

Plants provide hope for epilepsy initiative

Dr Matthew Gentry is an award-winning biochemist at the University of Kentucky, whose research into plant starch metabolism may unexpectedly lead to a cure for a fatal form of epilepsy – Lafora disease. His latest project is part of an ambitious NIH-funded programme known as the Lafora Epilepsy Cure Initiative, which involves a global collaboration of scientists and researchers, utilising state of the art technologies to determine potential treatments.

Lafora disease is a rare, congenital, neurodegenerative disorder that cuts seemingly healthy teenagers down in their prime. Over the course of a decade, sufferers develop increasingly severe and frequent seizures accompanied by headaches, dementia, hallucinations and finally death. A remarkable series of fundamental scientific findings led Dr Gentry and colleagues to uncover the cause of this condition: a faulty gene involved in the formation of glycogen, the main carbohydrate storage molecule in animals. He now hopes to build upon this research to find a cure for this most cruel of diseases.

GETTING TO GRIPS WITH GLYCOGEN

The main source of energy in animal cells is glucose – the simplest form of sugar. Animals store glucose molecules for future use by combining them into a carbohydrate ‘polymer’ called glycogen. Spherical glycogen molecules each contain around 55,000 glucose units, and are soluble in water, making them readily available for breakdown during bursts of energetic activity.

Glycogen is needed most in hard-working cells such as muscle, liver and brain cells. Brain cells are, however, acutely sensitive to changes in glycogen levels: too much glycogen, or any abnormal forms of the molecule, can induce damage and even cell death.

In the early twentieth century, a clinician called Dr Lafora noticed that certain brain cells of some teenage-onset epilepsy patients contained, instead of water-soluble spheres of glycogen, accumulations of a water-insoluble carbohydrate – now known as ‘Lafora bodies’. Dr Gentry’s collaborators have since demonstrated in laboratory animals that Lafora bodies cause brain cell death and Lafora disease. But how, and why, do these mysterious Lafora bodies form?

THE CRUCIAL ROLE OF PHOSPHATE

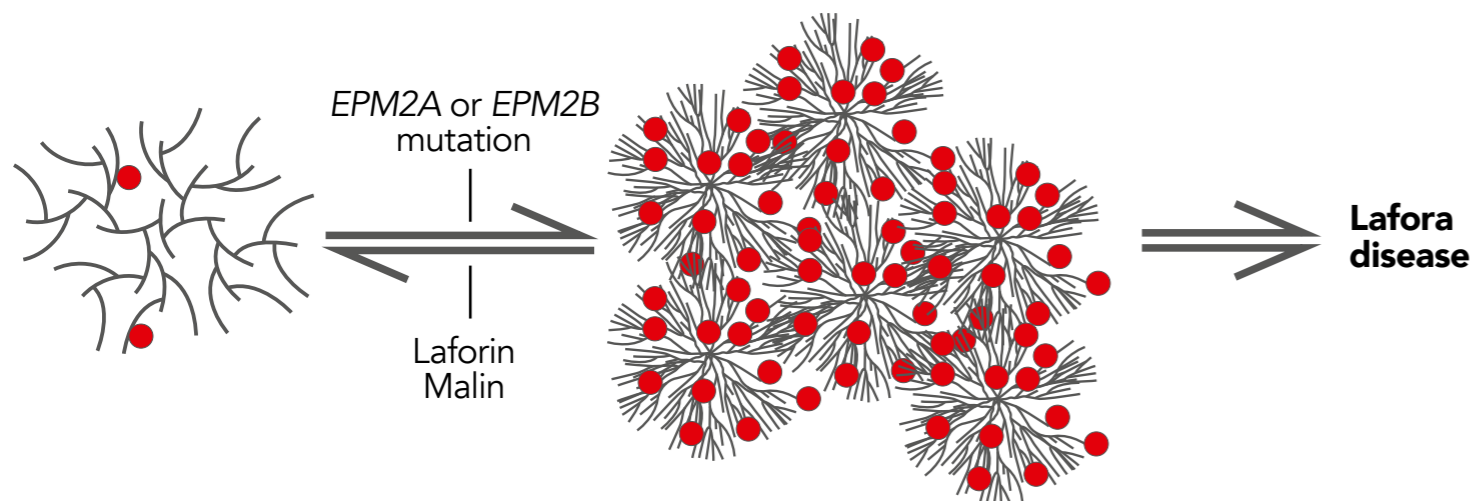
To answer this, we must turn to Dr Gentry’s work on carbohydrate metabolism in plants. Unlike animals, plants store glucose as an insoluble polymer called starch. To release energy from starch, enzymes called ‘dikinases’ ▶

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Glycogen

Lafora body



add small ions called phosphate groups to the outer surface of the polymer, which make it water-soluble and allow glucose molecules to be released. However, once the first layer of glucose molecules has been removed, the phosphate groups form an impenetrable layer preventing further glucose release. A second set of enzymes, 'phosphatases', are needed to remove the phosphate groups and allow more glucose molecules to be freed.

Early in his career, working with Dr Carolyn Worby, Dr Gentry identified one of the key starch phosphatases in plants, dubbed 'Starch-Excess 4' (SEX4), as well as an analogous enzyme in humans called 'laforin'. Dr Gentry proposed that laforin might also be able to remove phosphate groups from the surface of glucose polymers such as glycogen, ensuring it stays soluble and accessible for use as an energy source by cells, a hypothesis which was later proven by collaborators at Indiana University and the University of Toronto.

In the absence of laforin, glycogen molecules accumulate phosphate, turning them into insoluble and useless Lafora bodies, very

similar to plant starch. While Lafora bodies can accumulate in all cells, it is in the brain that they cause most damage, resulting in the symptoms of Lafora disease.

The gene that codes for laforin is known as *EPM2A*, and mutations in *EPM2A* – producing a defective laforin enzyme – account for around half of all cases of Lafora disease. Dr Gentry and his collaborators have determined the precise structure of laforin, which explains how it functions, and how mutations in different parts of the gene affect the molecule's activity in subtly different ways. The other half of Lafora cases, however, result from mutations in a related gene, *EPM2B*, which encodes the enzyme malin, the exact function of which is still not known and remains a key question for research.

DIAGNOSIS, TREATMENT AND CURE

This newfound understanding of the molecular and genetic causes of the condition may just give scientists the tools they need to find a cure. Prompted by the families of Lafora disease patients, Dr Gentry has teamed up with researchers from across the world who have helped to uncover the

causes of Lafora disease, to form the 'Lafora Epilepsy Cure Initiative' (LECI). With a grant from the US National Institutes of Health, their aim is to convert their fundamental scientific knowledge into early diagnosis, treatment and cure, before too many more teenagers tragically succumb.

Using mouse models, the team have shown that Lafora disease can be cured by inhibiting Lafora body formation. They now intend to use their knowledge of laforin and malin to diagnose precisely how different genetic mutations in different patients alter the activity of these molecules, resulting in the formation of Lafora bodies and onset of the disease. Their ultimate aim is to develop personalised treatments for the full spectrum of molecular and cellular causes of the condition.

The LECI will explore multiple treatment possibilities for Lafora disease, including state-of-the-art genetic and molecular approaches to reduce the amount of glucose stored as glycogen in Lafora patients. So, for instance, biological agents such as viruses can be used to modify gene activity, or additional enzymes could be used to degrade the Lafora bodies. The team also hopes to identify the ideal therapeutic 'window' following diagnosis in which there is the best hope that treatment will result in full recovery.

Lafora disease is one of several rare 'progressive myoclonic epilepsies', once thought to be neurological conditions but now, through the work of scientists like Dr

Q&A

Can you describe what Lafora bodies are and how they cause disease?

LBs are polymers of glucose. Glucose is typically stored as glycogen. Glycogen has a defined arrangement that includes 12–15 glucose molecules in a chain and then a branch occurs. This arrangement allows glycogen to be water-soluble. LBs have a disrupted or aberrant arrangement of branching and LBs resemble plant starch. Plant starch has longer glucose chains with less branching. This pattern allows the glucose chains to form helices (much like those seen with DNA) and these helices exclude water, making plant starch water-insoluble. LBs have fewer branches and longer glucose chains than glycogen, thus making them water-insoluble. As to why they cause disease, it is not entirely known. Two leading hypotheses are that: 1) they trap needed energy in the LBs and cells are energy deprived or 2) they literally clog up cellular trafficking.

When did you realise that your work in plants might have implications for the treatment of Lafora disease?

I'm a biochemist, and I'm interested in proteins. I was working on the LD proteins malin and laforin and trying to determine their biological substrates. I had a lot of failure and so I went back to the literature to read everything I could find about carbohydrates. I found papers on how plant starch has phosphate on it and that is when everything "clicked". I was

working on a disease where the patients have increased carbohydrate levels, the carbohydrates are hyperphosphorylated, and the protein is a phosphatase... so we thought that laforin could remove phosphate from glycogen and that the plant protein Starch-Excess 4 could be doing the same thing.

What is the importance of personalised diagnosis for Lafora disease patients?

Some mutations are more deleterious than others. So it will be more straightforward to treat the less deleterious mutations and more difficult to treat the more deleterious ones.

What do you mean by the 'therapeutic window' for Lafora disease and what is its significance?

There is likely a "window" where the body and cells can process Lafora bodies. There is probably a point of no return. We have to define where that point is and then we know we can treat up to that point. Then we have to figure out ways to move that point further so that we can treat all patients.

What is your ultimate vision for the LECI?

The LECI is the Lafora Epilepsy Cure Initiative, so our goal is that it ceases to exist and evolves into the cure for Lafora disease.

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Gentry, understood to be metabolic in origin. The impetus driving the LECI project may provide insights into many of these other heart-breaking disorders.

For Lafora disease, Dr Gentry is optimistic about the future, saying: "We see a clear track to develop effective methods to diagnose, treat, and ultimately cure Lafora disease. Our

vision is that one day a physician having diagnosed a child with this horrendous epilepsy would have the medicines and technologies to stop the disease in its tracks so that the child is able to resume development and live a healthy and normal life."

Detail

RESEARCH OBJECTIVES

Dr Gentry's research focuses on understanding how signalling failures within glycogen metabolism mechanisms cause the neurodegenerative epilepsy known as Lafora disease.

FUNDING

- National Institutes of Health (NIH)
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COLLABORATORS

- Peter Roach and Anna DePaoli-Roach, Indiana University
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- David Watt, University of Kentucky
- Antonio Delgado-Escueta, UCLA
- Tom Hurley, Indiana University
- Steve Johnson, Indiana University

BIO

Dr Gentry received his BS from the University of Evansville in 1996 before studying a PhD at Syracuse University until 2003. He later became a postdoctoral fellow at UC-San Diego before beginning his independent career in 2008. He is currently a Professor in the College of Medicine at the University of Kentucky.

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