QnAs with Hugo J. Bellen

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Hugo J. Bellen's path to genetics was circuitous. At first, he tried his hand at business engineering, economics, and veterinary medicine, but he eventually found his calling once he discovered that the fruit fly *Drosophila* could be used to disentangle the genetic underpinnings of development. Now a professor of molecular and human genetics at Baylor College of Medicine in Houston, Texas, Bellen has helped decipher how the nervous system develops and uncovered mechanisms underlying neurodegeneration. Developing ways to advance the field of *Drosophila* genetics, his laboratory has fashioned sophisticated genetic tools and numerous reagents that are now publicly available. In line with this endeavor, Bellen runs the Gene Disruption Project, which aims to target all known fly genes with new loss-of-function tools. Bellen's contributions to neurobiology and genetics led to his election to the National Academy of Sciences in 2020. His Inaugural Article (1) builds on a series of studies that establish a critical role for lipid metabolism in Alzheimer's disease (AD). The article describes a molecular pathway by which gene variants linked to AD affect lipid droplet formation in glial cells. The lipid droplets protect the brain from damage caused by reactive oxygen species (ROS), a main factor in aging brains, AD, and other neurodegenerative diseases. Bellen discussed his work with PNAS.

PNAS: Could you provide the context for your Inaugural Article (1)? What led to it?

Bellen: We started by doing a general genetic screen in *Drosophila* for mutations that cause neurodegeneration (2). We selected mutations that are lethal when homozygous and created flies that are heterozygous for the mutations but have homozygous mutant eyes. We then screened for flies that lost vision over time, telling us that we had identified a gene that is required for neuronal maintenance. Most pertinent for the Inaugural Article (1), among the genes we found was one associated with a rare neurodegenerative disease called Leigh syndrome. We showed that its loss caused a very significant accumulation of lipid droplets in glial cells. We then looked at a few other genes that encode mitochondrial proteins and saw the same phenotypes: lipid droplet accumulation in glia.

PNAS: So, you followed the lipid trail; where did it lead?

Bellen: Further study showed that all three genes encode proteins required for proper mitochondrial function. We then showed that neurons with defects in these mitochondrial genes exhibit high levels of ROS (3), which are often associated with neurodegeneration. From there, we worked out the mechanisms that lead from high ROS levels in neurons to lipid droplets in glia. In essence, the neurons get rid of the ROS by peroxidating lipids that are transported into the extracellular space, where apolipoproteins take them up to form lipoproteins. Glial cells then take up the lipoproteins and form lipid droplets. When there are too many lipid droplets, the glia can't cope; the lipid droplets burst and the glia get sick and die. Then the neurons die. We also found that preventing lipid droplet formation contributed to neurodegeneration because the peroxidated

Hugo J. Bellen. Image credit: Catherine Tasnier (photographer).

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lipids accumulate in the neurons and cause toxicity. Thus, this is an important homeostatic process that, when disrupted, is problematic.

PNAS: How did you connect these findings to AD?

Bellen: We found that a key gene in flies is required for lipid droplet formation, *APOD*, and the human homolog, *APOE*, can substitute in fruit flies for the loss of *APOD*. However, the *APOE4* allele that is a major risk factor for AD fails to do the job. These observations formed the basis of [the] current paper (1).

PNAS: Does your current work strengthen the link between lipids and AD?

Bellen: Correct. We started digging into the mechanism. What proteins are required for lipid secretion? What proteins are required for neuronal export? How are they captured? What are the key other players in that pathway? It turns out that many of the players are genes that other studies have shown predispose people to AD based on genome-wide association studies. This paper (1) provides molecular mechanisms to explain why these genes are causing AD. We knew that many of the genes encode proteins that are involved in synaptic transmission. But if you knock them out completely in all cells, instead of observing a slow degeneration like you see in AD, they are usually lethal. With our work we only reduce the proteins in one cell type, neuron or glial cells, to observe a slow progressive neurodegeneration. A 50% reduction of the proteins in one of these two cell types in most cases is sufficient to disrupt glial lipid droplet formation and cause neurodegeneration. This pathway is incredibly dose-sensitive and responds very quickly to a relatively modest reduction. That really tells us something about the mechanism and how it can be associated with AD.

PNAS: Do these findings establish lipid droplets as a key player in AD?

Bellen: Yes, we provide a function for genes that cause AD as part of a very specific pathway in the brain that is linked to high levels of ROS and the formation of lipid droplets in glia that are neuroprotective. If you impair that pathway genetically, you're predisposed to getting AD. When we started talking about this work in 2015/2016, the idea that lipid droplets were associated with neurodegenerative diseases was not well received. We received a lot of pushback. But that has completely changed, and there's a very significant set of publications now that support our findings. In fact, the NIH recently announced a [call for proposals to investigate links between] AD, lipids, and lipid droplets. In general, we're finding that lipids play a really important role in many neurodegenerative diseases. In AD it's the sequestration of peroxidated lipids in glia. In Parkinson's disease it's the elevation of ceramides that seems really important (4). Of course, the brain is 60% or more lipids, so it's not that surprising that lipids are going to be important.

1 M. J. Moulton *et al.*, Neuronal ROS-induced glial lipid droplet formation is altered by loss of Alzheimer's disease-associated genes. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2112095118 (2021).

2 S. Yamamoto *et al.*, A Drosophila genetic resource of mutants to study mechanisms underlying human genetic diseases. *Cell* **159**, 200–214 (2014).

3 L. Liu *et al.*, Glial lipid droplets and ROS induced by mitochondrial defects promote neurodegeneration. *Cell* **160**, 177–190 (2015).

4 G. Lin *et al.*, Phospholipase *PLA2G6*, a parkinsonism-associated gene, affects Vps26 and Vps35, retromer function, and ceramide levels, similar to α-synuclein gain. *Cell Metab.* **28**, 605–618.e6 (2018).