Report on analysis of the Folbigg CALM2 G114R mutation and its impact on calmodulin protein function

– prepared in connection with the 2022 Folbigg Inquiry

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Statements for the Inquiry:

Aalborg October 18, 2022

To whom it may concern:

I, Michael Toft Overgaard, acknowledge for the purpose of Rule 31.23 of the Uniform Civil Procedure Rules 2005 that I have read the Expert Witness Code of Conduct in Schedule 7 to the said rules and agree to be bound by it.

The opinions within this report are wholly or substantially based on my specialised knowledge as a professor in protein chemistry, and my experience within characterising the effect of genetic mutations at the protein level.

I have no prior experience with legal proceedings.

Yours sincerely,

Michael Toft Overgaard
Professor
Head of Department

…….

Aalborg October 18, 2022

To whom it may concern:

I, Mette Nyegaard, acknowledge for the purpose of Rule 31.23 of the Uniform Civil Procedure Rules 2005 that I have read the Expert Witness Code of Conduct in Schedule 7 to the said rules and agree to be bound by it.

The opinions within this report are wholly or substantially based on my specialised knowledge as a professor in personalised medicine, and experienced researcher within human genetics and linking variation in genes to diseases.

I have no prior experience with legal proceedings.

Yours sincerely,

Mette Nyegaard
Professor

Report on analysis of the Folbigg *CALM2* G114R mutation and its impact on calmodulin protein function

By Professor Michael Toft Overgaard and Professor Mette Nyegaard

This report has been made in accordance with a Letter of Instruction – 2022 Inquiry into the Convictions of Kathleen Folbigg, received on September 9, 2022 by email (copied in below for reference). The report is prepared in the form of answers to a list of questions given by the Letter of Instruction.

Fra: Rhanee Rego
Til: Michael Toft Overgaard; Mette Nyegaard
Emne: Folbigg Inquiry: Letter of Instruction
Dato: 9. september 2022 04:14:29
Vedhæftede filer: Letter of Instruction - Professor Michael Toft Overgaard and Professor Mette Nyegaard - 9 September 2022.pdf
Expert Code of Conduct.pdf
Legal Aid Expert Quote .docx

Dear Professor Toft Overgaard and Professor Nyegaard,

Please find **attached** a letter of instruction for your expert opinion for the Folbigg Inquiry. All details for your expert engagement are outlined in the letter. **Attached** is also the Expert Code of Conduct as referred to in the letter of instruction.

If there are any issues with the questions we have posed, please let me know.

We ask that you please provide your report to us prior to 19 October 2022 (we need to provide the report to the Inquiry on 19 October 2022).

The oral hearings relating to genetics and cardiology (of which you would fall into) will commence from 14 November 2022 for approximately two weeks. These hearings will be held in Sydney. Counsel Assisting the Inquiry is responsible for the calling of witnesses and as such, she will determine who comes to give oral evidence. Whether you will be called to give oral evidence will be a matter for determination in due course. We will communicate with you further on this point.

You will see in my letter it sets out payment details. **Attached** is a template you could use to provide us with a quote for payment. We ask that you please provide the quote as soon as you can, so we can submit it to Legal Aid and advise you of their decision.

Because you are engaged as an expert witness for this Inquiry, I would strongly recommend not speaking to media. Because there is a legal process underway, we are concerned to ensure the integrity of that process; we think experts talking to media at this stage may be negatively viewed by the Inquiry. The fact you have spoken prior to this time is fine, we just ask that you do not at this stage.

We look forward to receiving your quote and report in due course.

Kind regards
Rhanee

Rhanee Rego
Solicitor

Answers to questions from the Letter of Instruction

1. What is a calmodulin mutation?

A calmodulin mutation is a *change* in the calmodulin gene DNA leading to a *change* in one of the protein building blocks (amino acids), resulting in a calmodulin protein, that is different from the normal.

The body read the calmodulin gene DNA sequence as a recipe to make calmodulin protein. A calmodulin mutation is like a *typo* in the recipe, leading to an error in the produced protein. When we discover the typo (from sequencing), we can ***predict*** the effect of the mutation on the protein. When we manufacture the protein containing the mutation in the laboratory, we can ***measure*** the effect of the mutation on the protein.

In essence it is comparable to a typo in the recipe for making bread. Eg a typo changing *1 mg salt* to *1 kg salt (increasing the amount of salt 1 million times).* When we discover the typo we can, based on experience, ***predict*** how bad the bread will become. When we bake the actual bread, we can directly ***measure*** *(in this case taste)* how damaging the typo in the recipe actually is to the bread.

The discovery of the Folbigg calmodulin mutation in 2019 is similar to the first situation, where the damaging effect was ***predicted***. The *Europace* study in 2021 (Brohus et al., 2021) is similar to the second situation, where the damaging effect was directly ***measured***.

The Folbigg missense variant leads to a calmodulin protein, where amino acid residue number 114, a Glycine (abbreviated Gly or G), is changed into Arginine (abbreviated Arg or R). This is a dramatic change, as Glycine is the smallest of the 20 natural amino acid residues, with no side chain atoms and no charge, whereas Arginine is one of the largest amino acid residues and has a strong positive charge. This is illustrated in figure 3A in the *Europace* article (Brohus et al., 2021) – inserted with comments below for clarity (Figure 1).

The calmodulin mutations investigated in the *Europace* article was G114R, G114W and N98S. The effect of these mutations was determined by comparing the function with non-mutated normal calmodulin, included in all the tests performed (Brohus et al, 2021).

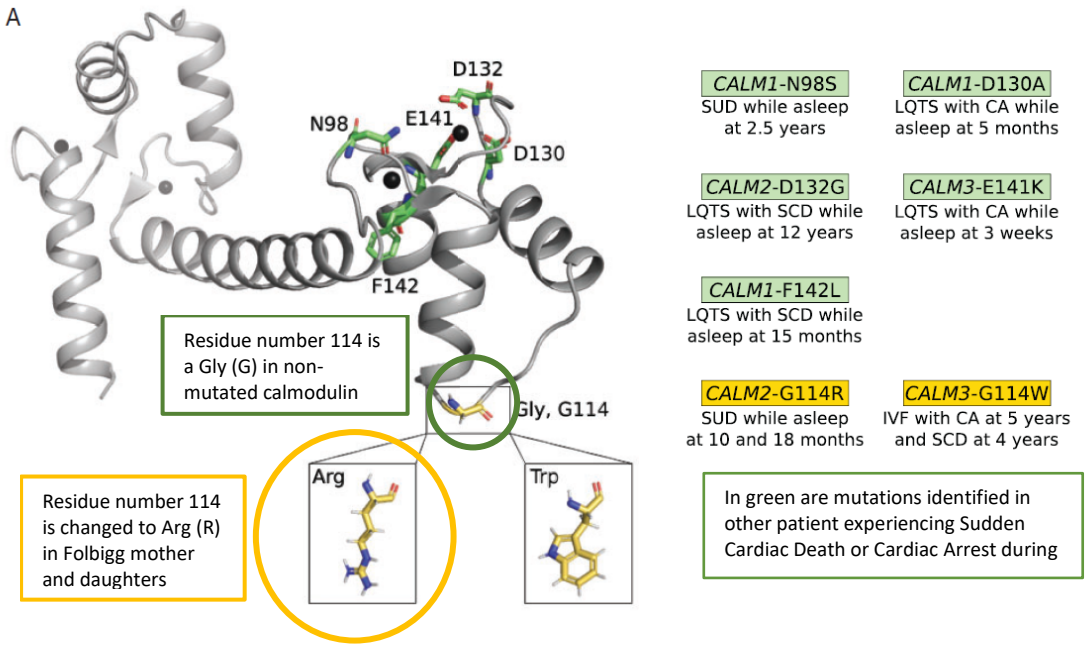

Figure 1. Three-dimensional structure of calmodulin in the calcium bound form (PDB 1CLL, www.wwpdb.org). The 4 calcium ions are shown as black spheres. Mutations at residues shown in green are associated with SUD/CA during sleep. The Folbigg mutation site, G114, is encircled in green. An Arginine residue (Arg, R) is encircled in yellow, for size comparison of the Folbigg mutation (G114R). A Tryptophan residue (Trp, W) is also shown for size comparison of the G114W mutation.

2. Are calmodulin genes unique? Why?

Yes, the calmodulin genes are unique for several reasons.

There are three calmodulin genes in the human genome, named as *CALM1, CALM2 and CALM3*. The calmodulin genes are special as they each independently encode the *exact same protein*. No other protein in the human body has, to the best of our knowledge, three different genes which encode the exact same protein.

I addition, the amino acid sequence of calmodulin is also the same ***in all vertebrate animals.*** It is highly unusual for so many species to have the exact same protein sequence. This demonstrates the importance of every single amino acid in calmodulin to be kept the same and demonstrates a strong selection pressure against any variation in calmodulin during evolution.

3. When was the first calmodulin mutation discovered? How was it discovered?

The first ever human calmodulin mutations were discovered by Mette Nyegaard, Michael Toft Overgaard and co-workers, published in American Journal of Human Genetics in 2012 (Nyegaard et al., 2012). It was discovered by investigating a large family from Sweden where several of the family members experienced seizures and adrenalin induced syncope's and two teenagers suffered sudden cardiac death during sport activities in school.

DNA is inherited as blocks. Using methods in the laboratory, we first identified the chromosome block that was inherited together with the disease. Subsequently, we sequenced this chromosome block, and we found a mutation in the *CALM1* gene (N54I). This was the first mutation ever identified in humans in any of the three calmodulin genes. During our attempt to publish our results, we had reviewer comments stating that they were sceptical about the finding, because calmodulin is so essential to the body, that mutations was considered incompatible with life in the scientific community.

After having established the new link between calmodulin and sudden cardiac death, we sequenced the *CALM1* gene in additional unrelated patients with unexplained cardiac arrhythmia and identified a second and different calmodulin mutation (N98S), causing a similar disease. This extra step - to find a second *unrelated* arrhythmogenic patient with a *different* mutation in the same gene - constitute the optimal and strongest genetic evidence when linking new genes to a disease.

4. Please detail each of your experience with calmodulin.

MTO: My expertise is in understanding and characterizing protein structure and function. I have particular experience in characterising the effect of calmodulin mutations on the calmodulin protein function, such as calmodulin binding to calcium, the calcium dependence of calmodulin binding to ion-channels - in particular the cardiac voltage gated calcium channel, Cav1.2, and the cardiac SR calcium release channel, RyR2. I have co-authored 15 scientific papers describing calmodulin mutations and how they impact the calmodulin protein.

MN: My expertise is within human genetics. I study how changes in DNA makes us different as human beings with respect to health and disease. I do that by studying the DNA from either families or large groups of people. Currently, I work to understand how other types of calmodulin mutations - for example mutations that turn the calmodulin on and off (called promoter mutations) - can cause disease. I also work to understand how some individuals can carry a calmodulin mutation without having arrytmia, by looking for modifier genes. It is through this work I have gotten an intimate understanding of how ***few*** calmodulin mutations, there are in the population.

5. What does 'rare' mean in nature with reference to genetic mutations?

We know that damaging genetic mutations are removed from the population by natural selection, with an efficiency that varies from gene to gene, depending on how critical the function of the gene is for the organism (Lek et al, 2016). Natural selection means that individuals carrying the mutation is less likely to survive or their offspring are less like to survive. The most important genes will become depleted in mutation, thus mutations become ultra-rare in the population.

When a gene has fewer mutations than expected, we say that ***the genes are constrained***. Highly constrained genes are generally dangerous to have a mutation in. Constrained genes are often involved in monogenic diseases, where a single mutation is enough to cause disease (Gardner et al, 2022).

It is known today, from having sequenced the DNA of more than half a million human individuals, that the three calmodulin genes are *highly constrained*. There is not a single calmodulin mutation, that has become frequent in the population. We can detect how constrained a gene is by comparing the *observed* number of mutations to the number of mutations that would be *expected* if there were no constraint on the gene (Lek et al, 2016; Samocha et al, 2017; Karczewski et al, 2020). Based on this measure, the three calmodulin genes are among the most constrained genes in the human genome. Our analysis shows that they in fact among the top 20 most constrained genes in our genome, out of 20,000 genes (unpublished). Thus, all three genes are among the top ***0.1% of most constrained genes*** in the human genome.

Taken together, this means that ***absence of variation*** is a powerful source of information about the consequences of gene mutations (Karczewski et al, 2020). So, with respect to genetic variation ***rare means bad.***

Because the calmodulin genes are highly constrained and mutations are ultra-rare, mutations in these genes are expected to be damaging.

6. Please describe the process undertaken when a previously unknown mutation is identified.

There are two situations.

Situation (1) The link between a gene and a disease is not known. Establishing a link for the first time between a gene variant and a disease often starts with a family, where the disease is clearly inherited. Researchers are searching for the exact block of DNA that is inherited as the same way as the disease. After having identified the DNA block (which often contain 50-100 genes), this block is sequenced to find the exact gene variant that is responsible for the disease. To be certain about the link, it is important to find other unrelated patient with the same disease, carrying a different variant in the same gene. This is the strongest genetic evidence for having identified a causal disease gene.
The work published by Nyegaard et al 2012 followed this procedure linking calmodulin variants with arrhythmia and sudden cardiac death.

Situation (2) The link between a gene and a disease is well-established. In this situation, the gene can be sequenced directly, searching for variants that are predicted to damage the protein. All three *CALM* genes are today well-established disease genes for severe cardiac arrhythmias such as CPVT and LQTS.
The work done by Professor Carola Vinuesa and her group, identifying a variant in CALM2, is similar to this procedure (described in Brohus et al, 2021).

To learn exactly what effect the variant has on the protein, two versions of the protein can be manufactured in the lab, one normal version (wildtype) and one version with the variant (abnormal protein). These proteins can be tested and compared. Sometimes other known faulty versions of the protein are also manufactured, to be able to compare to both the normal and several known pathogenic versions of the protein. See below for details *
The work published in Europace by Brohus et al includes this procedure.

* Details on laboratory work: in simple terms we insert a calmodulin encoding gene (with the mutation we want to study – or without any mutation for control) into bacteria. The bacteria produce the calmodulin protein, which we then harvest and purify to more than 99% purity. We use a protocol we have established and optimized since 2010. We then determine how the purified calmodulin can bind to calcium, and how it can bind to important calcium channels (such as the cardiac Cav1.2 and RyR2 calcium channels), using different calcium concentrations. This is done to mimic the different conditions calmodulin experiences in the

cardiac cells during a heartbeat (where the calcium concentrations vary: low calcium contraction in the relaxed cardiac muscle cell (diastole), high calcium concentrations in the contracted cardiac muscle cell (systole)).

7. What tests were used in the work done for the *Europace* article?

The tests employed for characterizing calmodulin mutations were:

1) Calmodulin's ability to bind calcium using fluorescence (Overgaard lab). The G114R mutation severely reduces the ability to bind calcium, at a similar level as the two cardiac arrhythmia mutations N98S and G114W (Illustrated in figure 3B in the *Europace* article (Brohus et al., 2021)). ***These tests demonstrated that the G114R mutation is damaging for calmodulin binding to calcium.***

2) The ability of calmodulin to bind the two cardiac calcium channels, Cav1.2 and RyR2, under different calcium concentrations using fluorescence polarization (Overgaard lab). The G114R mutation reduces the ability of calmodulin to bind to Cav1.2 and RyR2 at low and medium high calcium concentrations, in a manner similar to the two cardiac arrhythmia mutations N98S and G114W (Illustrated in figure 4 in the *Europace* article (Brohus et al., 2021)). ***These tests demonstrated that the G114R mutation is damaging for calmodulin binding to Cav1.2 and RyR2.***

3) Calmodulin effect on Cav1.2 function, tested using electrophysiology (in the laboratory of Professor Ivy E. Dick, University of Maryland). These tests demonstrated that the G114R mutation reduces the ability of calmodulin to sense the increase in calcium after the channel opens. Thus, the channel loses the ability to inactivate (close) properly in response to calcium – the so-called Calcium Dependent Inactivation (CDI) effect. The effect of the G114R mutation is identical to the effect of the G114W mutation, and slightly less severe than the impact of the N98S mutation (Illustrated in figure 5, A and B in the *Europace* article (Brohus et al., 2021)). ***These tests demonstrated that the G114R mutation is damaging for the Cav1.2 calcium channel function.***

4) Calmodulin effect on RyR2 function, using a so-called Store Overload Induced Calcium Release (SOICR) assay (in the laboratory of Professor S.R. Wayne Chen, University of Calgary). These tests demonstrated that the G114R mutation results in a decreased 'termination threshold' of RyR2 calcium channel opening, with an effect similar to G114W mutation (Illustrated in figure 5D in the *Europace* article (Brohus et al., 2021)). This is in contrast to normal calmodulin, where binding to the RyR2 channel increases the probability for the channel to close (seen as an increased 'termination threshold'). This mean that when G114R or G114W calmodulin binds to RyR2, each RyR2 opening releases an excess of calcium into the cardiac cell, compared to when non-mutated calmodulin binds (Illustrated in figure5 E in the *Europace* article (Brohus et al., 2021)). ***These tests demonstrated that the G114R mutation is damaging for the RyR2 calcium channel function.***

In summary, the biochemical and electrophysiological studies of the calmodulin G114R mutation show that it has deleterious effects on calcium binding and regulation of the two pivotal calcium channels involved in cardiac excitation contraction coupling, Cav1.2 and RyR2, in a similar manner to that of the pathogenic G114W and N98S variants (Brohus et al, 2021).

8. Had the tests in the work done for the *Europace* article been used before? Please provide examples.

1) The calcium binding test using fluorescence was initially described in the laboratory of Professor Madelaine A. Shea (VanScyoc and Shea, 2001). Since then, this assay has become one of the standard tests to determine the calcium binding ability of calmodulin, and in particular for human calmodulin mutations. At least 15 scientific articles have reported calcium binding effects of calmodulin mutations using this test (Some examples are: Crotti et al, 2013; Søndergaard et al, 2015a; Søndergaard et al, 2015b; Søndergaard et al, 2017).

2) The assay for determining the calcium dependent binding of calmodulin to binding domains of other proteins using fluorescence polarization, was first published in 2011 (Audran et al, 2013). The assay has been adapted and improved by our laboratory and used in 6 peer reviewed scientific articles, including the *Europace* article (Brohus et al, 2019; Brohus et al, 2021; Søndergaard et al, 2019; Søndergaard et al, 2020; Wang et al, 2018; Wang et al, 2020).

3) The electrophysiology test used for characterization of Cav1.2 function is a well-established technique for voltage gated calcium ion-channels. It was used in the seminal Nature article by the late David Yue's laboratory (first authored by Professor Ivy E. Dick) for demonstrating the absolute crucial role of calmodulin in mediating the calcium dependent inactivation effect (Dick et al, 2008). This assay was also the key experiment used in the article where the effect of human calmodulin mutations on Cav1.2 function was first demonstrated (Limpitikul et al, 2014 – co-authored by MTO and Professor IE. Dick). This particular article was the first to suggest that the calmodulin mutation LQT cardiac arrhythmia phenotype could be explained by a loss of Cav1.2 CDI.

4) The so-called SOICR assay, which evaluates the calcium channel RyR2 function, was established by Professor SR. Wayne Chen, initially for evaluating the effect of mutations in the RyR2 channel (Jones et al, 2008). The assay has been used in several high-profile scientific articles since then, and the assay was a key part in the article where Prof Chen demonstrated how important calmodulin is for correct termination of the RyR2 channel opening (Tian et al, 2013). It was also a key test in the recent Nature article, where intricate details on how calmodulin regulates RyR2 function was disclosed by several cryoEM structures combined with functional assays (Gong et al, 2019).

9. Can the tests used to determine the function of *CALM2* G114R be replicated?

Yes. All the experiments characterizing protein function were replicated at least three times for the *Europace* study, but the mutation effect on the calmodulin protein can be replicated in any other laboratory that can perform the same or similar tests. As detailed above, these tests have been used in several other scientific studies.

10. Is the *EP Europace* journal highly regarded?

The EP Europace journal is highly regarded and ranks among the top one third of all scientific journals within the topic of 'Cardiac and Cardiovascular Systems' (Web of Science, Journal Citation Report, 2021).

11. The article concerning *CALM2* G114R in the Folbigg family was accepted for publication in *EP Europace*. What significance does that have as a pointer to the validity and reliability of the tests used in the work done for the article?

The *Brohus et al.* article in *Europace* (Brohus et al., 2021) has been through a rigorous peer review process, where experts in the field anonymously review the methods, results and the conclusions drawn in the manuscript, and provide the editor their unbiased comments and recommendations. The published article can therefore be regarded as being of very high international standard in terms of the validity and reliability of the tests used and the conclusions drawn.

12. What is the concept of 'penetrance' in relation to genetic mutations?

Reduced penetrance can be exemplified as: If three individuals carry the same disease-causing mutation; two suffer from the disease, while the third is completely unaffected. Thus, if the penetrance is less than 100%, some carriers of a pathogenic gene mutation will not be affected by the disease. This is a very well-known phenomenon in genetics. It is relevant for essentially all mutations linked to disease. The more people carrying a particular mutation in the population, the more accurate we can calculate the penetrance.

13. What is the concept of 'variable expressivity' in relation to genetic mutations?

Variable expressivity can be exemplified as: If three individuals carry the same disease-causing mutation; two suffer a lot and one is only mildly affected. It is a very common and well-recognised phenomenon in genetics, that carriers of the same mutation are not affected to the same degree. It is seen for almost all known genetic diseases.

Reduced penetrance and variable expressivity of the phenotype is believed to be caused by ***modifier genes, epigenetics, or environmental factors*** or an interplay between these factors. If many people carry the same mutation, it is possible to calculate the penetrance of that particular mutation. In the calmodulin genes, mutations are so rare that it ***is not possible to perform any meaningful statistical calculations*** or estimates on the penetrance.

14. How does the concept of penetrance help us understand the *CALM2* G114R mutation in the Folbigg family?

It is already established that some calmodulin mutations have reduced penetrance. A reduced penetrance (lower than 100%) is seen for many other calmodulin mutations, in the small families in the calmodulinopathy register (Crotti et al, 2019). It is very likely that this is also the case for the *CALM2* G114R mutation. This would explain why the mother, who carries the mutation, is still alive.

15. How does the concept of variable expressivity help us understand the *CALM2* G114R mutation in the Folbigg family?

It is already established that some calmodulin mutations have variable expressivity (Nyegaard et al, 2012; Crotti et al, 2019). It is very likely that this is also the case for the *CALM2* G114R mutation. This would explain why the mother, who carries the mutation, has not experienced a cardiac arrest.

The multiple syncopes the mother has experienced, suggest that *CALM2* G114R mutation have variable expressivity with a milder expression. This is similar to the variable expressivity seen for the *CALM1* N54I mutation described in Nyegaard et al, 2012.

16. Is it accepted that mutations in *CALM* genes can cause varied expression including fatal arrhythmias?

Yes, this is seen in other cases and families. One example being the initial identification of the first human calmodulin mutation *CALM1* N54I, where the pathogenic mutation was present in a number of individuals in a large family. The mutation carriers in this family displayed a highly variable expressivity. The phenotypic picture was characterized by CPVT like features with symptoms including frequent syncope and three cases of sudden death or cardiac arrest. The affected family members displayed no other apparent clinical manifestations (Nyegaard et al, 2012).

17. Is it accepted that mutations in *CALM* genes can cause varied expression including less severe symptoms such as syncope?

Yes, see answer to question 15 and 16.

18. Can a person be alive, walking around and carrying an established pathogenic mutation, but not die?

Yes, this is possible. This is also what we see at the moment when we look at sequencing data from very large groups of adults from the general population. These cohorts are special as they contain individuals that are by definition alive at the time they sign up to have their DNA sequenced, so they will be enriched for people having strong modifier genes (also

called the healthy inclusin bias). These cohorts are very important for understanding the penetrance and variability of different pathogenic mutations (Kingdom et al, 2022).

19. Do we yet fully understand how this can be so?

We do not fully understand this at the moment. Understanding how mutations can cause disease in some people and not in others is an area of intense research for many monogenic diseases at the moment.

20. Are there examples of other families like the Folbigg family where people in the family are affected differently by the same mutation?

Yes, there are other examples of this in the scientific litterature. One example being the large four generation Swedish family from the initial calmodulin mutation identification (Nyegaard et al, 2012). Also, in the family where the *CALM3* G114W mutation was identified, the non-affected mother carried the mutation while her two children experienced cardiac arrest and sudden cardiac death (See pedigree in figure 2B in the *Europace* article (Brohus et al, 2021)).

21. Could the *CALM2* G114R variant have caused a lethal arrhythmia for either or both of the female Folbigg children?

Yes. Given the biophysical and functional impact of the *CALM2* G114R variant, we consider that the mutation likely caused the natural deaths of the two female Folbigg children.

In our opinion, based on the available research data and understanding of human genetics, the *CALM2* G114R mutation is enough to have caused the death of the two children.

Aalborg, October 18, 2022

Michael Toft Overgaard
Professor in protein chemistry

Mette Nyegaard
Professor in human genetics

Additional clarifying questions:

What is known about the mechanism for arrhythmogenic calmodulin mutations?
Following the initial discovery of the first two calmodulin mutations and the link to a form of severe cardiac arrhythmia (CPVT) and sudden cardiac death, several new calmodulin mutations in other positions in the protein have been identified in patients suffering from cardiac arrest, sudden cardiac death and/or cardiac arrhythmias including LQT syndrome and IVF. The most comprehensive overview of calmodulin mutations and resulting disease has been collected by Professor Peter Schwartz and co-workers in the so-called international calmodulinopathy registry (iCalmR) published in 2019 (Crotti et al, 2019). We were asked to write an editorial commentary to accompany this important work (Nyegaard and Overgaard, 2019). An overview of the iCalmR mutations is presented in Figure 2 below. Of note, the majority of mutations found in patients are in the right half (called the C-domain) of the calmodulin protein. This is also where the Folbigg mutation is found.

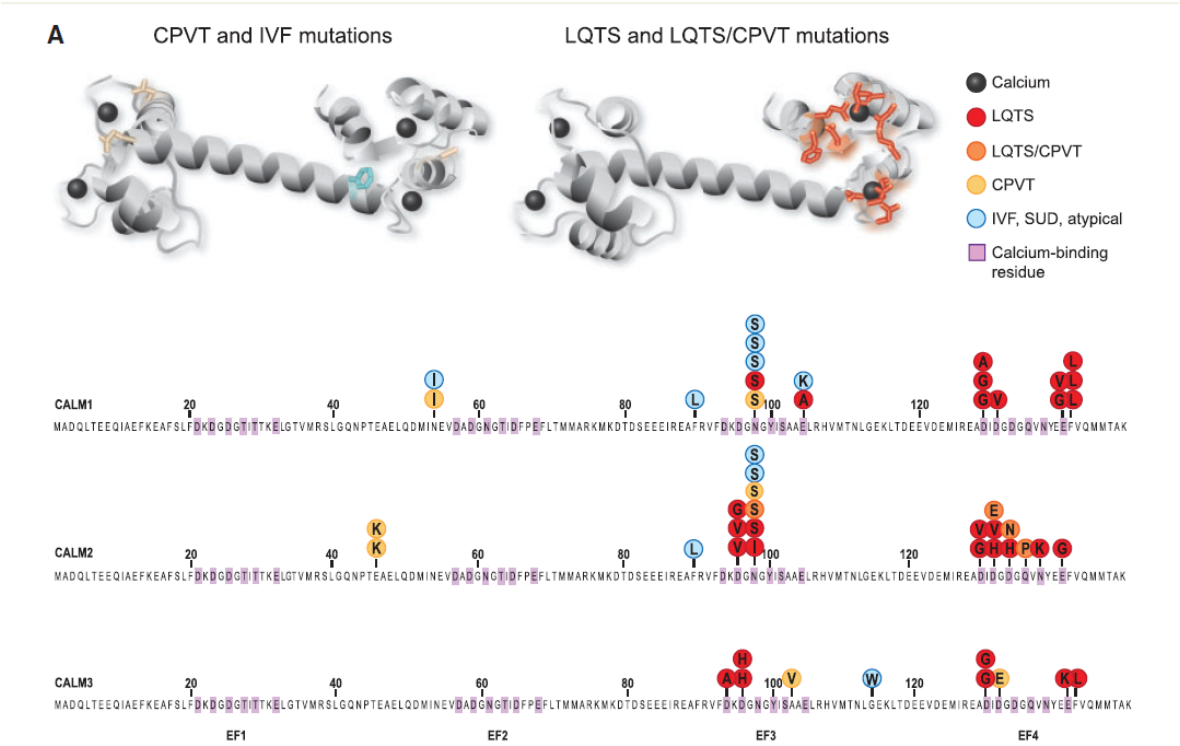

Figure 2. Position of arrhythmogenic calmodulin mutations.
Upper panel: pathogenic mutation sites shown in 'Stick' representation on the calcium bound calmodulin structure.
Lower panel: Individual founder mutations in *iCalmR* shown on the protein encoded from the representative *CALM* genes. (Nyegaard and Overgaard, European Heart Journal, 2019)

What is the known pathological mechanism for calmodulin mutations?
An increasing number of arrhythmogenic calmodulin mutations are discovered. The impact on calcium binding have been determined for some of these mutations. With the current knowledge available, there is a clear correlation between a mutation effect on calcium binding, and the length of the QT interval in the individual carrying the mutation.

- Calmodulin mutations that ***strongly reduces*** calcium binding have a ***very severe*** effect on the LQT interval causing early onset and severe disease (Figure 3). One example is the first three published LQTS mutations in *CALM2*: D96V, D130G, and F142L (Crotti et al 2013).
- Calmodulin mutations with a ***less severe effect*** on calcium binding affinity, results in a ***milder effect*** on the QT-interval. A good example of such a mutation impact is the N98S mutation (Limpitikul et al, 2014). In some individuals this mutation leads to mild LQTS. Other carriers of a N98S mutation display a mixed LQTS/CPVT phenotype, and yet others are diagnosed with CPVT (Crotti et al, 2019).
- Calmodulin mutations with ***minute or no measurable*** effect on calcium binding does not seem to affect the Cav1.2 channel function (Limpitikul et al, 2014) and have ***no effect*** on the QT interval. However, these mutations may still lose the ability to inhibit the cardiac RyR2 calcium release channel causing CPVT. The first identified calmodulin mutation, N54I, is an example of this (Nyegaard et al, 2012; Søndergaard et al, 2015a).

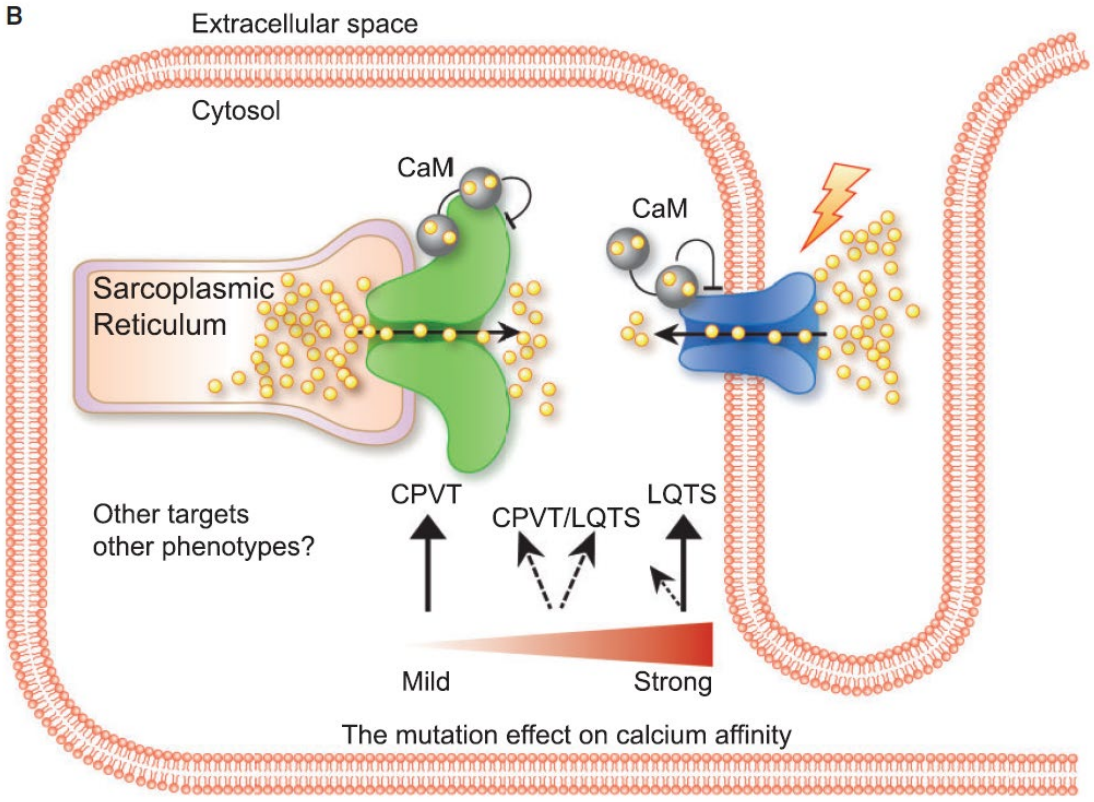

Figure 3. Schematic illustration of a cardiomyocyte and the main molecular mechanism for LQTS, CPVT and mixed phenotypes, indicating the main calmodulin targets (RyR2 in green and Cav1.2 in blue). Yellow spheres represent calcium ions. CaM: calmodulin. (Nyegaard and Overgaard, European Heart Journal, 2019)

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