NNPDF Research Fellowship #20

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Peter G. Pentchev NPC Research Fellowship

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PROJECT TITLE: Generation of humanized mouse models for Niemann Pick disease Type C
PROJECT DATES: September 1, 2020 – August 31, 2021

Lay Summary

In our project, *Generation of humanized mouse models for NPC*, we sought to establish more clinically relevant animal models for the study of NPC. Our previous research had shown that the pathogenicity of certain NPC1 mutations was not conserved between humans and mice; some mutations known to cause disease in humans do not cause disease when introduced into mouse models. Accordingly, to thoroughly understand how these mutations cause disease – and thus how we might develop effective therapies – we needed a mouse model that expressed the human NPC1 gene.

We used the CRISPR gene editing system to place the coding portion of the human NPC1 gene just before the mouse Npc1 gene. As a result, this mouse model expresses the human, rather than mouse, NPC1 protein. This initial mouse model expressed a healthy copy of the human protein, and we had planned to introduce a series of mutations to establish mouse models with various NPC1 mutations to begin understanding how these mutations might differentially impact disease phenotypes in an animal model.

Before making these mutant mouse models, however, we characterized our “humanized” mouse model which expressed a healthy copy of human NPC1 protein. We first quantified the expression of human NPC1 protein and found that in all tissues assayed – including the brain, liver, and spleen – and found a significantly lower expression level compared to healthy control mice. It has been well-documented in the field that expression level is a major determinant of pathogenicity of NPC1 mutations. In the case of the common NPC1 p.I1061T mutation, for example, artificial overexpression can lead to amelioration of disease phenotypes. For this reason, this model is unfortunately not viable to serve as a starting point for modelling patient mutations. Since the human protein levels are diminished in this model, if a mutation were introduced, our interpretation of the biological impact of that mutation would be confounded by artificially low expression levels.

This mouse model was helpful, however, in helping us gain insight into a longstanding question surrounding NPC disease: how much NPC1 protein is enough to protect against disease manifestations? Since NPC is a recessive disease, it is known that having one copy, or 50%, of healthy NPC1 is sufficient to protect against disease. It is not known, however, what the lower

threshold is for protecting against disease. Answering this question will be helpful in evaluating and developing therapeutics, such as proteostatic therapies, that aim to rescue partial function from mutated NPC1 protein and will improve our understanding of NPC disease more broadly.

We found that our mouse model, which expressed 10-15% of healthy NPC1, developed a unique disease presentation, distinct from that of classical NPC mouse models. Despite having markedly less NPC1 expression, the humanized mouse model did not lose body weight and had only a moderately shortened lifespan. While the humanized mouse model did develop mild neurological symptoms, they were vastly attenuated compared to typical NPC mouse models. This suggests that the lower threshold of healthy or functional NPC1 expression needed to greatly reduce and eliminate disease phenotypes is likely far less than 50%. This is a positive result for those developing therapeutics focused on partial rescue of mutant NPC1 protein; a clinical benefit may be achievable with relatively minor functional rescue.

As mentioned previously, this mouse model was generated by inserting the coding sequence of the human NPC1 gene into the mouse genome. We have now collected preliminary evidence that the issues we faced with low expression levels of human NPC1 can be corrected by including both the coding and non-coding portions of the human NPC1 gene. To this end, we have designed a new gene editing strategy where we will be replacing the entire mouse NPC1 gene with the entire human NPC1 gene, including both coding and non-coding regions. We are now in the early stages of generating this mouse model, which we anticipate will maintain normal levels of NPC1 expression, and thus can be used to generate a variety of mouse lines with different human mutations. These eventual mouse models will be critical in understanding and defining mutation-specific disease phenotypes found in the NPC patient population. These models will also represent more relevant systems to evaluate pre-clinical NPC therapies, since they will be tested in the context of the human, rather than mouse, NPC1 protein.