The importance of AGALopathy in Fabry disease

Welcome viewers, hello Fabrienne. It's been a while since Fabrienne last made a video. Part of the reason is that making videos is a tiring business, especially with a chronic genetic disease. But she also wanted to take her time to research some new topics. There is always the issue that we cannot research new data ourselves, so we have to rely on what is already published. But fortunately, that's a lot, and there's a lot of facts around that are not widely known.

Since our very first videos, we have mentioned endoplasmic reticulum stress as a secondary cause of Fabry disease, that is independent of lysosomal storage. Well, we had a research paper about this topic, but when we made our first videos, it was only a preprint and we couldn't quote it. But since, it has passed the peer review process and was officially published. In contrast to the preprint, the finished version included important data about Fabrienne's own D313Y variant. When she found out, she sat down with her home-made galactose lozenges to celebrate.

ER stress is not commonly explained in the context of Fabry disease, so let us explain. ER stress is a consequence of problems surrounding protein folding, which was extensively explained in Fabrienne's second video, healthy cells. A brief recap: Genetic information is stored in long strands of DNA, but the human body is 3D. That's why the body invented a method to convert the one-dimensional DNA information to 3D substances. Sections of DNA are copied to RNA and then translated to sequences of amino acids, which still form long strands, but the 20 different amino acids cause different folds and bends in the chain, producing a 3D protein. Some genetic mutations do not cause a complete loss of function of the proteins they encode, but only slightly affect protein structure. This may result in a moderate loss of activity or protein instability, meaning that the protein loses its shape much faster than a correctly folded protein.

Next, let's explain some of the relevant terms. Protein misfolding refers to the cause of the problem. As protein folding is generally an error-prone procedure, single misfolded proteins do not cause any problems. If, however, protein misfolding reaches a critical level, this condition is called endoplasmic reticulum stress, after the endoplasmic reticulum, the cell organelle where proteins are folded. Cells respond to ER stress with UPR, the Unfolded Protein Response. This reaction is threefold. One: downregulation of protein production in general through an enzyme called PERK. Two: upregulation of the folding capacity of the endoplasmic reticulum through the release of natural chaperones. And three: improved clearing of misfolded proteins through upregulation of Endoplasmic-Reticulum-Associated protein Degradation (ERAD). If the degradation mechanisms are overwhelmed, aggregates of misfolded proteins may form.[1] If normal protein production cannot be restored, cell death may be initiated.[2]

Now, what does the paper we mentioned earlier[3] say about the role of ER stress in Fabry disease? As part of a screening program of Czech patients undergoing kidney dialysis, 6 patients with the GLA variant L394P were identified. This variant causes a loss of enzyme function to around 15% residual blood α-galactosidase A activity, but no Gb3 deposits were

detected in the patients' tissue. That's because this level of enzyme activity still allows for much more Gb3 processing compared to classical Fabry mutations. At first, enzyme production in L394P patients appeared normal, but closer analysis revealed that only a fraction of the enzymes produced reached the lysosomes, while most were retained in early production stages. Both healthy and classical Fabry enzymes reached the lysosome normally. Additionally, several proteins involved in ER stress were found to be more abundant in L394P cells compared to healthy and classical Fabry cells. Treatment with a substance that facilitates protein transport to the lysosomes reduced the level of these ER stress markers in L394P cells, but not in healthy and classical Fabry cells. Treatment with migalastat improved protein production and trafficking in L394P cells, but also increased ER stress markers. Similar tests were conducted for other Fabry variants, and similar effects, in varying degrees of severity, were detected for most of these other Fabry variants.[3]

It has long been obvious that enzyme deficiency alone cannot explain everything we see in Fabry disease. A suggestion for the missing puzzle piece has been lyso-Gb3[4], but its usefulness, even as a biomarker is still being debated, even after 15 years.[5] As for understanding Fabry disease, it hasn't helped much. This new research might finally answer some of the open questions. The authors proposed a new name for ER stress in the context of Fabry disease: AGALopathy.[6] Other diseases of this kind are called proteinopathies, and as the protein in question is abbreviated AGAL, that yields AGALopathy. According to this concept, some Fabry variants are disease-causing mainly because of an enzyme defiency, others because of ER stress, and many missense variants may do a bit of both.[3]

According to the authors, AGALopathy might explain why women are often more severely affected than might be assumed based on enzyme deficiency alone.[3] Random X-chromosome inactivation might be part of the explanation, and cross-correction between healthy and Fabry cells doesn't really work[7], but adding AGALopathy to the mix would really make things easier to understand. Having a healthy copy of a gene might compensate for enzyme deficiency, which represents a loss of function, but does nothing to alleviate ER stress[3], when cells do more than they should, a negative gain of function. This is something we already mentioned in our video "Something is wrong in Fabry country". Additionally, the authors suggest that AGALopathy may explain why some patients don't respond to enzyme replacement therapy all that well, and why chaperone therapy sometimes shows adverse effects.[3]

As for Fabrienne, there are a lot more open questions surrounding Fabry disease, and this research offers whole new ways of answering them. The difference between classical and non-classical Fabry disease is hard to explain in terms of enzyme deficiency alone, as is the poor association between genotype and phenotype.[8] Factor V Leiden mutations have been described as one of the factors that influence the Fabry phenotype.[9] Is there a physiological system that is important for both Factor V Leiden and Fabry disease? Protein folding sure is.

The role of ER stress in Fabry disease might be greater than in other lysosomal storage disease, like Gaucher's disease, for two main reasons. For one, compared to Gb3 and Fabry disease, there are many more sphingolipids that degrade to glucocerebroside, the storage product in Gaucher disease's.[9] Gaucher's disease is like a traffic jam on a busy freeway, and

Fabry disease like a flat tyre on an offroad. Additionally, and this is highly unusual for a lysosomal storage disease, there is a second enzyme that can degrade Gb3.[10] In fact, this enzyme is so good at degrading α-galactose residues that it was initially called α-galactosidase B. It has since been determined that its primary substrate is α-*N*-acetylgalactosamine, and it has been accordingly renamed α-*N*-acetylgalactosaminidase, but the A in α-galactosidase A stuck. This second enzyme has some difficulty degrading short sugar chains, including Gb3, but nonetheless, Fabry cells with zero α-galactosidase A activity have a residual capacity for degrading Gb3 of around 15% compared to healthy cells.[10] For this reason, in laboratory tests α-galactosidase A activity is measured in the presence of an inhibitor of α-*N*-acetylgalactosaminidase.[11] Together, the relativily low abundance of Gb3 in comparison with other sphingolipids and the capability of α-*N*-acetylgalactosaminidase to cleave at least some amount of Gb3 mean that Gb3 is "a minor substrate that accumulates relatively slowly in αGAL-deficient cells".[12] And that is why we believe that the relative weight of protein misfolding is greater in Fabry disease than in Gaucher's disease, and research into ER stress in the context of Fabry disease is so important.

All in all, AGALopathy makes Fabry disease so much easier to understand that explaining Fabry disease without it feels like trying to explain plague without microbes. It's sure possible to invent ever new ways to adapt the data to the theory, but it's much easier to understand the complete picture of Fabry disease as described in scientific literature when taking AGALopathy into account.

Despite this, some authors question if AGALopathy is part of Fabry disease at all.[13] They mention theoretical reasons why the cell culture experiments that were used to prove the relevance of AGALopathy might not accurately represent what happens in actual patients. Questioning methods is a fair part of science, but if there are doubts, the proper way of going about is experimental research, not conjecture. Please note that this is not the first research regarding ER stress[14,15], and the previous ones were ignored as well. All in all, it looks like an attempt to prevent any understanding of Fabry disease beyond Gb3. That is dogma, not science. The following statement appears particularly questionable: "AGALopathy is thus not FD, which is based on an enzyme deficiency resulting in intracellular Gb3 accumulation. Thus, AGALopathy cannot be successfully treated with ERT."[13] Normally, a disease is defined by its cause, not its treatment. This is the kind of statement we'd rather expect from the pharmaceutical industry, not from clinicians with patient contact. But with the current push for substrate reduction therapy as a new treatment option for Fabry disease[16], no one wants to ask if Gb3 is the correct parameter to treat right now. And if all those patients with variants mentioned in the AGALopathy paper would be diagnosed with Fabry disease, an orphan status for new Fabry treatments would be out of the question, too. But if we look at the usual conflicts of interest[13], it appears very well possible that the authors' loyalty does lie with the pharmaceutical industry, not with their patients.

The question whether or not to include AGALopathy into the diagnosis of Fabry disease is not academic. There are real and practical consequences attached to it. Contrary to the statement that AGALopathy cannot be treated using ERT, we have heard of a number of D313Y patients reporting that ERT alleviated their gastrointestinal problems and pain. Possibly, ERT causes

cells to produce less α-galactosidase A of their own, thus alleviating AGALopathy. Likewise, Migalastat is not ideal, and might even have adverse affects, but we also have heard many positive reports about Migalastat treatment from D313Y and A143T patients. And to receive these treatments, a diagnosis is necessary. At the moment, AGALopathy is not a recognized diagnosis. There is no ICD code and no approved treatment. So, while neither ERT nor Migalastat is ideal for the treatment of AGALopathy, these are the best available treatment options for these patients. Denying them the diagnosis Fabry disease means denying them treatment. Again, this publication was written by clinicians with real and actual patient contact. We had hoped that they would find their satisfaction in alleviating their patients suffering, not in finding excuses to exclude those patients from treatment. And we have reliable information that the authors have repeatedly been told about D313Y patients with severe Fabry phenotypes, and that they are amongst the most dogmatic opponents of Fabry-specific treatment for these patients in Germany. And when we say excuses, we don't just refer to the exclusion of AGALopathy as an additional pathomechanism for Fabry disease. Additionally, the authors changed the original statement from "some patients do not respond to enzyme replacement therapy" to "AGALopathy cannot be successfully treated with ERT".

All in all, Fabry disease shows a strange disconnect between symptoms and diagnostic criteria. No patient visits a doctor because of zebra bodies in their kidneys, elevated lyso-Gb3 levels in their blood or Gb3 deposits in their heart muscle cells. However, in practice, such biomarkers weigh more than treatment success in actual patients. This is not a new phenomenon. In the initial clinical trials, TKT (Transkaryotic Therapies, later acquired by Shire) tried to prove that Replagal improves neuropathic pain.[17] Because of the slow changes in Fabry disease, no meaningful change could be established in the 6 month trial. Genzyme, instead, established Gb3 clearance from kidney tissue as the benchmark for the efficacy of Fabry treatment, based on an expected direct correlation with future clinical benefit[17], that still hasn't been proven[18,19,20]. In this video we see that this disconnect is extended even further, defining Fabry disease as "a condition that can be successfully treated using ERT".[13]

Fabry patients live with Fabry disease, Fabry experts live off Fabry disease. That means that both groups have different interests. For patients, it's best if Fabry were a trivial disease, whilst experts need an aura of mystery surrounding there field of interest. Or, Fabrienne, how many experts for common cold do you know? Me neither. But what Fabry disease is is not determined in academic disputes; it's what happening inside the cells of Fabry patients that defines this disease. Therefore, Fabry disease belongs to the patients, and the discourse regarding Fabry disease should be determined by the patients as well.

Progress in our understanding of Fabry disease requires openness for all factors that contribute to the whole complex of symptoms and other characteristics we observe. Protein misfolding has been suggested as a part of Fabry disease almost twenty years ago, and has repeatedly been covered up. We cannot let that happen again. All Fabry patients and patient organisations must communicate to their respective contacts on the professional side that they want AGALopathy to be included in future considerations of what Fabry disease is. Ask about the role of protein misfolding for your specific mutation when talking to your physician,

ask about AGALopathy in patient webinars, explain what you know to other patients. Only then can Fabry research move forward.

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