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REVIEW

of the PhD thesis by Magda Lewandowska, MSc, entitled "Variation of Polish and Lithuanian gene pool over time – alleles predisposing to infectious diseases"

The PhD thesis presented by Magda Lewandowska is of general interest for experts in the field of medicine and molecular and evolutionary biology, as ancient DNA (aDNA) studies have the capability to reveal insights into the presence and evolution of infectious diseases and the mechanisms and genetic background of host-pathogen interaction. Despite that there are many studies available on the occurrence of TB in various populations of different time periods and geographic locations, there was only very few work done dealing with susceptibility genes and genetic predisposition to infectious diseases in ancient populations. Therefore, the PhD thesis makes an important contribution to this research field and helps to bridge the gap between the current knowledge on the presence of infectious diseases in the past and the genetic makeup of the human host.

In this PhD thesis molecular methods were used to analyze aDNA from ancient human skeletons with a focus on alleles predisposing to infectious disease. Within the last three decades, it has become possible to extract minimal amounts of degraded DNA from bones and mummified tissue and amplify and sequence human as well as bacterial DNA. This has allowed to proof the presence of certain diseases in ancient population, collecting information on the ancestry of a population, e.g. by determining the mitochondrial haplogroups, and to unravel the susceptibility and predisposition to diseases. The applied analytical methods in this work as well as the statistical analysis are fully adequate to answer the main research questions of this PhD thesis.

It should be noted that the presented PhD thesis has been realized at the Department of Molecular Biology, Medical University of Łódź, the first and oldest Polish research center for the study of ancient DNA, founded and led by Prof. Henryk W. Witas.

The PhD thesis is generally were well written and the text preparation is consistent and characteristic for an experimental study. It includes an abstract, a theoretical background and introduction, a detailed description of the applied methodology, a results section and discussion followed by the main conclusions. At the end of the thesis a list of publications by M. Lewandowska, MSc, is included. The work is supplemented with seven concise appendices. The graphical illustration of the thesis has been developed appropriately, all figures and tables are informative and well structured.

The chapter **Introduction** represents a good and detailed description of the field of ancient DNA, explaining perfectly the challenges and limitations, but also the potential of aDNA research. It mainly focuses on ancient human DNA, thereby reflecting the main topic of the thesis. The guidelines for working with ancient DNA as defined by Cooper and Poinar (2000) are described and

explained in detail. This is a very important pre-requirement for any successful work in this research area, and particularly important for aDNA studies that are based on PCR amplification and direct sequencing. In other studies, using next-generation sequencing (NGS), new criteria have been introduced, such as damage pattern or measuring of fragment lengths, which was described in the thesis accordingly (see pages 25-26).

The chapter **Genetics of infectious diseases** provides a profound introduction to the complex topic of host-pathogen interaction and the co-evolution of infectious diseases and humans. The most important aspects are described and Mrs Lewandowska also manages to underline the complexity of the topic and the various factors that are influencing the occurrence and spread of infectious diseases in populations. The polymorphisms used in this PhD work are described in sufficient detail, including the relevant genetic changes.

The following chapter **Population genetics** contains a very detailed description of the evolutionary mechanisms with regard to the topic of the PhD thesis. In order to understand the complexity of the topic of this study, it is important to describe the main factors, such as evolutionary forces (mutation, gene flow and drift, etc.) or genetic variation, that are involved in the interplay between pathogen evolution and human adaption. Some parts of the description could have been more condensed as they have to be considered as basic knowledge.

The **Aim of the study** is well and clearly described and puts the focus on the six studied polymorphisms and the comparison to data from modern populations from Poland and Lithuania.

In the chapter **Material and methods,** the analysis of the samples was described with regard to the specific requirements when working with ancient DNA. The sample size (n=327) is within a very good range for aDNA studies, given the huge efforts that are necessary for extracting and analyzing ancient specimens. The material is very well selected from different periods, including samples from the 1st century to the 16th-18th century, .
Extensive efforts were undertaken to avoid contamination and authenticating the results. The analyses were done in a specialized lab that allows the avoidance of external contamination with modern DNA or cross-contamination of the samples during handling. The DNA extraction followed a well-established protocol for ancient skeletal specimens and the various DNA amplification protocols were well designed and adapted to aDNA characteristics, such as short PCR fragments and high PCR cycle numbers. The PCR and RFLP products were visualized by gel electrophoresis and sequenced with the conventional Sanger method.
The results underwent a detailed statistical analysis, in order to calculate and compare allele frequencies with modern populations from Poland and partly Lithuania and test for possible evolutionary or demographic processes. It must be highly appreciated that Mrs Lewandowska applied this wide variety of statistic test in her PhD work, as this represents an important step for the validation of the results of this study. This is in contrast to several other studies in this field of research, where results are often not validated with the same thoroughness.

The **Results** first present a summary of the quality measurement for the presence of aDNA in the samples, that is described as DNA yield. The DNA preservation in the samples appears to be surprisingly high with values ranging from 54% to 100% for the DNA yield and 26% to 89% for the allele yield. The allele distribution in the ancient populations and the comparison to modern populations revealed statistical significant differences of the six studied polymorphisms in almost all different time periods, except for the group of the 2nd – 7th century in Lithuania. Moreover, the study successfully shows a high level of homozygosity within the different subpopulations that was further substantiated by F_{ST} analysis. The study further points toward a significant contribution of external gene pools including Neolithic haplogroups, such as N1a. Another important result of this

study is shown by allele fluctuations that indicates four of the studied polymorphisms were subjected to genetic drift, while the two remaining were under purifying or balancing selection.

The results are followed by as very good and thorough **Discussion** of the achieved results, considering both the limitations of the study, such as small sample numbers, difficulties of assessing allele frequencies, etc. and the perspectives of this work. Further analyses could extend the sample size and add more ancient specimens from pre- and post-Neolithic times, in order to better understand the complex interaction of population size and evolutionary processes regarding pathogen resistance. It has to be acknowledged that the PhD candidate is fully aware that the work with ancient DNA and in particular with human DNA is very challenging. Therefore, the results have to be interpreted with great caution, as the determination of alleles could be hampered by degradation effects, such as sequence modifications, dropout effect, etc.

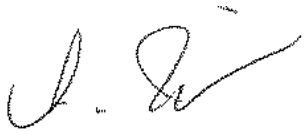
Finally, the **Conclusions** provide a consistent summary of the main findings of this PhD work. This chapter underlines the enormous efforts that were undertaken for the study of the 327 skeletons from Poland and Lithuania. The PhD thesis shows in an excellent way how aDNA studies can contribute to our understanding of the co-evolution of pathogens and humans and it could serve as a model for further similar studies in this field. A better understanding of the evolution of infectious diseases may further contribute to the continuing challenges in fighting diseases, such as tuberculosis and malaria, and looking for new ways to treat, prevent and avoid these deadly diseases.

The PhD thesis is generally very well written and illustrated sufficiently. There a just a few minor issues that could be improved. In some parts, the English could need some improvement and I would recommend to use the help of an English native speaker for final corrections. In the introduction, the PhD candidate should consider some recent work on the use of petrous bone for sampling, as it seems to contain the highest amount of endogenous DNA. The description of some parts of the population genetics section are too basic and could be shortened. The possible use of NGS for further analysis could be mentioned and discussed in the Discussion. It could help to improve the allele determination and further rule out possible contamination.

The minor comments and few suggestions mentioned above do not diminish the achievements of this study and my positive evaluation of the PhD dissertation of Magda Lewandowska, MSc. The dissertation meets all the requirements for a doctoral thesis and represents a comprehensive and competent approach to the presented scientific problem. The reviewed PhD work contains new and important scientific findings that were achieved by thorough application of appropriate research methods.

Taking into account the research concept and the amount of new knowledge achieved by the results, I submit to the High Council of the Faculty of Biomedical Sciences and Postgraduate Education a request to admit Magda Lewandowska, MSc, to further stages of the doctoral defense.

Bolzano, April, 18th 2017



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