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Lay Summary

The cerebellum is the part of the brain that typically co-ordinates balance and movement/motor control. In NPC1, this area of the brain is severely affected, with the neurons at the core of the cerebellum – Purkinje cells – one of the earliest and hardest hit in this disease, although there are a subset of Purkinje cells that show less pathology than all the others.

At the start of the fellowship we had a number of research aims,

- Characterise the precise sequence of biochemical changes in the NPC1 cerebellum
- Better understand the differences between vulnerable and disease-resistant areas of the cerebellum
- Determine whether there were any unreported problems in the NPC1 cerebellum
- Establish criteria with which to assess the effect of potential therapies on neuropathology
- Provide proof of concept that the NPC1 cerebellum is amenable to the transplantation of new neurons

We have analysed the storage lipids in the mouse NPC1 cerebellum. The aim was to determine how cerebellar lipid storage progresses over time. Sphingosine (one of the lipids stored in NPC disease) was elevated as early as one week of age in the NPC1 mice where as glycosphingolipid levels rose later in disease, and cholesterol levels did not increase in this part of the brain over the lifespan of the mouse. Evidence from a previous study suggests a defect in lysosomal calcium in NPC1 cells (Lloyd-Evans et al, Nat Med. 2008. 14(11):1247-55). We tested slices of NPC1 mouse cerebellum and found this defect is also present in neurons. The young age of the slices indicate that the calcium defect is likely an early event in NPC neuropathology.

It is also important to know where in the cerebellum the lipids are accumulating. Not all Purkinje cells are affected in the same way in NPC1, and can be looked at as three distinct groups, one group that dies off fairly early in disease, a second group that dies off significantly later in disease, and a third group that, over the lifespan of the NPC1 disease mouse are resistant and survive. We studying these groups of Purkinje cells, as the differences between them will give us insights into what aspects of the disease process are killing these important neurons, and what is different about the Purkinje cells which survive. This information will be key to inform future therapeutic approaches aimed at maximising Purkinje cell survival.

Staining of GM1 (a glycosphingolipid stored in NPC1 disease) was optimised and then used together with the cholesterol-label filipin to study Purkinje cells. Cholesterol accumulates in Purkinje cell bodies that die early in pathology, but also in the disease-resistant subset of Purkinje cells. The same is true for GM1. In agreement with another recent report (Elrick et al, Hum Mol Genet, 2010, 19(5):837-47), this indicates that while cholesterol and GM1 storage is part of neuropathology, it is unlikely that the accumulation of these lipids is the cause of Purkinje cell neurodegeneration.

We noted a very early increase in cerebellar levels of sphingosine, an early pathology noted in non-neuronal NPC1 cells. To see if there are differences in how sphingosine is stored in vulnerable and disease-resistant areas of the cerebellum, we need a way of analysing the different cerebellar areas independently. We have developed a way of micro-dissecting the mouse cerebellum in such a manner that we can collect these different areas, and are now working on measuring their sphingosine levels.

There are also very striking differences in brain inflammation between these different groups of Purkinje cells, with higher levels of damaging inflammation in the areas which die sooner in disease, and a lack of inflammatory progression in areas which survive the longest. This tells us that the different groups of Purkinje cells are not just dying off at different times but are interacting with the brain immune system differently.

At the NNPfF conference in Toronto, there was a presentation that described the use of Calbindin as a potential biomarker in NPC1. Calbindin is a protein naturally expressed at high levels in Purkinje cells. According to our work so far, calbindin levels in NPC1 Purkinje cells are initially lower than normal. The levels of Calbindin then seem to

recover dramatically, and even exceed what is found in normal Purkinje cells. Higher levels of calbindin correlate with the neurons that survive the longest, indicating that calbindin may not only be important as a biomarker, but also have a role to play in the disease itself.

The combination of an early rise in sphingosine levels and an early defect in lysosomal calcium regulation in the NPC1 cerebellum begged the question, “if these things are going wrong early in the development of the mouse NPC1 cerebellum, is there anything else going wrong?” We have noted a small but consistent number of Purkinje cells that seem to fail to develop properly. Normally, after these neurons are generated deep inside the brain, they move upwards until they reach the correct position. Instead, this small pocket of Purkinje cells seems to have gotten stuck halfway. While this is not the case for the majority of NPC1 Purkinje cells, it does indicate that there may be problems in neurons far earlier than previously thought, as the transition of these neurons to the correct position in the brain normally occurs before the mice are born.

Together with the early storage of sphingosine, the early calcium defect and the initially lower amount of calbindin, this indicates there are a number of NPC1 phenotypes that occur very early in disease progression. Understanding these phenotypes could suggest new targets for biomarkers. Understanding these disease processes better, such as the ways the different subsets of Purkinje cells are affected in NPC1 and the nature of neuroinflammation, are also giving us better criteria with which to judge the effectiveness of novel therapy combinations on the brain in our mice studies.

While drug treatments may slow the progression of disease, they cannot replace the neurons that die. Normally the mature brain is poor at accepting transplanted neurons, but initial research shows that a diseased brain affected by inflammation may be better at incorporating new neurons. We trialed the transplantation of healthy mouse cerebellar stem/progenitor cells in symptomatic 6-week old NPC1 mice to see whether the transplantation of new cells was viable and if the NPC1 cerebellum could support their development. Transplanted cells survived and developed into Purkinje cells suggesting that the NPC1-brain can accept new neurons through transplantation. The hope is that the transplanted cells are able to develop correctly into neurons and help replace the function of neurons lost in the disease, however whether they can be delivered in high enough numbers or prove useful in delaying or reducing symptoms remains to be tested.