morphology. While for the EVCs determination and prediction the progress in genomics, triggered by recent developments in massively parallel sequencing (MPS) technologies proved essential, for the prediction of age and age-related characteristics, both genomic and epigenome approaches have become indispensable. Indeed, since the discovery that DNA methylation (DNAm) changes during aging, a number of differentially methylated CpG sites have been suggested as potential biomarkers for prediction of age itself and age-related physical features.

In the submitted PhD thesis, Ms. Rezvan Noroozi, aware of the necessity of combining of both genomic and epigenomic markers in aging research, tackles an interesting issue of DNA sequence and methylation variation in age-related human appearance traits, including facial skin and scalp hair aging. Doing so, she undoubtedly addresses the current trends and innovations in research of human aging. Moreover, she fills in the specific gaps in the knowledge on mutual relationships between genetic, epigenetic and environmental factors in the development of age-related EVCs.

The PhD candidate aimed to provide more insight into genetic and epigenetic background of age-related EVCs, as well as their association with a number of environmental factors and individual rate of aging. To accomplish this ambitious goal, she collected 741 blood and 221 buccal swab samples from unrelated Polish individuals of both sexes, accompanied by their lifestyle data and 3D facial images. She subjected the

samples to the analysis of DNAm to determine their individual epigenetic aging rates and their correlation with several EVCs. She also verified the association between the epigenetic age acceleration (EAA) with single nucleotide polymorphisms (SNPs) and various measures of skin and hair aging. Based on genomic and epigenomic data, she finally developed several prediction models for perceived age and age-related EVCs.

Ms. Noroozi's thesis has essentially correct structure characteristic of experimental studies. It contains abstract in both English and Polish, a short glossary, a concise introduction, an appropriate theoretical background, a comprehensive characteristic of biological material, an adequate description of molecular and bioinformatic methods, a detailed presentation of the results and an appropriately structured and critical discussion, justified by the data. The thesis is accompanied by a short appendix including two figures with summary statistics of different DNAm measures and one table with a list of differentially methylated positions (DMPs) detected for various EVCs in the epigenome-wide association analysis (EWAS). The study's reference list contains as many as 210 correctly selected positions. One minor issue on the dissertation's structure concerns the actual lack of the study's objectives, at least in the form of a separate subsection. Also, the study lacks formal conclusions, usually required in PhD theses. Instead, the objectives are scattered thorough the text, being mostly presented in the "Abstract". Some elements of the objectives were also indicated in the last sentence of the Chapter 2 ("Theoretical background"). Meanwhile, four conclusions are implicitly presented in the Abstract in the form of "validated hypotheses", and "Discussion" (Chapter 5) is accompanied by very general concluding remarks only. Such structure resembles the one usually employed in original scientific articles rather than PhD dissertations. While that is of minor importance, it seems that the study, being complex and rich in the experimental data, would benefit from classical structure.

"Theoretical background" of the PhD thesis (Chapter 2), preceded by a short "Introduction" (Chapter 1), is concise, but appropriate and perfectly understandable. The author presents essential information on the complex process of aging itself, followed by its epigenetic background, with special emphasis on methylation changes. That logically leads a reader to considerations on different DNA methylation-based estimators of aging, including consecutive DNAm Age clocks, clearly displayed on Figure 1. In the final sections of Chapter 2, more specific information of biology of facial skin and hair aging is presented, together with its genomic and epigenomic background. Practical applications of the studies on human appearance traits are also emphasized. Importantly, they are not restricted to forensic science, but also to more general medical and cosmetic research, putting the thesis in a broader perspective. Summarizing, Chapter 2 possesses appropriate structure and contains information essential for complete understanding of the

dissertation's research. Most importantly, all threads introduced in this chapter are further continued and developed in the discussion section (Chapter 5).

"Materials and Methods" section (Chapter 3) constitutes a painstaking and very informative characterization of biological material. Special emphasis was put on detailed reconstruction of facial aging characteristics of the study's participants, which is justified in the light of further genomic and epigenomic association analyses. A minor remark on this part concerns the actual number of blood samples – in the subsection 3.1 and further parts of the study dedicated to molecular analyses the sample number is 741, while in the tables presenting the participants' descriptive, lifestyle and facial aging characteristics, (Tables 1, 2 and 3, respectively), the number is 735. Molecular, bioinformatic and statistical methods were described in the manner detailed enough to be repeated by other researchers. It is worth noting that the thesis employs advanced and complex tools of modern genomics and epigenomics, including epigenome- (EWAS) and genome-wide association analyses (GWAS). In- silico, statistical and prediction modelling methods are sophisticated and relevant for the data obtained.

In the „Results" section (Chapter 4), the PhD candidate presented the results of DNAm age estimations, correlations, both epigenomic and genomic associations, in-silico functional analyses, and performance evaluations of the newly developed prediction models in an absolutely understandable fashion, in the form of informative tables and figures. Irrespective of the multiplicity and complexity of the data, the section is concise and devoid of unnecessary repetitions. The latter particularly applies to the association data of different age-related EVCs (subsection 4.3), which are not easy to read themselves, but their presentation is clear and detailed enough to understand the main message. Moving the list of DMPs for various EVCs detected in EWAS analysis to Appendix (Table 1) was a good decision, since that data are particularly extensive. To sum up, Chapter 4 is perfectly organized and I actually have no issues to raise, neither on its structure nor its contents.

In the „Discussion" section (Chapter 5), the candidate maturely and critically interpreted the results of her own research, referring appropriately to the other groups' observations. The study itself contains many novel and original findings of considerable importance for both general aging and more applied forensic science. In particular, the thesis points to the specific DNAm Skin&Blood clock as the most accurate in predicting chronological age. Moreover, the study reports a number of novel associations between EVCs (e.g. hair loss and greying, various measures of skin aging) and various DNAm and EAA measures. The thesis also presents new association of one particular SNP located in the *SOCS2* gene, previously implicated in longevity. Another two SNPs reported in the GWAS were found to be associated with skin wrinkles and perceived age. Interestingly,

DMPs identified for facial aging were mapped to as many as 162 unique genes, including *EDAR*, involved in disease and hair, teeth and skin development. Finally, very promising models were suggested for estimating perceived age and for predicting facial wrinkles. As for minor critical remarks are concerned, the author emphasized relatively low statistical power and too small sample size for the GWAS analysis (p. 70). While this particular GWAS really appears to be underpowered due to the relatively low number of samples, the actual statistical power might had been estimated precisely before conducting the analysis. Another issue of minor importance concerns the statement that the age estimations obtained from PC clocks generally showed lower mean absolute error (MAE) values than their original epigenetic age clocks (p. 62). The contents of Table 5 (Chapter 4), as well as a narrative of the Discussion itself prove the opposite.

The references were presented consistently, with one exception of position no. 51 (p. 87), where some clerical error probably appeared. The thesis in general is correctly written and uses excellent English; some minor errors (e.g. "extremely" instead of "externally" in the Glossary, p. 1) can be easily corrected.

Summarizing, the findings of the PhD thesis by Ms. Rezvan Noroozi are interesting and original, significantly contributing to the complex fields of aging and forensic sciences. There is no doubt that the candidate correctly formulated and validated her own research hypotheses using up-to-date laboratory, bioinformatic and statistical methods. Ms. Noroozi unequivocally has theoretical knowledge in biomedical sciences and practical skills necessary for conducting her own scientific research.

Therefore, I officially state that Ms. Rezvan Noroozi, MSc, deserves the award of a doctoral degree in biomedical sciences at the Jagiellonian University of Kraków. Moreover, owing to the scientific originality and excellence of the submitted thesis, I suggest the candidate to be rewarded for her dissertation with a specific distinction, according to the University's policy.

With best regards,

Professor Tomasz Grzybowski, PhD, DSc