New Daicel Arbor Biosciences myBaits® Publication: "*Evolution of immune genes is associated with the Black Death*" - Nature - October 2022

The Black Death was a devastating pandemic caused by the bacterium *Yersinia pestis* that resulted in severe mortality throughout Europe and beyond in the mid 1300's. To identify regions of human genome associated with the immune response that were impacted by natural selection as a direct result of the Black Death, a team led by researchers from McMaster University and the University of Chicago used ancient DNA techniques including *myBaits® target* enrichment to sequence immune genes from populations of individuals in London, England and across the country of Denmark that died before, during, and after the Black Death. The team then applied population and functional genetics to delve deeper into the effects of the variants that were found to have the strongest signals of selective pressure due to the Black Death.

Several researchers currently employed by Daicel Arbor Biosciences participated in this study, including one of the joint lead authors **Dr. Jennifer Klunk**. Dr. Klunk currently leads the myReads® NGS services group at Daicel Arbor Biosciences, and this paper was a major part of her doctoral research at the McMaster Ancient DNA Centre.

The Arbor team was able to sit down with Dr. Klunk this week to ask her some questions about this fascinating new research study!

Arbor: *"Congrats to you and your co-authors on the new paper! What does this new study illuminate about the impacts of the infamous Blank Death pandemic on the human population?"*

Dr. Klunk: "Thank you! This work was a major team effort and it would not have been possible without my co-lead author, Dr. Tauras Vilgalys, who lead the bioinformatics analyses. In this new study, we used a tightly dated ancient DNA sample set to determine whether there exists a signal of selective pressure on immune-related loci due to the Black Death – and the answer was yes! Then, we looked to identify variants that exhibited a strong signal of selective pressure. We found four strong candidates and our top candidate variant was in a gene called *ERAP2*, which is a known contributor to immune response to modern day diseases. The allele that we identified as protective appears to have increased the chances of survival during the plague by as much as 40%, which is a really dramatic effect. Our next step was to confirm that the variant that we identified is directly involved in the immune response to *Yersinia* pestis and what that involvement looks like. We performed *in vitro* experiments to evaluate the effect of the *ERAP2* variant on gene expression and cytokine response of cell lines infected with *Y. pestis.* What we found is that the protective allele increases *ERAP2* expression in relation to the deleterious allele

and that more copies of the protective allele allow for better restriction of intracellular replication of *Y. pestis.* With these results, we were able to determine that we had found a variant that was under selective pressure due to the Black Death and show that it is involved in the immune response to *Y. pestis.* This is an exciting result because it is empirical evidence that connects selective pressure to a specific disease event. The protective *ERAP2* variant is still around today, and in fact, it is present at pretty high frequencies. However, this variant has been linked to autoimmune and inflammatory disorders in modern populations, suggesting that there is a cost to maintaining this variation in the population. What we are seeing, then, is balancing selection in action – the benefit of defense against pathogens like *Y. pestis* seems to be counter-balanced against the cost of immune disorders."

Arbor: *"How was myBaits target capture used in this research, and how did this approach compare with alternative options that you may have considered?"*

Dr. Klunk: "We applied several myBaits Custom targeted capture kits to over 300 ancient DNA samples from individuals who died before, during, and after the Black Death in order to sequence a set of putatively neutral loci and immune-related loci that may have been under selection due to the Black Death. When we started this project, we screened a small number of individuals to assess DNA preservation to see if whole genome sequencing would be a feasible approach to answer our questions. We found that the samples contained, on average, about 2% human DNA. We would have had to sequence billions and billions of reads from each sample to get good coverage of our loci of interest, which was just not feasible for our project's sample size – or our budget – not to mention that the complexity of an ancient DNA library is typically exhausted long before it hits the billion read mark. With myBaits, we were able to sequence each individual to about five million reads to get the data we needed, which let us include many more individuals in our study than we would have been able to do if we went with a whole genome sequencing approach."

Arbor: *"What was your favorite part of this project?"*

Dr. Klunk: "I was fortunate enough to be able to travel to London to collect small samples from the skulls of some of the individuals who died during and after the Black Death. Handling these individuals' remains not far from where they were originally buried really put the work we were doing into context for me."

Arbor: *"How is your current work with the myReads team informed by your experiences in ancient DNA research during your Ph.D.?"*

Dr. Klunk: "Over the course of my Ph.D., I handled hundreds and hundreds of ancient samples from subsampling through data analysis. From optimizing extraction techniques to nailing down appropriate capture conditions, I learned how to deal with difficult samples and how to troubleshoot each piece of the NGS pipeline. I'm lucky that my position with the myReads team here at Arbor allows me to put those skills in project planning and execution to good use for other researchers who are also working with difficult or unusual samples. I like to say that when you are used to getting ancient DNA samples to work, fresh samples are no problem!"

The full research article was published in Nature on October 19, 2022, and is available at the following link: https://www.nature.com/articles/s41586-022-05349-x

For additional information about the implications of this fascinating research, please check out the following scientific news:

- https://brighterworld.mcmaster.ca/articles/black-death-immunity-genes/
- https://www.sciencenews.org/article/black-death-immunity-gene-crohns-disease-health
- https://www.statnews.com/2022/10/19/black-death-may-have-influenced-evolution-of-genes-involved-in-immune-responses-study-finds/
- https://www.nytimes.com/2022/10/19/science/bubonic-plague-black-death-genetic-protection.html

Daicel Arbor Biosciences provides myBaits Custom enrichment kits for any targeted sequencing application, including degraded DNA and other sensitive sample types. In addition, our myReads NGS services team can handle DNA/RNA extraction, library preparation, target capture (if applicable), sequencing, and bioinformatics for any sample type, even ancient DNA.

Contact our team today if you are interested in deploying similar techniques in your research program!

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