

**PODCAST 1:****What inspired you to work in the field of NMD and more especially myotubular myopathy\* (\*MTM or X-Linked Myotubular Myopathy)?**

I got interested in working in neuromuscular disease (NMD) and in particular myotubular myopathy while I was a child neurology resident at Children's Hospital Philadelphia (CHOP). When I went there I thought I was going to be interested in epilepsy, like most neurologists, but in my first week as a Neurology Fellow we took care of a patient with myotubular myopathy and got to meet the family and the child, basically from diagnosis.

The clinic at CHOP is a very special one in that we had this very strong group of neuromuscular clinicians. I was with one of them on service and we met the patient, and then we immediately went to see the muscle biopsy in pathology and then we had the genetic results. Seeing everything together got me really interested from a scientific perspective. Very quickly with this family, and subsequent other families, the ability to really work with the family and with the child was something that really appealed to me as a paediatrician. The bond that forms with patients and families of neuromuscular disease is something that I think is very unique in medicine and neurology in that you really form this relationship with the families and with the patient, for their life. That really appealed to me as a clinician, somebody to really be able to make an impact not just at one point but also moving forward.

Also in a lot of neurologic disease you don't necessarily have a great interaction with the child, especially if the brain is affected in a way that doesn't enable good communication. Obviously there's always opportunities for having an interaction. But there was something very specific about taking care of a child who had a neuromuscular disease and the interaction that happens with that, that I found just unique and that also inspired me to want to work more with the children to find some way to help. The scientist in me thought well, there is something that's not known, and knowing this might ultimately impact how this child is able to move around the world and appreciate what's already a very good quality of life in a better way. It was very easy to see how children with neuromuscular disease, even though they have motor disabilities, have great quality of life, but also what the potential is with making some improvements. Then, if you could have this person be a little stronger that would make a big impact. That seemed like something that was a challenge but also something that could really make a big difference. That motivated me from the science end as well as the clinical end.

**Can you explain what myotubular myopathy is?**

Myotubular Myopathy - I always call it MTM\* - is a x linked genetic condition that's caused by a mutations in a gene called myotubularin. So as you probably know, a gene mutation leads ultimately to a problem with a protein. In this case it's the myotubularin protein. The myotubularin protein doesn't function properly and that results in the condition.

Because it's an x-linked gene, it affects primarily boys as there isn't a balancing copy of the gene – women have two X chromosomes. In boys since there is only one x-chromosome, the mutations result in clinical symptoms in boys.

**What causes myotubular myopathy?**

Now, the bigger question of what causes myotubular myopathy I think is interesting and I would say is still unknown. I think we know a lot about what myotubularin does, but we don't necessarily know why what it does, causes a muscle disease. And actually one of the things that my lab and others have really tried to understand is how to link what we know myotubularin does with why the muscle doesn't work right. What we know myotubularin does is act as an enzyme that breaks down a very specific lipid product in muscle and presumably other cells, but primarily muscle. That's this 'PI3P' (phosphoinositide 3 phosphate) - which is a very long term! I didn't understand anything about these things when I first started working on MTM: the term is phosphoinositide 3-phosphate (PI3P). So you've probably heard this, but it's an incredibly confusing long word. It has to do with a very specific type of lipid that has a specific modification on it. And the way I like to think about the phosphoinositides, or PIPS, is that they are like molecular zip codes/post codes. If you think about the cell from your old cell biology classes, it's like a fried egg shape- and you know you have the different components of that - you have the nucleus, you maybe have the golgi apparatus or the endoplasmic reticulum. Every one of these structures has a different zip code and that zip code is set by the PIPS. So the nucleus has specific PIPS around it, the membrane has specific PIPS around it, and there's a lot of communication between parts of the cell. Actually my son and I have been talking about this because he's been doing the cell right now, and in every text book it's like the cell has a nucleus and it's like a big bag of jelly. It's actually not true at all really. The structure is very highly organised. There is communication, always, between different parts of the cell. You'd have ways of bringing things back and forth that are really important. Shuttle, cargo that you move, and you need to do in a very productive way. And the PIPS are the things that set, 'okay, if I'm a golgi, I'm going to bring something there: it's got to have to have the right PIP in order to get there'. So basically it tells you - that's where you are going to go and once you get there you want to remove it because you have your old communication label. And so what, on some basic level, myotubularin does is control one part of this zip code. And the part of the postal code that it works on is something called the endosome - which is a communication station

inside the cell. Things come from the cell membrane and then when they get inside - whether it's a receptor, or protein or some kind of molecule - it comes into the structure in the middle of the cell called the endosome. Then that's the decision-making point. 'Do I want to get rid of it? - Do I want to bring it back to the cell? - Do I want to shoot it out and give it to another cell?' The endosome is what decides that. And for reasons we don't totally understand myotubularin is what helps make that decision - by removing this PIP. And so from a construction of that old fried egg cell, that is what it does - it influences its communication. And when you lose myotubularin - if you look at the cells of patients for example, this communication isn't done properly. If the cell doesn't know whether or not it's supposed to bring something and have it broken down, or send it back to the membrane, it gets trapped in this endosome.

### **What does that mean for the muscle?**

So the question is - and this is still my question - what does this mean for the muscle? I think we still don't understand why it is that this communication being abnormal causes a muscle disease, that causes muscle to be weak.

On the other hand we've done a lot of work to describe why the muscle doesn't work right and we've focused really on a specific structure in the muscle called the Triad. And it is called 'Triad' because it has three components. It's got something called a T-tubule, which is shaped like a 'T' and it comes down from the outside of the muscle membrane and it's linked up with two other things called the sarcoplasmic reticulum. And this Triad structure is responsible for releasing calcium inside the muscle, and for every muscle contraction you have to have this release of calcium first. This triad controls that release of calcium - if you don't have it you don't get a muscle contraction. If you look at muscle fibres from patients with MTM you see either no Triads or a reduced number of them. You can imagine if you have to have this structure in order to do a muscle contraction and you don't have the structure, then you won't have normal muscle contractions and that you'll be weak. At least my understanding, and I think that is accepted in the field now, is that's why muscle is weak.

I think my little side note would be that this was first discovered in our zebrafish. It speaks to the power of using animal models as a way to understand disease because this was something that was obviously occurring in the patient muscles, always, from the first description of the disease but hadn't been recognised before. And it took looking in our fish - because we didn't know what to look for - we were just looking around to see what was not normal about the muscle, to see this structure not being right. Then we went back to the patient muscle biopsies and saw also that it's not right there as well. And that's how we linked it. So now what we're trying to do from a disease perspective is link this - what we know myotubularin does on these endosomes and the PI3P - with

what's wrong with the muscle, and try to understand how to bridge that gap. I still think we don't understand yet how those things interact. But we have made, despite that, I think a lot of progress in terms of coming up with some strategies for therapy.